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Article

Response of Winter Wheat (*Triticum aestivum* L.) to Selected Biostimulants under Drought Conditions

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Abstract: To prevent the staggering degradation of the environment, restrictions in the use of plant protection products and fertilizers are being strengthened every year. Therefore, methods for improving plant tolerance to unfavorable environmental conditions are sought to positively affect both plants and the natural environment. Here, we evaluated and compared the efficacy of four commercial biostimulants on the tolerance of winter wheat to drought stress. The effects of the following biological agents: *Bacillus* sp., soil bacterial strains, free amino acids, and humic substances on winter wheat were assessed in a pot experiment under full hydration soil moisture and under drought. Among the studied biostimulants, the two based on bacterial strains had the strongest beneficial effects on improving the tolerance of wheat plants to drought. In plants treated with either of these two, the highest level of CO_2 assimilation was recorded under drought. Moreover, in the same plants, the decrease in transpiration value due to drought was the smallest. The highest stomatal conductance under drought was also noted in these same plants. The results of chlorophyll fluorescence also indicate the smallest damage to the photosynthetic apparatus in the plants on which these bacterial biostimulants were used. Under drought, the lowest initial fluorescence values were noted for these bacterial preparations, as were the highest values of maximum fluorescence. On the other hand, a parameter indicating stress was reduced due to drought in all plants, except for those treated with one of these preparations. Another parameter showing the efficiency of the use of light photons in the photosynthesis process increased only in plants treated with one of these preparations, whereas for other plants it decreased due to drought, with the smallest decrease observed in plants treated with the other preparations. The most effective work of the photosynthetic apparatus in such treated plants was observed by the fastest transport of electrons through photosystems under drought. Additionally, under drought, the highest grain yield was obtained in plants treated with one of these bacterial preparations. The drought stress resistance index indicated that among all tested formulations, plants treated with either of these bacterial preparations scored the best. The use of these two biostimulants is recommended for comparative efficacy studies in the field, to help combat the drought-related yield losses of wheat.

Keywords: abiotic stress; biostimulation; chlorophyll fluorescence; photosynthesis

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1. Introduction

The sowing crops in Poland are predominantly represented by cereals. Wheat and other basic grains such as rye, barley, or oats can be grown in any part of Poland owing to the favorable climatic conditions [1]. Among about 20 subspecies of wheat, the cultivation in Poland is dominated by common wheat (*Triticum aestivum* ssp. *vulgare*), which belongs to a profitable crop [2]. 'Bogatka' is a bread variety of common wheat belonging to the technological group B. 'Bogatka' is characterized by a very good yielding potential under Polish climatic conditions, is characterized by high weight of a thousand grains, frost tolerance, and gluten content, as well as good resistance to fungal pathogens and lodging [3,4].

Plants grown under natural environmental conditions are exposed to the adverse effects of many stress factors. One of such most common abiotic stress factors is drought [5–7]. Water deficit in plants is caused by atmospheric drought, which may derive from high temperature, low air humidity, or increased water transpiration. The dates of agrotechnical treatments should be adapted to the prevailing climatic conditions, as a result of which the conditions for growing thermophilic plants may be improved [8]. It is also recommended to use biologically active substances, including fertilizers, plant protection products, and biostimulants. The use of plant biostimulants can improve flowering, fruit setting, and nutrient use efficiency, as well as stimulate plant growth and increase tolerance to abiotic stresses [9,10]. Biostimulants are classified as per the biologically active substances present, which are to improve the tolerance of plants to abiotic environmental stresses [11]. Biostimulants act on plants' metabolic and enzymatic processes to improve productivity and crop quality as well as assist plants to cope with abiotic stress [12]. The effects of the biostimulators may be multifaceted and the effects of their activities vary depending on the type of biostimulant used and the plant variety, but in the end most of them have a beneficial effect on crops [13]. There is no legal or regulatory definition of plant biostimulants anywhere in the world, which precludes a categorization of the substances and microorganisms covered by the concept. However, some major categories are widely recognized by scientists, regulators, and stakeholders [14]. According to the same study, the main categories of plant biostimulants include humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds, and beneficial fungi and bacteria.

One of the biostimulants used in the experiment was the 'Naturvital®-Plus', a preparation containing humic substances of equal proportions and purity. The content of this biostimulator is 21% of total humic extract, or 14% of humic acids and 7% of fulvic acids. Humic and fulvic substances are the main components of lignites, soil, and peat, which are produced by the biodegradation of organic matter resulting in a mixture of acids and carboxyl groups. Fulvic acids are humic acids with a higher oxygen content and lower molecular weight [15]. As a biostimulant, humic acids exert many effects on plant growth, yield, and protection under several analyzed abiotic stresses [16]. The key mechanisms targeted by humic and fulvic acids contain preparates which are roots targets; nutrient availability and metal chelation, as well as whole plant responses; reactive oxygen species (ROS) scavenging, osmoprotection, membrane stability, and ion homeostasis [17].

Another two preparations used in our experiment were from the bacterial group of biostimulants. Bacteria with the capacity to fix atmospheric nitrogen (N_2) symbiotically belong to many different genera, *Bacillus* being only one of those potentially used in biostimulation. Several mechanisms have been reported for how specific microbial inoculants stimulate plant growth and nutrient uptake, including asymbiotic nitrogen fixation, solubilization of nutrients, sequestering of iron by production of siderophores, and production of volatile organic compounds (VOCs) [18]. 'BaktoKompleks' is a natural biopreparation composed of five strains of soil bacteria of the *Bacillus* genus (1,000,000,000 in mL) selected from Polish soils. The use of bacterial strains from the place/climate where they will be used is extremely important for the effectiveness of the preparation. Moreover, the use of bacteria in the form of spores enables a wide spectrum of product activity and achieves a rapid effect. According to the manufacturer's information, the 'BaktoKompleks' preparation accelerates the decomposition of organic matter, as it catches crops, manure, or harvest residues in the soil. It affects the production of plant hormones and stimulates the development of microflora accompanying root growth. Its application improves the soil structure, allows plants to use previously unavailable nutrients present in the soil and is a catalyst for soil-localized biochemical reactions that affect the balance of nitrogen, phosphorus, and sulfur compounds. The second bacterial biostimulator used in our experiment was the 'Biomega' preparation, containing strains of *Bacillus velezensis* bacteria. These bacteria create biofilms, are responsible for the production of antimicrobial metabolites, and induce plant immunity. *Bacillus velezensis* has been shown to be active against fungal pathogens [19,20]. Moreover, similar to other *Bacillus* sp. species, they produce endospores that are highly resistant to adverse environmental conditions and support the survival of vegetative cells under field conditions [21]. According to the manufacturer's information, 'Biomega' provides nitrogen bound from the atmosphere, supports the metabolic activity of plants at the root level, and unlocks phosphorus in the soil.

Another biostimulator used in our experiment was the 'Raiza-Mix', a preparation that contains brown algae (*Ascophyllum nodosum*) in which the presence of cytokinins, gibberellins, and auxins was observed. Phytohormones derived from algae stimulate the production of amylase and the seed germination process, induce root formation, and stimulate cell growth [9,14]. According to OOsten et al. Ref [17], the key mechanisms targeted by algal-based preparations focus at the same time on root targets (root zone water availability and root ethylene and auxin levels), shoot targets (stomatal regulation and xylem hydraulic conductance), as well as on whole plant responses (ROS scavenging, membrane stability, and osmoprotection). In addition, the preparation contains free amino acids, including proline and serine, as well as macro- and micronutrients. There is considerable evidence that the exogenous application of a number of structural and non-protein amino acids, including glutamate, histidine, proline, and glycine betaine, can provide protection from environmental stresses or are active in metabolic signaling and have roles in plant defense [18]. Biostimulants containing free amino acids, including glutamine, betaine, or proline, increase the nitrogen assimilation and stimulate the plant metabolism of carbon and nitrogen [11]. The micronutrients contained in the preparation, such as zinc, molybdenum, manganese, copper, iron, or boron, affect the proper development of the root system and improve nitrogen management, the efficiency of the photosynthesis process, and pathogen resistance.

The aim of this study was to evaluate the effectiveness and compare the effects of the selected preparations based on the following biological active substances: soil bacteria, strains *Bacillus* sp., free amino acids, and humic substances, to enhance the winter wheat tolerance to drought stress.

2. Materials and Methods

2.1. Plant Material and Growing Condition

The pot experiment was carried out in the garden (the periods from sowing seeds to the induction of drought stress and from the end of physiological measurements to harvest), as well as in the greenhouse (during drought stress) and phytotron (during physiological measurements) that belong to the Department of Agronomy at the University of Life Sciences (Poznań, Poland). The experiment was carried out in 2021 as a two-factor experiment with three replications. The first analyzed factor was presence of drought stress. Control plants were kept at optimal hydration of 20–22% of soil volumetric moisture, whereas in the drought-stressed plants soil moisture was reduced to 6–8% of soil volume moisture. The second analyzed factor was the use of biostimulants (Table 1).

Table 1. Active substances and trade names of biostimulants used in the study.

No.	Active Substances of Preparation	Method of Application	Trade Names of Biostimulants
1	Control	spray with distilled water	-
2	5 strains of soil bacteria of the genus *Bacillus* (1,000,000,000 per mL)	soil spraying after sowing	'BaktoKompleks'

Table 1. *Cont.*

No.	Active Substances of Preparation	Method of Application	Trade Names of Biostimulants
3	Humic acids: 14%, fulvic acids 7%, potassium (K_2O) soluble in water 6%	foliar spraying	'Naturvital®-Plus'
4	Algae Ascophyllum nodosum, 20 free amino acids including proline and serine (14.16% by volume), phytohormones including cytokinins, auxins and gibberellins, N (2.27% by volume), B (0.24% by volume), Cu (0.12% by volume), Fe (1.30% by volume), Mn (0.59% by volume), Mo (0.024% by volume), Zn (0.24% by volume)	seed treatment	'Raiza-Mix'
5	Strains of soil bacteria *Bacillus velezensis*	seed treatment	'Biomega'

Pots with a capacity of 6 L were filled with the ready substrate, KRONEN® BIO soil for herbs and vegetables. A ready-made, commercial medium, which was used in many previous experiments, was used [5,22,23]. Soil quality specification: pH (5.2–6.3); Electrical conductivity (EC): below 90 mS·m^{-1}; Particle size: fraction 0–5 mm. Seeds were treated before sowing with 'Raiza-Mix' and '*Bacillus velezensis*' at a dose of 200 mL·100 kg^{-1} of seeds in a standard water dose. Then, 10 grains per pot of winter wheat of the 'Bogatka' variety were sown. The 'Bogatka' wheat used in the experiment is a Polish variety bred by DANKO Hodowla Roślin sp.z o.o. and registered with KRO in 2004. After sowing, the soil was sprayed with the 'BaktoKompleks' preparation at the dose of 1 L·ha^{-1}. At the tillering stage, foliar spray was applied with the preparation 'Naturvital®-Plus' at the dose of 3 L·ha^{-1}. The treatment was carried out using a Kwazar Venus Space 2 L pressure hand sprayer, at a working pressure of 0.2 MPa. At the end of tillering stage, the seedlings were thinned, leaving 5 plants per pot with the greatest morphological uniformity assessed visually.

Abiotic stress was carried out in the BBCH 69 phase in the greenhouse (60% to 80% relative humidity, 20 to 25 °C, 16 h day and 8 h night). The soil volume moisture content was monitored daily using ThetaProbe probe (Eijkelkamp, Giesbeek, The Netherlands). At 12 days after the abandonment of watering, the soil humidity of the drought-treated pots was at the level of 6–8% by volume. That humidity makes the water difficult for plants to access, resulting in a visible loss of turgor in the leaves. For the control plants, an even, optimal soil humidity of 20–22% was maintained.

2.2. Physiological State of Plants

The physiological status of wheat plants after the application of selected biostimulants was assessed both in plants that were exposed to drought stress and in plants that grew under optimal hydration (Figures S1 and S2).

Measurements of the physiological status of plants were made after placing them in a phytotron (at a constant air temperature of 25 °C and 70 ± 5% relative humidity), in the dark for 6 h in order to suppress photosynthesis. The measurement was carried out in the same order of objects and repetitions. Control plants were measured alternately with the stressed plants. Gas exchange and chlorophyll fluorescence were measured on the same, youngest, and fully developed leaf.

2.2.1. Gas Exchange

Photosynthetic activity was assessed by measuring gas exchange with the instrument LCpro-SD (ADC BioScientific Ltd., Hoddesdon, UK) using the parameters: A—CO_2 Assimilation Level (µmol·m^{-2}s^{-1}), E—Transpiration Level (mmol·m^{-2}s^{-1}), Gs—Stomatal Conductance (mol·m^{-2}s^{-1}), Ci—Intercellular CO_2 Concentration (vpm).

The LCpro-SD instrument settings were matched to the experiment according to the manufacturer's instructions [24]. The air flow to the measuring chamber (u) was kept at 200 µmol s^{-1}. The concentration of H_2O (reference H_2O) was set to ambient, i.e., the concentration actually encountered in the environment. The PPFD-photosynthetic photon flux density, which is the light intensity in the measuring chamber emitted by the red and blue LEDs of the spectrum in the proportion of 10:1, respectively, was 400 µmol·$m^{-2}s^{-1}$ (LCP Narrow Lamp, ADC BioScientific Ltd., Hoddesdon, UK). Gas exchange measurements were run in triplicate.

2.2.2. Plant Chlorophyll Fluorescence

A score of parameters related to fluorescence were measured with a Fluorometer OS5p (Optisciences Inc., Hudson, NH, USA) using a PAR clip. In the experiment, a kinetic protocol was chosen that combines measurements in light with measurements of dark-adapted plants. It allows for the determination of parameters confirming the occurrence of stress in plants. We measured the following indices: F0—minimum fluorescence, Fm—maximum fluorescence, Fv/Fm—maximum PSII quantum yield in the dark-acclimated state, Y (PSII)—PSII quantum yield in the light-acclimated state, ETR—electron transport rate. The fluorimeter settings were selected for the experiment according to the manufacturer's instructions (OS5p User's Guide, The standard in Plant Stress Measurement, Opti-Sciences, 040113), and our previous experiments on wheat plants [23]. Fluorescence measurement was performed in triplicate, analogous to the measurement of photosynthetic activity.

2.3. Relative Water Content (RWC)

Using *f.m.* (i.e., fresh mass of the leaves), the *RWC* measurements were carried out in accordance with the Weatherly method [25]. *RWC* was calculated based on the formula:

$$RWC = \frac{f.m. - d.m.}{f.m.\ in\ full\ turgor - d.m.}$$

where *f.m.* = fresh mass of leaves, *d.m.* = drought mass of leaves.

Two-centimeter cuttings of leaves were sampled both from drought-stressed and control (optimally hydrated) plants and weighed thrice. The first weight assessment was conducted immediately after sampling (*f.m.*). After distilled water soaking at room temperature for 4 h, a second weighing was made (*f.m.* in full turgor). Subsequently, the plant samples were dried for 4 h at 70 °C and the last weighing was carried out (*d.m.*). Measurements were made in triplicate per pot.

2.4. Estimation of Drought Resistance Index (DRI)

Using the results of physiological measurement parameters, an equation using a 0 to 1 scale was developed for all biostimulants, based on which the plant tolerance to drought stress was assessed. The following input data were used in the following equation: A—CO_2 assimilation level, E—transpiration level, Yield—quantum yield of the photochemical reaction in PSII, all weighted equally [26].

The plant physiological activity (PPA) index was calculated from the following equation:

$$PPA = [(0.33 \times \text{A}) + (0.33 \times \text{E}) + (0.33 \times \text{Yield})] \times 100\ [\%]$$

Based on the *PPA* index calculated for the control plants and for the drought-stressed plants, *DRI* was calculated as per the following formula:

$$DRI = \frac{PPA\ drought}{PPA\ control}\ [\%]$$

2.5. Yield and Yield Parameters

The collection of spikelets and the cuttings from plants were carried out after the wheat plants reached physiological maturity (BBCH 92). Then, the following yield and yield components were determined: grain yield (g per pot), spikelets yield (g per pot), mass of thousand grains (MTGs; g), plant height (cm), and spikelets length (cm). The height of the plants and the length of the spikelets were measured using a tape measure and expressed in centimeters. The cleaned spikelets were weighed, and the spikelet yield was determined; then, they were threshed, and the grain yield was determined using an analytical balance. The MTGs was determined by taking a sample, which was divided into three samples of 100 grains. The samples were weighed. The final value was the product of the average weight of the samples and 10.

2.6. Statistical Analysis

The experiment was conducted once, from 19 April 2021 to 29 July 2021. Throughout the experiment, the data were collected based on three biological replicates (pots) and 3 technical replicates (measurements), unless stated otherwise. We collected the data at germination stage, at application of the drought stress stage, at post-drought recovery, and at full turgor. To analyze the effects of drought presence/absence and of biostimulant formulation on the physiological status of the seedlings, we applied the two-way ANOVA, type I, on the data for each parameter whose methods of assessment are described above. Both ANOVA and the subsequent Fisher's least significant difference (LSD) test at $\alpha = 0.05$ were implemented in Statistica 13.3 (Dell Software Inc., Round Rock, TX, USA) and were used to assess the significance of differences among the means. For each given parameter, if the means differed at the significance level of 0.05, they are marked using different letters. The values of the LSD $_{\alpha = 0.05}$ are presented for each parameter with single-factor effects disregarded, as we detected the factorial interactions throughout.

3. Results and Discussion

3.1. Physiological State of Plants

3.1.1. Gas Exchange

The level of carbon dioxide fixation is an important indicator for measuring the photosynthetic activity of plants [27]. The essential physiological response to drought is a decrease in CO_2 assimilation and photosynthetic activity as a result of stomata closure. Have the used biostimulants influenced the physiological defense mechanisms against the effects of drought? Many studies revealed commonalities among the plant responses to different biostimulants; these included the increased root development, the enhanced nutrient uptake, and the abiotic stress responses [18,28,29]. In our experiment, we did not investigate the tolerance mechanisms, but tried to determine whether the applied biostimulants significantly improved wheat tolerance to drought. The level of CO_2 assimilation differed significantly between the control plants and those subjected to drought stress, as well as between the biostimulants used. Both examined factors also significantly influenced the remaining gas exchange parameters: Gs, E, and Ci. A significant decrease in the level of CO_2 assimilation under drought was noted for all tested biostimulants. The strongest response to stress was observed in plants biostimulated using the 'Raiza-Mix', where the value of parameter A decreased by 71.8%. In turn, for control plants, in which the biostimulator was not applied and the weakest reaction to stress was noted, the decrease in the value of parameter A was 60.4%. However, in the control plants, the values of this parameter, both under irrigation conditions and under drought, were the lowest. The highest values of the A parameter, both under drought and under optimal hydration, were recorded in plants treated with the 'BaktoKompleks'. Plants biostimulated using *Bacillus thuringiensis* showed much higher rates of net assimilation as well as stomatal conductance compared to the non-treated controls, regardless of the drought imposition [30]. Additionally, wheat seedlings treated using ACCd containing the *Bacillus subtilis* strain LDR2 exhibited a better photosynthetic efficiency under drought stress [31]. We observed significant decreases in the values of the E parameter in all plants tested (under drought) in our study. The strongest reduction in transpiration was noted for plants where the 'Raiza-Mix' biostimulator was used (by 63.8%), whereas the smallest decrease was observed for plants treated with the '*Bacillus velezensis*' (decrease by 41.7%). The imposition of drought caused significant decreases in the Gs parameter for all tested plants, compared to their non-stress exposed controls. The largest decrease was recorded for plants where the 'Raiza-Mix' biostimulator was used (by 70%), and the lowest decrease was recorded for plants treated with '*Bacillus velezensis*' (by 8.0%). The plants treated with the 'BaktoKompleks' biostimulator were the only ones among the examined plants that did exhibit significant increases in the Ci parameter under drought (an increase of 2.7%) (Table 2). Inoculation of the wheat seed with beneficial plant growth promoting rhizobacteria (PGPRs) improved Fv/Fm, net CO_2 assimilation, stomatal conductance, and transpiration rate under drought conditions. Such enhancements of photosynthesis lead to increased production of biomass, assessed as dry weights and lengths of shoots and roots [31]. The *Bacillus* ssp. effect on improving drought tolerance is based on the enhanced uptake of nutrients, both macro- and micronutrients. Previously suggested indirect mechanisms behind this phenomenon included root biomass increase, area of root surface increases, or number of root hair increases [18]. *Bacillus* treatment provided systemic effects that involved metabolic and regulatory functions supporting both growth and stress management [32]. Taking into account that all plants had similar soil moisture, higher values of CO_2 assimilation and transpiration in plants treated with bacterial biostimulators prove their greater ability to manage water. This may be due to the improved growth of the root system, due to which the stomata did not close as a result of drought, and the plants did not inhibit gas exchange.

Table 2. Effects of application of biostimulants and imposition of drought on the photosynthesis-related parameters: A—level of CO_2 assimilation, E—level of transpiration, Gs—stomatal conductance, Ci—concentration of intercellular CO_2.

Biostimulant	A (μmol·m^{-2}s^{-1})		E (mmol·m^{-2}s^{-1})		Gs (mol·m^{-2}s^{-1})		Ci (μmol·mol^{-1})	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Control	4.483 ^{e}	1.777 ^{j}	1.200 ^{cd}	0.517 ^{f}	0.067 ^{d}	0.027 ^{f}	241.0 ^{e}	263.0 ^{cd}
BaktoKompleks	10.58 ^{a}	3.940 ^{f}	2.173 ^{a}	1.090 ^{d}	0.167 ^{a}	0.075 ^{cd}	244.0 ^{e}	250.5 ^{de}
Naturvital®-Plus	8.475 ^{c}	2.083 ^{i}	1.785 ^{b}	0.653 ^{ef}	0.120 ^{b}	0.043 ^{ef}	239.5 ^{e}	299.0 ^{a}
Raiza-Mix	10.19 ^{b}	2.873 ^{h}	2.047 ^{a}	0.740 ^{e}	0.160 ^{a}	0.047 ^{e}	242.3 ^{e}	274.7 ^{bc}
Bacillus velezensis	6.557 ^{d}	3.170 ^{g}	1.343 ^{c}	0.783 ^{e}	0.087 ^{c}	0.080 ^{cd}	248.7 ^{e}	285.7 ^{b}
LSD $_{\alpha = 0.05}$	3.811		0.178		0.019		0.294	

Different letters $^{a–j}$ indicate statistically different mean values (α = 0.05).

3.1.2. Fluorescence of Plant Chlorophyll

The measurements of chlorophyll fluorescence are usually modified depending on the type of stress, its duration, and intensity; as such, these measurements allow for the detection of stress-related changes in PSII [23]. Differences in the values of F0, Fm, and Fv/Fm were observed both among the applied biostimulators and between plants growing under optimal hydration and under the imposed drought. Increases in the F0 values (minimum fluorescence) ranged from 13.0% for plants treated with the 'BaktoKompleks' to 40.0% for plants where the biostimulator was not applied. The F0 of the dark-adapted leaf allows for the assessment of the loss of excitation energy during the energy transfer to the PSII [33]. Higher values of F0 under drought in the non-treated control plants may indicate a lower efficiency of their excitation energy transfer between the photosynthetic reaction centers [24]. Under drought, the significantly lowest minimal fluorescence values were noted for the bacterial preparations '*Bacillus velezensis*' and 'BaktoKompleks', which indicated the lowest excitation energy losses in energy antennas in these objects. Fm lacked significant differences between the irrigated and drought-stressed plants, with the exception of the 'BaktoKompleks'-treated plants, for which the highest Fm value of 813.3 units was recorded in plants under full hydration, and it was higher than the stressed plants by 14.2%. The Fm parameter determines the potential yield of PSII and can be used as a proxy to the photochemical activity of the photosynthetic apparatus [34,35]. For all tested biostimulants except '*Bacillus velezensis*', we noted significant decreases in Fv/Fm between plants growing under optimal water conditions and those stressed by drought. The decrease in the Fv/Fm value ranged from 0.3% for plants treated with the '*Bacillus velezensis*' biostimulator to 5.3% for plants treated with 'Raiza-Mix' (Table 3). The Fv/Fm parameter determines the potential yield of PSII and can be used as another indicator of the photochemical activity of the tested plants' photosynthetic apparatus [34,35]. Previous reports documented decreases in Fv/Fm observed for the dark-acclimated state and due to drought [27,36,37]. Therefore, the indicated bacterial preparation likely contributed to the protection of PSII functions.

Table 3. Effects of biostimulants and drought on the chlorophyll fluorescence parameters after dark adaptation: F0—minimum fluorescence, Fm—maximum fluorescence, Fv/Fm—maximum PSII quantum yield in the dark-acclimated state (not-nominated units).

Biostimulant	F0		Fm		Fv/Fm	
	Control	Drought	Control	Drought	Control	Drought
Control	142.5 ^{cd}	199.5 ^{a}	565.3 ^{c}	623.2 ^{bc}	0.789 ^{a}	0.753 ^{b}
BaktoKompleks	146.7 ^{cd}	165.8 ^{b}	813.3 ^{a}	698.2 ^{b}	0.790 ^{a}	0.764 ^{b}
Naturvital®-Plus	166.0 ^{b}	196.8 ^{a}	615.5 ^{bc}	633.0 ^{bc}	0.802 ^{a}	0.763 ^{b}
Raiza-Mix	149.4 ^{bcd}	196.2 ^{a}	631.7 ^{bc}	641.0 ^{bc}	0.790 ^{a}	0.748 ^{b}
Bacillus velezensis	134.0 ^{d}	156.3 ^{bc}	668.8 ^{b}	691.0 ^{b}	0.795 ^{a}	0.793 ^{a}
LSD $_{\alpha = 0.05}$	19.01		93.60		0.018	

Different letters $^{a–d}$ indicate statistically different mean values (α = 0.05).

Plants treated with the 'Naturvital®-Plus' and 'Raiza-Mix' biostimulators significantly exhibited the highest chlorophyll content compared to other objects when fully hydrated. On the other hand, the lowest CCI value was found in plants that were not treated with biostimulants. The values of the CCI parameter decreased significantly in all stressed plants. The highest decrease was recorded for the non biostimulated plants (by 91%), whereas the lowest decrease was recorded for plants where the 'Naturvital®-Plus' biostimulant was used (by 71%) (Table 4). Under drought, there was a chlorophyll content decrease due to the oxidation and degradation of chloroplasts, as reported in a study to evaluate the application of foliar and soil silicon application to spring wheat plants growing under drought stress [38]. In an experiment carried out on bread wheat varieties, the content of chlorophyll decreases due to drought stress [39]. Similarly, in our studies, a decrease in the chlorophyll content in leaves of plants stressed by drought was noted as compared to plants under full hydration. The highest relative chlorophyll content was found in plants where the 'Naturvital®-Plus' biostimulant was used. This formulation containing fulvic acids had an overall desirable beneficial effect on chlorophyll content in plants when applied as a foliar treatment. In turn, the algae extract used as a biostimulant exerted a similarly beneficial effect on the chlorophyll content in durum wheat plants growing both under control conditions and under the influence of salt stress [40]. Additionally, in our studies, the use of biostimulants resulted in increased relative chlorophyll content for both well-hydrated and drought plants, compared to control plants.

Table 4. Effect of application of biostimulants and drought stress on CCI—chlorophyll content index and chlorophyll fluorescence parameters after light adaptation: Y (PSII)—PSII quantum yield in the light-acclimated state, ETR—electron transport rate (not-nominated units).

Biostimulant	**CCI**		**Y (PSII)**		**ETR**	
	Control	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Control	24.70 b	2.187 d	0.371 bc	0.212 f	28.78 a	14.13 c
BaktoKompleks	26.44 b	3.370 d	0.369 bc	0.404 a	28.40 a	23.85 b
Naturvital®-Plus	32.06 a	9.270 c	0.364 c	0.221 ef	27.73 a	14.75 c
Raiza-Mix	32.48 a	5.978 cd	0.379 abc	0.246 de	29.52 a	16.03 c
Bacillus velezensis	26.42 b	3.350 d	0.392 a	0.260d	30.54 a	20.72 b
LSD $_{\alpha = 0.05}$	4.508		0.027		3.811	

Different letters a–f indicate statistically different mean values (α = 0.05).

Values of both fluorescence parameters measured in the light Y (PSII)and ETR significantly differed between the tested biostimulants and as a result of the introduced stress. The Y (PSII) parameter informs the ratio of the light excitation quantum necessary for the photochemical transformations to the total of PAR quanta absorbed. The ETR parameter describes the rate of flow of electrons through the photosystems. Both parameters are lowered as a consequence of damage or reduction in the photosynthetic apparatus efficiency due to drought [27]. Plants biostimulated using the 'BaktoKompleks' showed under drought the highest efficiency of the quantum yield in the light-acclimated state (0.40 units), with this parameter's lowest values observed for plants where the biostimulant was not used under drought (0.21 units). For plants where the 'BaktoKompleks' preparation was applied, the Y (PSII) parameter significantly increased in its value under drought (by 9.5%), whereas for the remaining plants, the value of this parameter decreased significantly and ranged between 35.1% for plants treated with 'Raiza-Mix' and 42.9% for plants where no biostimulants were used. Damage to photosystem II as a result of drought resulted in a significant decrease in the electron transport rate (ETR) for all tested biostimulants. The decrease in the value of the ETR parameter between the watered plants and those under drought ranged from 16.0% for plants treated with the 'BaktoKompleks' biostimulant to 50.9% for plants in which the biostimulant was not used (Table 4). Analyses of chlorophyll fluorescence in wheat under drought indicated that the decreases in the Y (PSII) and ETR parameters could indicate a regulation at a physiological level of the electron transport, possibly by the enhanced excitation energy quenching in the PSII antennae [27]. The authors designated as a cultivar with greater drought tolerance, with regard to the photosynthetic activity, a cultivar in which Fv/Fm and Y were much less affected by the stress. Based on our data, this indicates the greatest acquired drought tolerance due to the use of the 'BaktoKompleks' biostimulant.

3.2. Relative Water Content

The RWC informs of the relative water content in leaves and is directly influenced by the soil water content. A lowered RWC indicates grater impediments to water flow already at the soil–root interface. Alternatively, it may be due to the decreases in soil hydraulic conductivity under low soil moisture [41]. It is a sensitive variable that rapidly reacts to environmental conditions such as temperature, light, and humidity. A plant with an inherent ability to maintain the leaf turgor under drought stress minimizes the resultant stress impacts, allowing for a continuation of turgor-dependent processes including plant growth, stomatal activity, or activity of complexes of photosystems I and II [42]. The plants with the significantly highest RWC value included those treated with the 'BaktoKompleks' both under optimal hydration (0.78%) and stress (0.78%), as well as watered control plants (0.77%). Significantly, the lowest value of this parameter was recorded in stressed plants treated which '*Bacillus velezensis*' (0.35%). In plants where 'BaktoKompleks' or 'Raiza-Mix' were used, no decrease in RWC due to drought was found, whereas the strongest decrease was noted for plants treated with '*Bacillus velezensis*' (by 52.7%) (Table 5). The lack of a decrease in RWC indicates the ability of plants, as a result of the biostimulant used, to regulate osmotic action and thus to actively accumulate dissolved substances as a response to the reduction in the water potential in the soil, which reduces the harmful effects of water deficit [41]. In their experiment on drought wheat biostimulated with amino acids and yeast, the authors also obtained a higher RWC in biostimulated plants compared to control plants.

Table 5. Effect of biostimulants and drought stress on RWC.

Biostimulant	RWC (%)	
	Control	**Drought**
Control	0.77 [a]	0.72 [bc]
BaktoKompleks	0.78 [a]	0.78 [a]
Naturvital®-Plus	0.71 [c]	0.65 [d]
Raiza-Mix	0.74 [b]	0.74 [b]
Bacillus velezensis	0.74 [b]	0.35 [e]
LSD $_{\alpha = 0.05}$	0.03	

Different letters [a–e] indicate statistically different mean values ($\alpha = 0.05$).

3.3. Drought Resistance Index (DRI)

The values of the plant physiological activity isndex for plants in full hydration (PPA_c) ranged from 433.0% for plants in which the 'BaktoKompleks' biostimulant was used to 199.8% for plants where no biostimulant was used. Similarly, the values of the PPA_{wd} index for plants stressed by drought ranged from 179.3% for plants where the 'BaktoKompleks' was used to 82.70% for control plants. The highest DRI value was observed for plants treated with '*Bacillus velezensis*' (0.508%), whereas the lowest value was obtained for plants treated with 'Naturvital®-Plus' (0.278%) (Table 6). Physiological results were also used to estimate the water deficit resistance in blueberry cultivars using a water deficit resistance index; this allowed for the assessment of the physiological parameters and comparison of the cultivars' reactions under drought [26]. The DRI conversion factor was also successfully used in the earlier studies and indicated the spelled wheat varieties as more tolerant to drought [23].

Table 6. Effect of biostimulants and drought stress on DRI—drought resistance index.

Biostimulant	PPA_C (%)	PPA_{wd} (%)	DRI (PPA_{wd}/PPA_C)
Control	199.8	82.70	0.410
BaktoKompleks	433.0	179.3	0.414
Naturvital®-Plus	350.6	97.58	0.278
Raiza-Mix	416.3	127.3	0.306
Bacillus velezensis	273.6	139.0	0.508

3.4. Crop Yield and Yield Parameters

3.4.1. Grain Yield

Significantly, the highest grain yield for plants fully hydrated was recorded for those treated with the 'Raiza-Mix' biostimulant (11.9 g per pot), and the lowest for plants where no biostimulant was used (8.7 g per pot). Under drought, the grain yield was highest for plants where 'BaktoKompleks' was used (7.2 g per pot), and the lowest for plants treated with 'Raiza-Mix' (3.8 g per pot) (Table 7). The biostimulant containing growth hormones, amino acids, and enzymes of biological origin significantly increased the productivity of common wheat by 8% compared to the control [43]. The responses of spring wheat to the biostimulants indicated that the application of microorganisms in the form of soil spraying increased the grain yield by 23.1% as compared to the control [44]. Our study confirmed that the use of any biostimulant increased the grain yield significantly under full hydration conditions. The highest grain yield under full hydration (37% higher than control) was obtained for plants where the 'Raiza-Mix' preparation was used: its composition is also based on algae, amino acids, growth hormones, and microelements. On the other hand, under drought, the highest grain yield was obtained in plants where the 'BaktoKompleks' bacterial preparation was used (38% higher compared to control plants).

Table 7. Effect of biostimulants and drought stress on grain yield, MTG—mass of a thousand grains and plant height.

Biostimulant	Grain Yield (g per pot)		MTGs (g)		Plant Height (cm)	
	Control	Drought	Control	Drought	Control	Drought
Control	8.7 ^{bc}	5.2 ^{de}	51.7	45.3	39.50 ^{d}	42.93 ^{bcd}
BaktoKompleks	11.2 ^{ab}	7.2 ^{cd}	58.0	53.7	45.28 ^{ab}	41.14 ^{cd}
Naturvital®-Plus	10.8 ^{ab}	5.6 ^{de}	58.0	55.3	45.94 ^{ab}	43.22 ^{bcd}
Raiza-Mix	11.9 ^{a}	3.8 ^{e}	57.7	47.7	45.78 ^{ab}	42.25 ^{bcd}
Bacillus velezensis	9.6 ^{abc}	5.4 ^{de}	54.7	49.7	47.23 ^{a}	43.45 ^{abc}
LSD $_{\alpha = 0.05}$	2.60				3.90	

Different letters $^{a–e}$ indicate statistically different mean values ($\alpha = 0.05$).

3.4.2. The Mass of a Thousand Grains

The mass of a thousand grains for all tested objects did not differ significantly (Table 7). Amino acids-based biostimulants increased the average weight of a thousand grains of winter wheat in comparison with the control group, in which such preparations were not used [45]. Wheat plants treated with a biostimulant containing microelements, chelating compounds, and nitrogen compounds increased the MTGs [43]. Our studies confirmed the overall beneficial impacts of biostimulants on the MTGs, both in plants growing under optimal irrigation and in those subjected to drought. However, similar to other studies [45], there were no significant differences.

3.4.3. Plant Height

In all the tested plants, except for the controls, a decrease in plant height was observed and it ranged from 2.72 cm (5.0%) for plants treated with 'Naturvital®-Plus' to 4.14 cm (9.1%) for plants treated with 'BaktoKompleks'. The control plants, in which no biostimulant was used, exhibited a height higher by 8.7% under drought than under optimal hydration (Table 7). Winter oilseed rape plants in which a biostimulant containing marine algae extract was applied, were 12% higher than the control plants [46]. Under control conditions, maize plants treated with mycorrhiza seed material were 39.6 cm (30%) higher compared to plants that were not treated [47]. Under drought, the plants with treated seeds were 1% taller than the non-treated controls, respectively. Similarly, in this study, hydrated plants in which biostimulants were applied were taller than the non-treated controls. Plants treated using the '*Bacillus velezensis*' bacterial preparation were the tallest (19.6% taller than the controls), whereas the control plants were the smallest. Under drought, the smallest plants were those sprayed with 'BaktoKompleks', and the tallest ones these treated with '*Bacillus velezensis*' (1.2% taller than the control plants).

4. Conclusions

All of the applied biostimulants had a positive effect on the physiological state and the yield of winter wheat under full hydration. The use of the biostimulants also improved the tolerance of winter wheat plants to drought, based on the analyzed physiological parameters. Of the tested preparations, 'BaktoKompleks' and '*Bacillus velezensis*' had the greatest positive impact on improving the tolerance of wheat plants to drought. Based on their strong positive effects, we recommend these two preparations to be assessed for expected similar effects in the follow-up field studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13010121/s1, Figure S1: Photographs of wheat grown under optimal water conditions. From the left: 'Control', 'BaktoKompleks', 'Naturvital®-Plus', 'Raiza-Mix', '*Bacillus velezensis*'; Figure S2: Photographs of wheat grown under drought stress. From the left: 'Control', 'BaktoKompleks', 'Naturvital®-Plus', 'Raiza-Mix', '*Bacillus velezensis*'.

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