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Influence of SDHI Seed Treatment on the Physiological Conditions of Spring Barley Seedlings under Drought Stress

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Abstract: Seed treatments help reduce the pathogen load and thus improve the condition of plants from their earliest developmental stages, but they can have impacts beyond their basic fungicide protection role. In this study, we investigated how seven spring barley seed treatments affected the plants' physiological state. The tested seed treatments differed significantly in their impacts on the vigor parameters of barley seeds and on the physiological state of seedlings under drought stress and after regeneration. Seed treatments based on substances from the succinate-dehydrogenase-inhibitors (SDHI) group did not cause inhibition of seedling growth and also display by the highest vigor index values. Using the analysis of photosynthesis-related parameters, we showed that seed treatments from the SDHI group provided a superior tolerance of the imposed drought in spring barley than other treatments. In addition to protection against abiotic stress, SDHI treatments also rendered a higher efficiency of photochemical reactions in the treated plants.

Keywords: abiotic stress; chlorophyll fluorescence; fluorescence; *Hordeum vulgare* L.; photosynthesis; succinate-dehydrogenase-inhibitor

1. Introduction

Both seeds and soil can be a source of pathogens potentially weakening the plant development, and consequently reducing the quantity and quality of the crop [1]. Seed treatment helps reduce the pathogen load and thus improves the condition of plants from their earliest developmental stages [2–5]. The beginning of the use of fungicides, especially seed treatments, dates back to 1600, when copper sulphate ($CuSO_4$) was used for the first time in a very high concentration to protect wheat from the common bunt fungus [6]. Methionine demethylation inhibitors affecting sterol biosynthesis in fungi (especially triazoles, imidazoles, and pyrimidines) are the most important group of systemic fungicides currently used to treat seeds [7]. Förster et al. [8] noted a strong growth inhibition of barley root, shoot, and coleoptile after treating the seeds with triadimefon and triadimenol. A delay in the extension of the primary leaf after seed treatment with substances from the triazole group has been described in many

crops [9,10]. Reduction of the coleoptile length, primary leaves, and internode length due to the use of triadimenol and triticonazole as a treatments of wheat seed was also well documented [3,11,12]. It was thus shown that seed treatments can have a number of impacts beyond their basic fungicide protection role. Carboxamide fungicides (succinate-dehydrogenase-inhibitors; SDHIs) act on the pathogens by inhibiting succinate dehydrogenase in the mitochondrial respiration. The site of SDHI action is the succinate dehydrogenase complex in the respiratory chain (complex II), at the ubiquinone binding site (SQR). As a result of the electron transport interference, mitochondrial respiration is inhibited [13–15]. The first generation of SDHIs dates to the end of the 1960s. These compounds were mainly used for controlling plant diseases caused by *Basidiomycetes* [16]. The currently used second generation of SDHIs exhibit a broad spectrum of activity against pathogenic fungi for many crop species [17,18].

Carboxamide fungicides' (SDHIs) biological activity has been assessed in this study. Those agents are included in formulations blocking the succinate dehydrogenase (SDH) activity. Active substances classified in the SDHI group are derived from various ranges of chemistry and have protective, translaminar, or systemic activity. Sedaxane, as the literature indicates, is a broad-spectrum substance that controls seed-borne pathogens such as *Ustilago nuda*, *Tilletia caries*, *Microsochium nivale*, and *Pyrenofora graminea*, as well as soil-borne fungi: *Rizoctonia solani*, *Rizoctonia cerealis*, and *Typhula incarnata*. The discovery of this substance was the result of an intensive chemical synthesis and a biological research program that included hundreds of carboxamide analogues. Sedaxane was chosen by Syngenta for use only as a seed treatment, because it combines the optimal physicochemical properties, a wide spectrum of activity and renders an excellent plant tolerance. It specifically inhibits the enzyme SDH, which catalyzes an important stage in the trichloroacetic acid cycle and the respiratory chain of pathogens [19–21]. According to Ajigboye et al. [22], the fact that Sedaxane inhibits the SDH complex II in fungal mitochondria may suggest that it exerts a similar effects on the plant mitochondrial complex as well.

Fluxapyroxad, a second generation SDHI fungicide, is an active substance developed by BASF in 2012 to control a wide spectrum of plant fungal pathogens. The active substance are two acids: 3-(difluoromethyl)−1-methyl−1*H*-pyrazole-4-carboxylic (C−1) and 3-(difluoromethyl)−1*H*-pyrazole-4-carboxylic (C−2) and 3-(difluoromethyl)−1-methyl−1*H*-pyrazole-4-carboxamide (C-7) [23]. One of the formulas based on this substance is BAS 700 05 F, a seed treatment solution that protects cereals from seed-borne and soil-borne pathogens such as *Ustilago* spp., *Pyrenophora* spp., *M. nivale*, and *Fusarium* spp. and against early leaf infections especially in barley cultivation caused by *Puccinia hordei*, *P. teres*, *Rhynchosporium secalis*, and *Ramularia collo-cygni* [24].

An emerging limiting factor in plant growth beyond the pathogens is the abiotic stress caused by water deficit and extremely high temperatures at low humidity, leading to drought stress [25–27]. Due to the changing climatic conditions, tolerance to abiotic stress is becoming one a particularly desirable characteristic in plant crops. Analyses of gene expression and measurements of gas exchange and fluorescence of chlorophyll indicated that fungicides from the SDHI group increase the photosynthesis and photosystem II (PSII) efficiency in plants under drought stress [22].

Photosynthesis is unquestionably the most important physiological process carried-out by plants. It is the basic factor underlying the biomass creation, and thus the production of the final crop [28–30]. Strong drought often associated with elevated temperature and high light intensity limits CO_2 assimilation and causes increased photoexcitation energy in PSII. This in turn disturbs the balance between the supply of assimilation force produced in photochemical reactions (ATP and NADPH) and reduces the related demand of the Calvin-Benson cycle. The outcome forces the plant to various processes of scattering the excess energy absorbed by chlorophyll. One of such processes is an increased fluorescence. Chlorophyll fluorescence is a measure of the energy of absorbed light quanta that has not been used in photosynthesis and has not been emitted as heat [31–33]. Fluorescence intensity, therefore, measures how strongly the processes related to the functioning of PSII are disturbed. As a physiological parameter, it allows to determine whether the plant has become stressed and to assess the time it needs for complete regeneration.

Abiotic stress is considered to be the main limitation of crop failure worldwide, which can potentially reduce the average yield of most field crops by more than 50% [34]. Variations in environmental conditions, such as photoperiod, nutrient status and solar radiation, can affect plant growth and development [35], however drought-related stress is one of the most important abiotic factors affecting the reduction of seed yield and biomass in global agriculture. In the face of climate change, an increase in the frequency of insufficient precipitation and the resulting dryness in many parts of the world is forecast [36,37], therefore, improving drought tolerance was an important goal of crop improvement programs.

It is well known that water deficiency can disturb a wide range of basic plant physiological and metabolic reactions that are involved in regulating crop growth and yield. In addition, drought stress can lead to increased production of reactive oxygen species (ROS), including hydrogen peroxide, hydroxyl radical and superoxide anion [38]. Generated ROS can cause the oxidation of photosynthetic pigment particles, membrane lipids, proteins and nucleic acids, and thus interfere with the normal functioning of the cell. To alleviate the destructive effects of ROS, plants have developed several adaptations and protective mechanisms that include stimulation of effective enzymatic [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX)] and nonenzymatic (phenolic acids, carotenoids, flavonoids, ascorbic acid, proline, etc.) pathways of antioxidant defense [39,40].

Barley (*Hordeum vulgare* L.) is a crop often grown in dry and semi-dry areas. Water deficit in these areas and improper distribution of precipitation reduce the germination and growth of barley [41]. Barley is one of the most important cereal crops in many developing countries, where it is often subject to extreme drought stress, which significantly affects its yield [42]. It is grown in a wider environmental range than any other cereals with an unfavorable climate. Under these conditions, barley encounters drought stress during seed germination and early stages of growth. These stages are most exposed to drought stress and pose a challenge in barley production. For instance, in the studies described by Nosalewicz et al. [43] it has been shown that by exposing barley to drought-related stress during reproductive stages, it reduced the ratio of shoots:roots and the number of thick roots in the F1 generation.

Naturally, drought stress affects the interaction of the plant with the pathogen when both stress occurs simultaneously, therefore, the aim of this study was to assess how seven seed treatments of spring barley under drought stress conditions affect the plant physiological state. Those treatments contained active substances from the fungicide groups of phenylpyrroles, carboxamides, and triazoles.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

As the plant model in our study, we used spring barley (*Hordeum vulgare* L. conv. *distichon*) cultivar 'Penguin' purchased from Danko Plant Breeding (Choryń, Poland). Plants were grown for 30 days in potted cultures in a greenhouse (60% to 80% relative humidity, 20 to 25 °C, 16 h day/8 h night). Natural sunlight was supplemented with sodium lamps light (HPS) with a capacity 400 W (Elektro-Valo Oy Netafim, Avi:13473/, Uusikaupunki, Finland). Ten barley seeds of equal size were sown in triplicate, in each 1.0 dm^3 pot filled with the same amount of soil substrate (universal substrate, pH 5.5). Seven various seed treatments were used before sowing (Table 1). The control group consisted of the untreated seeds.

Soil moisture was maintained at a constant level of 12% and regularly watered (100 mL H_2O/vase every 48 h) and was monitored daily with a probe (ThetaProbe, Eijkelkamp, The Netherlands). Drought stress was imposed in the second leaf phase, by stop the watering. A 10 days after drought imposition, soil moisture reached 4% to 5% *v/v*, and became hardly available to plants. All the leaves clearly lost their vigor. At that time, the physiological state of plants was determined in both the watered control and the drought stressed plants.

Table 1. Active substances and trade names of seed treatments used in experiment.

Treatment ID	Active Substances of Seed Treatments	Trade Name of Treatments
1	Control – untreated	
2	Fludioxonil 25 g/L	Maxim® 025 FS/ Celest® 025 FS
3	Fludioxonil 25 g/L + sedaxane 25 g/L	Vibrance® Duo / Maxim® Power
4	Fludioxonil 25 g/L + sedaxane 25 g/L + triticonazole 20 g/L	Vibrance® Star
5	Triticonazole 50 g/L	Triter 050 FS
6	Prothioconazole 150 g/L + tebuconazole 20 g/L	Redigo® Pro 170 FS
7	Fludioxonil 33.3 g/L + fluxapyroxad 33.3 g/L + triticonazole 33.3 g/L	Kinto® Plus
8	Fludioxonil 50 g/L	Madron 50 FS

Before the measurements, the plants were placed in in the dark for 9 h to silence photosynthesis. The entire measurement was carried out in a phytotron at a constant air temperature of 25 °C. After measurements, the plants were transferred back to the greenhouse and the watering regime was applied again to reach soil moisture of 12% and watered as mentioned previously. After 7 days, as the plants regained the turgor, a second measurement of the physiological state of the plants was carried out. Both measurements of the physiological state of plants (during drought stress and after plant regeneration) were carried out in an analogous manner. The same order of measurements was maintained. Drought-stressed plants and watered plants were measured alternately. For the measurements of the photosynthesis rate and chlorophyll fluorescence, the youngest fully developed leaf was used.

2.2. Assessment of Barley Seeds Quality

The assessment of seed sowing quality was carried out in accordance with the protocols described in the Main Inspectorate of Plant Health and Seed Inspection of Poland and the International Seed Testing Association [44,45], which include: germination energy, germination capacity, and vigor index. In addition, the root length was determined. The seedling growth test was performed on a roll test basis, on a 25-grain sample, in four replications. Each roll consisted of 3 layers of wetted filter paper 30 cm × 45 cm (quality paper type R with a retention time of 30 s). The rolls were placed in a thermostatic cabinet 'ST 5+' (Pol-Eko-Aparatura, Wodzisław Śląski, Poland) at a temperature of 19 °C and the barley germination energy was determined after 5 days, and germination capacity after 7 days. After this period, the length of normal seedlings was measured.

The vigor index was calculated according to the following formula:

$$\text{Vigor index (VI)} = [\text{seedling length (cm)} \times \text{germination (\%)}].$$

2.3. Physiological State of Plants

2.3.1. Plant Photosynthesis

Plant photosynthesis intensity was assessed based on rate of CO_2 exchange – A (v), transpiration rate – E (μmol × m^{-2} × s^{-1}), sub-stomatal CO_2 – Ci (μmol × mol^{-1}) and stomatal conductance – Gs (mol × m^{-2} × s^{-1}) in the leaf chamber of single leaves. The measurements were taken for the first young fully mature healthy leaf using a portable photosynthesis system (LCpro-SD, ADC BioScientific Ltd., Hoddesdon, UK) with a narrow leaf chamber (area: 5.8 cm^2). Photosynthesis measurements were carried out in triplicate for each factorial combination. The CO_2 concentration (Reference CO_2) in the leaf chamber was kept at 360 vpm, leaf chamber temperature was (Tch) at 25 ± 1 °C, the flow rate of air (u) was kept at 200 μmol × s^{-1}, and ambient H_2O concentration (Reference H_2O). Photosynthetically

active radiation (PAR) was kept at 400 µmol × m^{-2} × s^{-1}, adjusted automatically by a red-blue light-emitting diode light source (LCP Narrow Lamp, ADC BioScientific Ltd.).

2.3.2. Plant Chlorophyll Fluorescence

Chlorophyll fluorescence was measured at the same leaf and at the same light intensity as photosynthesis (PAR = 400 µmol × m^{-2} × s^{-1}), with Multi-Mode Chlorophyll Fluorometer (OS5p, Opti-Sciences, Inc., Hudson, NY, USA) with a PAR Clip that allows the measurement of PAR and leaf temperature. A kinetic test mode that combines the measurements under the light conditions and the measurements after dark adaptation was selected. Fluorescence measurements were carried out in six replicates for each combination. The fluorometer settings protocol followed our previous study [46]: Modulation Source: Red, Modulation Intensity: 29, Detector Gain: 06, Saturation Flash Intensity: 30, Flash Count: 001, Flash Rate: 255 (sec). The following parameters were measured: F_0—minimum fluorescence, F_m—maximum fluorescence, Y—Quantum Yield of Photosynthetic Energy, ETR—Electron Transport Rate.

2.4. Statistical Analysis

The effect of two factors (presence of drought stress and type of seed treatment) on the physiological state of plants was examined using two-way ANOVA with replicates. The bidirectional ANOVA model for factor interactions was as follows:

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$$

where y_{ij}—estimated value of variables (photosynthesis parameters) in the presence or absence of drought stress (i = 1, 2) and using the chosen type of treatment (j = 1, 2,..., 8), µ—overall average, a_i—the effect of the occurrence or absence of drought stress, β_j—the effect of j-type treatment, $(\alpha\beta)_{ij}$—effect of interaction of drought stress and treatment and e_{ij} – random error.

In the case of rejection of null hypotheses about the lack of influence of the factors analyzed or their interactions, Tukey's procedure was used for multiple comparisons.

In order to determine the mutual relations between the analyzed parameters of the physiological state of plants (parameters Ci; Gs; A; E; Y; F_0; F_m; ETR), the principal components analysis was carried out and biplots were used to illustrate the results obtained [47]. The linear combinations of the active variables listed above maximizing the amount of total variance explained were used as the main axes (main components) of the biplots. The vectors coming from the center of gravity of the set of points represent selected photosynthesis parameters. Two biplots were presented in the study. On both biplots, the points represent all seed treatment combinations (eight variants) and the presence or absence of drought stress (control/drought), a total of 16 combinations, with the first biplot presenting the results obtained during drought and the second during regeneration.

3. Results

3.1. Effect of the Seed Treatments on Germination Energy and Germination Capacity

Seed germination energy differed significantly depending on the seed treatment used (Table 2). Significant differences were also noted for germination capacity. Seeds treated with prothioconazole plus tebuconazole showed the lowest germination energy and germination capacity as compared to other analyzed seed treatments. The seed treatments used also significantly changed the values of the calculated vigor index. Barley seeds treated with treatments containing sedaxane (treatments No. 3,4, 7 and 8) had the highest values of the vigor index. The lowest value of this parameter was noted for variant 6, in which prothioconazole and tebuconazole were used.

Table 2. Impact of applied treatments on germination energy, germination capacity, vigor index, root and shoot length of spring barley seeds.

Treatment	Germination Energy	Germination Capacity	Vigor Index	Root Length	Shoot Length
1. Control – untreated	92.0 ± 0.1^{a}	92.7 ± 1.2^{bc}	5263 ± 544.8^{a}	60.4 ± 3.5^{ab}	57.2 ± 6.4^{ab}
2. Fludioxonil	92.0 ± 0.1^{a}	95.3 ± 2.3^{abc}	4796 ± 154.5^{a}	51.0 ± 3.8^{b}	52.1 ± 2.2^{ab}
3. Fludioxonil + sedaxane	88.0 ± 4.0^{a}	95.3 ± 2.3^{abc}	5832 ± 480.8^{a}	59.6 ± 6.9^{ab}	66.3 ± 5.0^{a}
4. Fludioxonil + sedaxane + triticonazole	93.3 ± 4.6^{a}	94.0 ± 3.5^{abc}	5497 ± 436.4^{a}	70.1 ± 8.7^{a}	58.9 ± 4.1^{ab}
5. Triticonazole	92.0 ± 6.9^{a}	97.3 ± 2.3^{a}	4211 ± 496.5^{ab}	56.4 ± 2.7^{ab}	44.8 ± 2.5^{b}
6. Prothioconazole + tebuconazole	80.0 ± 4.0^{b}	91.3 ± 2.3^{c}	3192 ± 165.4^{b}	57.4 ± 4.4^{ab}	40.0 ± 3.2^{b}
7. Fludioxonil + fluxapyroxad + triticonazole	92.0 ± 6.0^{a}	94.7 ± 4.2^{abc}	5005 ± 652.2^{a}	67.2 ± 1.4^{a}	54.2 ± 15.97^{ab}
8. Fludioxonil	93.3 ± 4.6^{a}	96.0 ± 2.0^{ab}	5285 ± 897.1^{a}	56.9 ± 7.8^{ab}	56.5 ± 8.1^{ab}
LSD	7.3	4.6	1138	15.1	20.7

Different letters indicate statistically different mean values $p < 0.05$.

Analysis of the average length of barley sprouts showed that treatments containing prothioconazole with tebuconazole most strongly reduced the length of the seedling among all the analyzed preparations. The longest shoot length, 66.3 ± 5.0 mm long in average, were observed in treatment No. 3 (Table 2). The root length test showed that sprouts from the seed treatment No. 4 containing fludioxonil + sedaxane + triticonazole were the longest (70.1 ± 8.7 mm), whereas they were the shortest (51.0 ± 3.8 mm) for combination No. 2, based on fludioxonil.

3.2. Effect of the Seed Treatments on the Physiological State of Plants During Drought Stress

A significant impact of the applied seed treatments on the efficiency of photosynthesis measured during drought stress was recorded. The use of any seed treatment significantly increased CO_2 assimilation in plants growing under drought stress compared to the untreated seeds. Only treatment No. 8 resulted in no increase of CO_2 assimilation under well-watered conditions. The highest levels of CO_2 assimilation under well-watered conditions were found for plants with seeds subjected to treatments No. 4 and 7, for which the increase of CO_2 assimilation in relation to untreated control was: 83% and 76%, respectively, whereas in relation to plants growing under drought −41% and 38%. Photosynthesis measurement under drought stress showed the highest CO_2 assimilation in plants treated with treatment No. 3 (Table 3).

Significant impact of the seed treatments used on the level of transpiration rate (E) was also recorded. The most intense transpiration under well-watered conditions was observed for plants with seeds subjected to treatment No. 4 and it exceeded the untreated control by 63%, whereas under drought stress by 13%. Under drought, the highest level of transpiration was observed in plants treated with seeds subjected to No. 6 (exceeding the untreated control by 26%), and the lowest to No. 7 (lower than the untreated control by 17%) (Table 4).

The highest values of stomatal conductance (Gs) under well-watered conditions were observed in plants with seeds subjected to treatments No. 3, 4, and 7, exceeding the untreated control by 70%, 67%, and 63%, respectively. Contrastingly, during drought stress, the Gs parameter values indicated smaller differences among the treatments used, and the highest level of Gs was observed in plants with seeds subjected to treatment No. 4 (higher than untreated control by 21%) (Table 5).

Similar results were obtained for the measurements of sub-stomatal conductance of CO_2 (Ci). Under well-watered conditions, the highest value of Ci was recorded for plants with seeds subjected to treatment No. 4 (20% higher than the untreated control). In contrast, the highest values of Ci under drought were recorded for plants with seeds subjected to treatments No. 3, 4, and 6 (higher than the untreated control by 15%, 15%, and 13%, respectively) (Table 6).

Based on the analysis of chlorophyll fluorescence, we found a significant effect of the applied seed treatments on both parameters measured after plants adaptation to darkness: minimum fluorescence (F_0) and maximum fluorescence (F_m), and both parameters measured under luminescence: quantum yield of photosynthetic energy (Y) and electron transport rate (ETR) (Figure 1).

The highest F_0 values (Figure 1A), both during measurement under drought and under well-watered conditions, were found for plants with seeds subjected to no treatment. High values of the F_0 parameter were also noted in plants with seeds subjected to seed treatments No. 2, 6, and 8 under well-watered conditions, and No. 6 and 8 under drought. The lowest values of the F_0 parameter were recorded in plants under drought with seeds subjected to treatments No. 3, 4 and 7. Marginally larger differences between the analyzed seed treatments were noted in the case of maximum fluorescence (F_m) (Figure 1C). The highest values of this parameter under drought were recorded for plants with seeds subjected to treatment No. 3, whereas the lowest—for the untreated plants under drought stress with their F_m 34% lower than the value for plants with seeds subjected to treatment No. 3. The highest values of ETR (Figure 1E) and Y (Figure 1G) parameters were found for plants under well-watered conditions, with seeds subjected to treatment No. 7. The increase in the value of both parameters in relation to untreated and unstressed plants was 21%. Under drought, the highest values of ETR and Y

parameters were recorded for plants with seeds subjected to treatment No. 4, and the increase of their value in relation to untreated plants, under drought stress, was 14%.

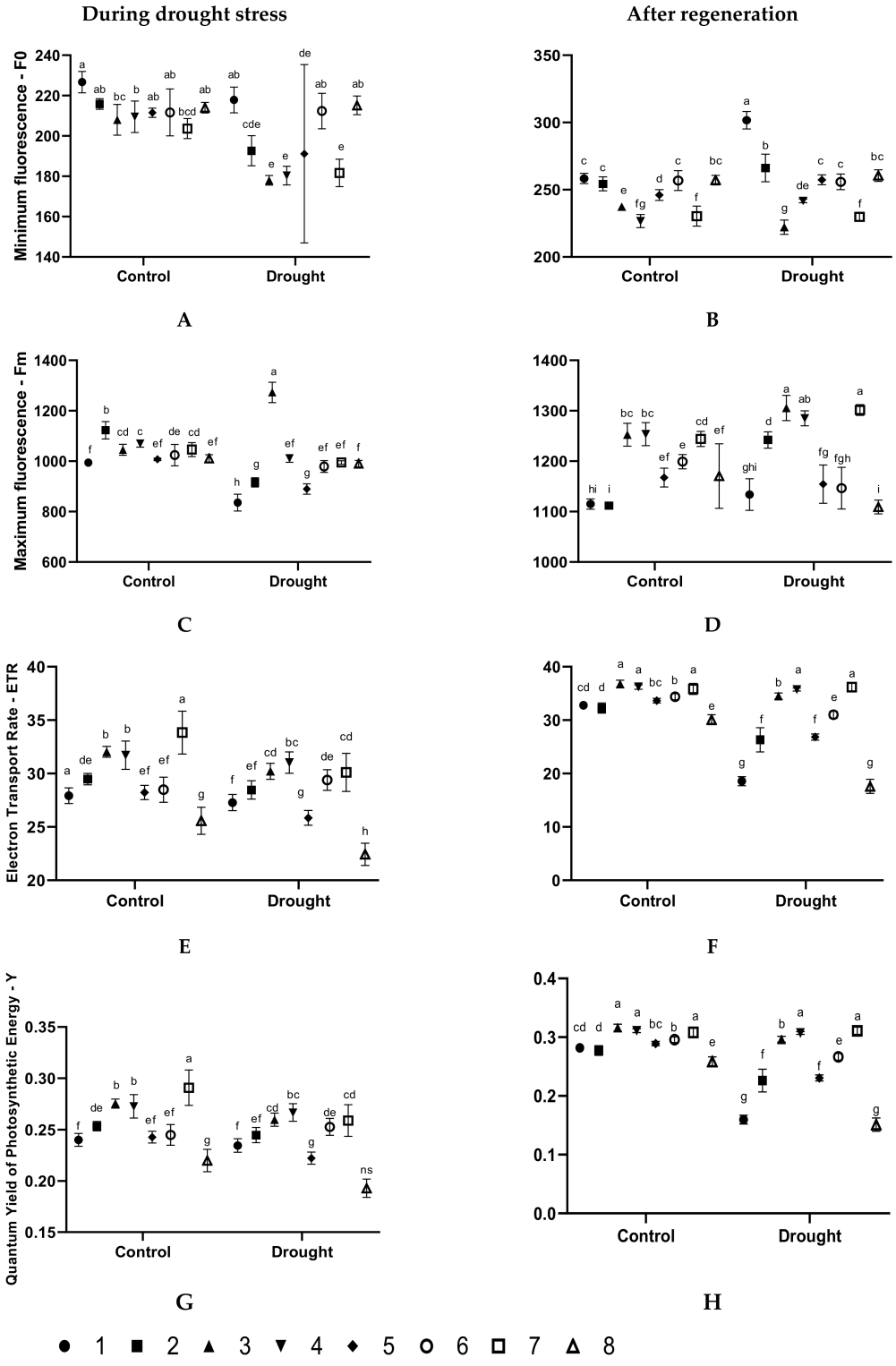

Figure 1. Parameters of chlorophyll fluorescence (non-nominated units): left column graphs imaging measurements during drought stress and right column graphs imaging measurements after regeneration. Letters a-i indicate statistically different mean values ($p < 0.001$).

Table 3. The influence of the seed treatment and drought stress on photosynthesis rate (CO_2 assimilation)—A (mmol × m^{-2} × s^{-1}) during drought stress and after regeneration.

Treatment	During Drought Stress		After Regeneration	
	Control	Drought	Control	Drought
1. Control – untreated	6.440 ± 0.290 ^{j}	6.767 ± 0.416 ^{ij}	9.627 ± 0.340 ^{h}	10.553 ± 0.125 ^{g}
2. Fludioxonil	8.930 ± 0.061 ^{d}	8.643 ± 0.174 ^{def}	8.005 ± 0.065 ^{i}	12.180 ± 0.100 ^{cd}
3. Fludioxonil + sedaxane	10.580 ± 0.370 ^{b}	8.803 ± 0.280 ^{de}	13.797 ± 0.237 ^{a}	13.387 ± 0.270 ^{b}
4. Fludioxonil + sedaxane + triticonazole	11.760 ± 0.340 ^{a}	8.350 ± 0.165 ^{efg}	13.077 ± 0.112 ^{b}	13.807 ± 0.329 ^{a}
5. Triticonazole	7.210 ± 0.288 ^{hi}	8.060 ± 0.017 ^{g}	9.970 ± 0.05 ^{h}	11.013 ± 0.012 ^{f}
6. Prothioconazole + tebuconazole	9.390 ± 0.250 ^{c}	7.387 ± 0.352 ^{h}	13.107 ± 0.122 ^{b}	11.880 ± 0.180 ^{de}
7. Fludioxonil + fluxapyroxad + triticonazole	11.307 ± 0.124 ^{a}	8.197 ± 0.196 ^{fg}	12.527 ± 0.072 ^{c}	11.553 ± 0.278 ^{e}
8. Fludioxonil	6.560 ± 0.110 ^{j}	7.570 ± 0.208 ^{h}	9.623 ± 0.035 ^{h}	10.640 ± 0.288 ^{g}
LSD	0.454		0.350	

$^{a-j}$ different letters indicate statistically different mean values $p < 0.0001$.

Table 4. The influence of the seed treatment and drought stress on transpiration rate – E (mmol × m^{-2} × s^{-1}) during drought stress and after regeneration.

Treatment	During Drought Stress		After Regeneration	
	Control	Drought	Control	Drought
1. Control – untreated	1.903 ± 0.046 ^{e}	1.550 ± 0.010 ^{f}	1.990 ± 0.010 ^{j}	3.020 ± 0.010 ^{gh}
2. Fludioxonil	2.205 ± 0.085 ^{d}	1.523 ± 0.040 ^{f}	2.567 ± 0.227 ^{i}	3.217 ± 0.091 ^{fg}
3. Fludioxonil + sedaxane	2.717 ± 0.115 ^{b}	1.350 ± 0.040 ^{gh}	3.793 ± 0.114 ^{a}	3.673 ± 0.051 ^{ab}
4. Fludioxonil + sedaxane + triticonazole	3.110 ± 0.040 ^{a}	1.463 ± 0.105 ^{fg}	3.403 ± 0.086 ^{cdef}	3.617 ± 0.110 ^{abc}
5. Triticonazole	1.983 ± 0.178 ^{e}	1.523 ± 0.143 ^{f}	2.923 ± 0.118 ^{h}	3.523 ± 0.021 ^{bcd}
6. Prothioconazole + tebuconazole	2.190 ± 0.085 ^{d}	1.953 ± 0.064 ^{e}	3.247 ± 0.168 ^{efg}	3.370 ± 0.392 ^{cdef}
7. Fludioxonil + fluxapyroxad + triticonazole	2.523 ± 0.131 ^{c}	1.280 ± 0.066 ^{h}	3.013 ± 0.038 ^{gh}	3.493 ± 0.095 ^{bcde}
8. Fludioxonil	2.110 ± 0.098 ^{d}	1.390 ± 0.066 ^{fgh}	2.497 ± 0.129 ^{i}	3.347 ± 0.093 ^{def}
LSD	0.166		0.256	

$^{a-h}$ different letters indicate statistically different mean values $p < 0.0001$.

Table 5. The influence of the seed treatment and drought stress on stomatal conductance – Gs (mol × m^{-2} × s^{-1}) during drought stress and after regeneration.

Treatment	During Drought Stress		After Regeneration	
	Control	Drought	Control	Drought
1. Control – untreated	0.100 ± 0.001 ^{de}	0.067 ± 0.006 ^{gh}	0.130 ± 0.010 ^{g}	0.180 ± 0.010 ^{e}
2. Fludioxonil	0.130 ± 0.010 ^{bc}	0.073 ± 0.006 ^{fgh}	0.157 ± 0.015 ^{f}	0.193 ± 0.015 ^{e}
3. Fludioxonil + sedaxane	0.170 ± 0.020 ^{a}	0.083 ± 0.006 ^{efg}	0.267 ± 0.015 ^{a}	0.227 ± 0.006 ^{bc}
4. Fludioxonil + sedaxane + triticonazole	0.167 ± 0.021 ^{a}	0.087 ± 0.006 ^{ef}	0.243 ± 0.006 ^{b}	0.223 ± 0.015 ^{c}
5. Triticonazole	0.110 ± 0.010 ^{d}	0.070 ± 0.001 ^{fgh}	0.217 ± 0.006 ^{c}	0.193 ± 0.006 ^{e}
6. Prothioconazole + tebuconazole	0.140 ± 0.010 ^{b}	0.077 ± 0.006 ^{fgh}	0.213 ± 0.006 ^{cd}	0.196 ± 0.004 ^{de}
7. Fludioxonil + fluxapyroxad + triticonazole	0.163 ± 0.015 ^{a}	0.077 ± 0.006 ^{fgh}	0.233 ± 0.006 ^{b}	0.227 ± 0.012 ^{bc}
8. Fludioxonil	0.117 ± 0.015 ^{cd}	0.063 ± 0.006 ^{h}	0.147 ± 0.015 ^{fg}	0.197 ± 0.006 ^{de}
LSD	0.019		0.028	

$^{a-h}$ different letters indicate statistically different mean values; for measurement during drought stress $p < 0.0003$, for measurement after regeneration $p < 0.0001$.

Table 6. The influence of the seed treatment and drought stress on sub-stomatal CO_2—Ci (µmol × mol^{-1}) during drought stress and after regeneration.

Treatment	During Drought Stress		After Regeneration	
	Control	Drought	Control	Drought
1. Control – untreated	212.7 ± 3.786 ^{f}	171.0 ± 4.583 ^{i}	221.7 ± 2.517 ^{h}	228.0 ± 8.660 ^{gh}
2. Fludioxonil	242.3 ± 9.019 ^{bc}	188.3 ± 3.055 ^{gh}	236.7 ± 1.528 ^{fg}	238.0 ± 1.000 ^{f}
3. Fludioxonil + sedaxane	253.0 ± 4.359 ^{ab}	196.7 ± 6.658 ^{g}	262.3 ± 3.512 ^{a}	247.6 ± 7.767 ^{cde}
4. Fludioxonil + sedaxane + triticonazole	256.0 ± 5.000 ^{a}	196.7 ± 5.686 ^{g}	260.0 ±4.359 ^{ab}	252.0 ± 2.646 ^{bcd}
5. Triticonazole	220.7 ± 10.017 ^{ef}	179.0 ± 2.646 ^{hi}	239.0 ± 2.646 ^{ef}	239.3 ± 2.082 ^{ef}
6. Prothioconazole + tebuconazole	232.3 ± 10.970 ^{cd}	193.7 ± 9.292 ^{g}	237.3 ± 12.423 ^{f}	238.6 ± 1.155 ^{f}
7. Fludioxonil + fluxapyroxad + triticonazole	245.3 ± 4.509 ^{ab}	190.3 ± 3.512 ^{gh}	253.0 ± 0.000 ^{bc}	243.7 ± 3.215 ^{def}
8. Fludioxonil	230.0 ± 4.582 ^{de}	170.0 ± 7.810 ^{i}	237.0 ± 5.568 ^{f}	237.7 ± 2.309 ^{f}
LSD	11.628		8.915	

$^{a-i}$ different letters indicate statistically different mean values; for measurement during drought stress $p < 0.026$, for measurement after regeneration $p < 0.015$

3.3. Effect of the Seed Treatments on the Physiological State of Plants after After Regeneration

The assessment of the physiological state of plants at regeneration, after previously imposed drought stress, was carried out analogously to that under that same stress. Photosynthetic activity and chlorophyll fluorescence were measured.

A significant influence of the applied seed treatments on the efficiency of photosynthesis measured after plant regeneration was observed. The highest values of parameter A were found for plants with seeds subjected to treatment No. 4, which had also been subjected to drought stress (exceeding the untreated drought-stressed control by 31%) and for plants with seeds subjected to treatment No. 3, growing under well-watered conditions (exceeding untreated the unstressed control by more than 43%) (Table 2). The highest values of the parameters E, Gs, and Ci were found in plants previously growing under well-watered conditions, for which seed treatment No. 3 was used, and the increase in their values in relation to the untreated unstressed control was 91%, 105%, and 18%, respectively. Among plants previously subjected to drought stress, the highest level of transpiration was recorded for plants with seeds subjected to treatment No. 3 (higher than untreated by 22%) (Table 3), the highest stomata conductivity in plants with seeds subjected to treatments No. 3 and 7 (higher than untreated by 26%) (Table 4), whereas the highest concentration of sub-stomatal CO_2 concentration in plants with seeds subjected to treatment No. 4 (higher than untreated by 11%) (Table 5). The lowest values of transpiration and sub-stomatal concentration of CO_2 after previous drought stress were recorded for the untreated plants, and stomatal conductance additionally in plants with seeds subjected to treatments No. 2 and 5.

Measurement of chlorophyll fluorescence after regeneration indicated that majority of the seed treatments used had a positive effect on the photosystem II regeneration process. The differences for the measured fluorescence parameters values of the plants growing under well-watered conditions and the plants with their seeds treated with any treatment were smaller than these for the respective measurements under drought. Notably, no such effect was found for plants with seeds subjected to treatment No. 8.

Similar to the measurements under drought, the highest values of the F_0 parameter after regeneration were noted for the untreated plants (Figure 1), followed by the plants with seeds subjected to treatments No. 2, 8, 5, and 6, and the lowest in plants with seeds subjected to treatment No. 3. Measurement of the F_m parameter indicated the highest maximum fluorescence values in the group of plants subjected to drought stress with seeds subjected to treatments No. 3 and 7 (higher than the non-treated by 15%), and the lowest in the plants with seeds subjected to treatment No. 8. Analysis of fluorescence-related parameters after regeneration indicated the largest Quantum Yield of Photosynthetic Energy (Y) and Electron Transport Rate (ETR) in plants with seeds subjected to treatments No. 3, 4, and 7 in both drought-stressed plants and those under well-watered conditions. The values of the parameter Y in plants subjected to drought stress with seeds subjected to treatments No. 7 and 4 exceeded those of the untreated plants by 94% and 92%, respectively, whereas the ETR parameter by 95% and 92%, respectively. Notably, plants with seeds subjected to treatments No. 3, 4, and 7 and stressed with drought have shown a higher Quantum Yield of Photosynthetic Energy and Electron Transport Rate than plants not previously stressed, with seeds subjected to treatments with other treatments. The lowest values of both fluorescence parameters were found in plants with seeds subjected to treatment No. 8 and untreated control.

3.4. Principal Component Analysis

To assess the relationship between the analyzed parameters of the physiological state of plants (Ci; Gs; A; E; Y; F_0; F_m; ETR) and the seed treatments used (1–8), under drought (Figure 2A) and after plant regeneration (Figure 2B), an analysis of the main principal components (PCA) was carried out. Such analysis allows to determine the variables that have the greatest impact on the individual major components. It also facilitates the interpretation of the impact of the seed treatment used on barley tolerance to drought, which in turn may have an impact on the yield. Each of the vectors represents one

variable, and their sizes and directions describe the effect they exert on the main components. Based on this analysis, plants treated with seed treatments from the SDHI group (No. 3, 4, and 7) showed significantly higher tolerance to drought. In addition, after the stress factor has ceased (after apply watering), the plants returned faster to better physiological conditions.

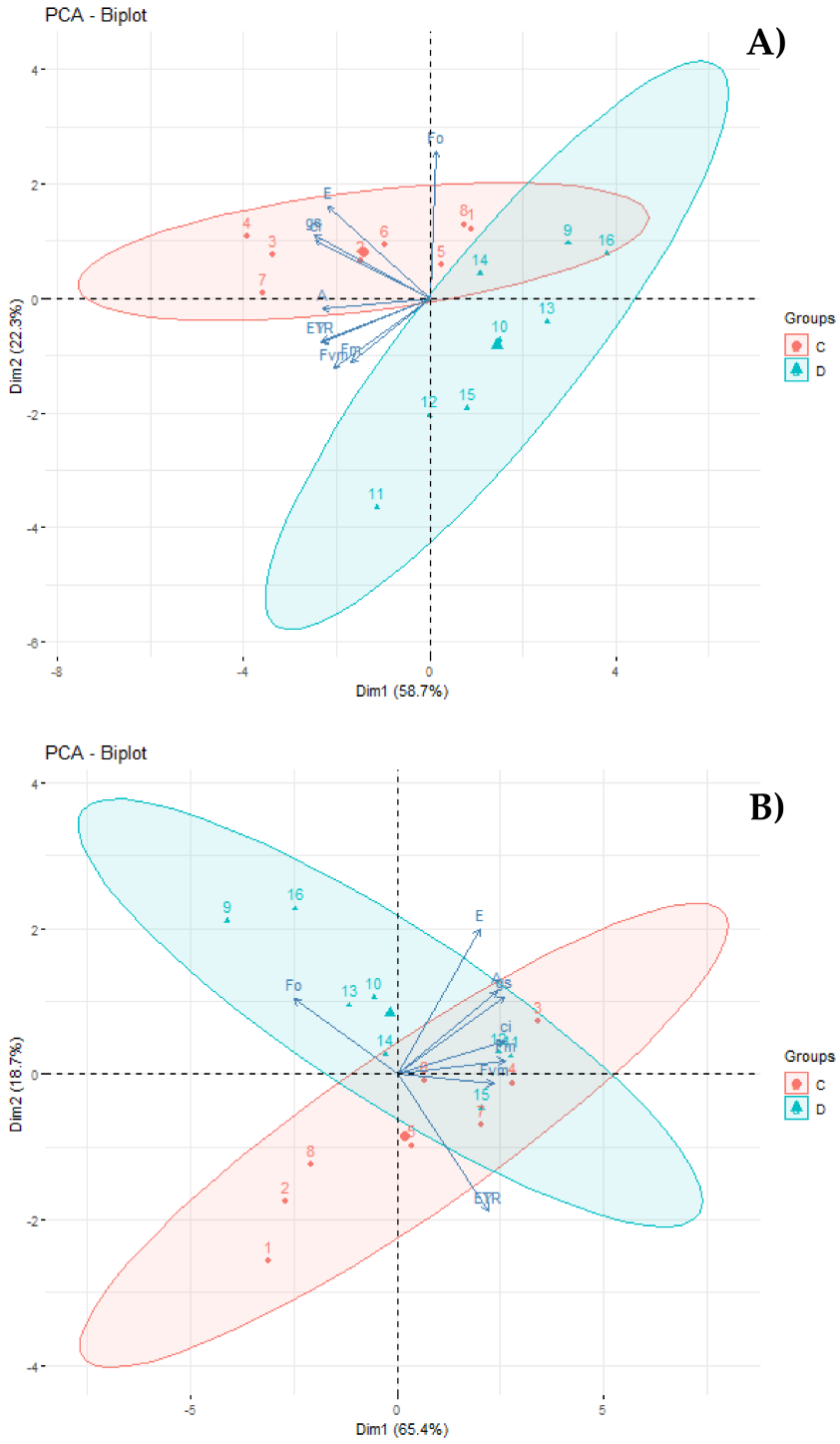

Figure 2. Projection of the variables on the component plane (1 × 2): (**A**)—during drought stress; (**B**)—after plant regeneration. C: control; D: drought stress; 1–8: seed treatments (well-watered); 9–16: seed treatments (drought stress); Dim: dimension.

4. Discussion

Seed treatments may affect many aspects of plant growth and development. In the case of triazoles, literature data indicate one negative impact — inhibition of growth in roots and plant seedlings. This is especially true for two substances: triadimefon and triadimenol [8]. Delay in primary leaf elongation is another negative effect noted in several crops after application of triazoles as seed treatment [9]. In the case of triadimenol and triticonazole, available experimental evidence points to the reducing effect of the length of coleoptiles, first leaves, and first internodes in wheat seedlings [3,12]. Triazoles, *i.e.*, prothioconazole, tebuconazole, and triticonazole, were also tested as seed treatments in this study. Prothioconazole and tebuconazole in its composition (No. 6) had the biggest impact on the reduction of seedling growth, but the lowest vigor index was also observed. Seed treatments with the most stimulating effect on barley seedlings contained sedaxane and fluxapyroxad, and that effect was particularly evident in the root length analysis. Longer roots can take up more water, which helps mitigate the drought in critical plant growth stages and results in higher harvest rates and reduced water loss under drought [48]. During drought, the roots signal to the leaves, to reduce the transpiration and growth rate, while increasing the efficiency of water consumption [49]. Efficient or larger root systems contribute to adaptation in dry environments, particularly when plants rely on seasonal rainfall, but they are less effective in the environments where crop growth depends on the surface soil water [50]. Therefore, a well-developed root system in the early stages of plant development is extremely important. Carefully chosen seed treatment can be helpful in ensuring that.

Facing the climate change, one possible way to increase barley yield under adverse climatic conditions is to improve the plant photosynthetic efficiency [51]. Modern, non-invasive measuring instruments that conduct measurements based on the difference in the concentration of CO_2 and H_2O in the air and the amount of air flowing through the plant material in a unit of time, allow to assess the degree of CO_2 assimilation and transpiration. Particularly valuable results in assessing the physiological state of plants under the abiotic stress and after regeneration can be obtained as a result of combining gas exchange assessment with chlorophyll fluorescence testing [52]. Experiments conducted on many crop species indicated the possibility of a wide application of chlorophyll fluorescence parameters in the detection of metabolic disorders also caused by plant protection products [53]. According to Berdugo et al. [54] the decrease in photosynthetic activity in the aging leaves is associated with the reduction of photochemical reactions happening in the PSI and PSII, therefore the use of chlorophyll fluorescence is an accurate method for detecting changes in the physiological state of plant tissue resultant from fungicide application. Our results lead to similar conclusions. The values of winter barley chlorophyll fluorescence indices, such as F_0, F_m, Y, and ETR, depended on the type of seed treatment used. The use of treatments, especially those containing sedaxane and fluxapyroxad, had a positive effect on mitigating the damage to the photosynthetic apparatus caused by drought. This stress is considered one of the most important environmental factors limiting growth, plant metabolism, and crop productivity around the world [55–57]. Photosystem II (PSII) is the most important protein-pigment complex in chloroplasts, but also the most susceptible to drought [32]. Minimal fluorescence (parameter F_0) of dark-adapted leaf is an indicator of the excitation energy loss during its transfer energy to the PSII reaction center [58]. Higher values of that parameter due to drought stress in the untreated plants may indicate their lower efficiency of the excitation energy transfer between photosynthetic complexes. We recorded the highest values of the F_0 parameter in the untreated plants, both under drought stress and unstressed, and the lowest in treated plants with seeds subjected to treatments No. 3, 4, and 7 subjected to drought stress. After plant regeneration, the highest value of the F_0 parameter measured was found in the untreated plants previously exposed to drought stress, whereas the lowest value in plants with seeds subjected to treatment No. 3. The F_m parameter, dependent among others on the chlorophyll contents in plant tissue, indicates the maximum fluorescence intensity of plants adapted to darkness, and the reduced values of this indicator can be caused by damage that prevents the total reduction of electron acceptors in PSII. Daszkowska-Golec et al. [51] observed in their studies of barley photosynthesis that the value

of the F_m parameter decreased significantly as a result of drought only as a result of prolonged stress. In our studies, the lowest values of F_m under drought were recorded for untreated plants subjected to drought, whereas the most favorable values were recorded for plants subjected to drought with seeds subjected to treatment No. 3. Plants with seeds subjected to treatments No. 3, 7, and 4 (all of the tested preparations containing active substances from the SDHI group) showed the highest values of the F_m parameter after regeneration, whereas the lowest values were found for plants stressed by drought with seeds subjected to treatment No. 8.

Significant differences were also shown for two photosynthesis-related parameters measured under the light: Y and ETR. The Y parameter specifies the ratio of the quantum used in photochemical transformations to the total number of PAR quanta absorbed [59], whereas the ETR parameter is the product of the value of Y and the value of light photons during the measurement multiplied by 0.5 (to transport 1 electron by both photocircuits, they need the absorption of 2 PAR quanta). Here, under drought, the highest ETR value, and thus the highest quantum yield of photochemical reactions in PSII, was shown by the unstressed plants with seeds subjected to treatments No. 7, 3, and 4. In these plants, however, a decrease in the value of both parameters was noted as a result of drought stress. This can be considered an indicator of the physiological regulation of electron transport by increased quenching of excitation energy in the PSII antennas [60]. This assumption was confirmed by the results of both parameters measured after regeneration, when the highest values of both parameters were recorded for the previously unstressed plants, followed by drought stress treated plants with the same active substances from the SDHI group (isopyrazam). In the research of Ajigboye et al. [61], with sprays using three fungicide preparations in winter wheat plants, under drought the application of substances from the SDHI group resulted in a significant increase of the maximum photochemical efficiency of PSII and the speed of electron transport. In their subsequent studies, Ajigboye et al. [22] have shown that the sedaxane used as seed treatment caused a significant increase in the efficiency of excitation energy capture by open PSII reaction centers in plants exposed to drought stress. This is in line with another study [54], which showed an increase in the effective quantum yield of PSII under drought compared to the untreated control at a significant level only in the group of plants treated with a substance from the SDHI group (bifaxene).

Analysis of gene expression and measurements of chlorophyll fluorescence and gas exchange showed that fungicides from the group of SDHI increase photosynthesis and PSII efficiency in wheat plants under drought stress conditions [22]. Sedaxane used as seed treatment for wheat grain later cultivated under drought redirected the metabolites from stress defense reactions to plant adaptation processes. This has now been confirmed in our research on barley; here the gas exchange in plants stressed by drought and growing under well-watered conditions was also measured, both under imposed drought and after regeneration. Plants treated with substances from the SDHI group reduced water transpiration under drought stress conditions, with the lowest values of parameter E recorded for such treatments. But, high values of transpiration after regeneration indicate that the reduction of water evaporation was the result of a defense mechanism against excessive water loss. Drought tolerance is the ability to maintain a relatively high carboxylation to maintain high photosynthesis efficiency [30]. In response to abiotic stress, such as unfavorable temperature, humidity, light intensity, or CO_2 concentration in the atmosphere, plants regulate the number of stomata in the developing leaves. Owing to this specific adaptation, plants can minimize the water loss and optimize CO_2 assimilation, thereby increasing their water use efficiency [62]. Assessment of the photosynthetic activity in soybeans growing 7 days under drought showed a decrease in CO_2 assimilation in the stressed plants compared to unstressed plants by 25%, transpiration by 48%, stomatal conductance by 66%, sub-stomatal CO_2 conductance (Ci) by 26% [63]. Daszkowska-Golec et al. [51] in their studies on barley photosynthesis noted a significant quadruple decrease in the Gs parameter value at the first stage of drought, which decreased three times more as result of the stress prolonged for the next 10 days. Notably, among the analyzed plants under drought, the highest values of Ci and Gs parameters were also shown for plants treated with substances from the SDHI group. In addition,

measurements after regeneration showed the highest Ci and Gs values for non-stressed plants with seeds subjected to treatments No. 3, 4, and 7, but also for those that were previously under drought stress treated with the same substances. Winter wheat sprayed with three fungicide preparations from the SDHI group (isopyrazam) exhibited comparable values of the photosynthesis intensity and chlorophyll fluorescence under drought, the highest values of CO_2 assimilation, transpiration, stomatal conductivity, and PSII efficiency [61]. Contrastingly, in our studies there was a significant reduction in transpiration under drought in plants with seeds subjected to treatments with the SDHI group agents, with comparatively high values of the other parameters—A, Ci and Gs. Inhibition of the SDH activity by partial reduction of SDH subunits has been shown in tomatoes and *Arabidopsis* as an action to increase the photosynthetic efficiency of leaves by increasing stomatal conductance [64,65]. Other studies that observed an improvement in photosynthesis and an increase in biomass in wheat seedlings treated with sedaxane, suggested that it may have contributed to maintaining the function of stomata under drought [22].

5. Conclusions

The seed treatments tested here differed significantly in their impacts on the vigor parameters of the barley grain, as well as on the physiological state of seedlings under drought and after regeneration. Seed treatments based on the substances from the SDHI group (sedaxane and fluxapyroxad) did not cause inhibition of seedling growth, which was also confirmed by the highest values of the vigor index. Substances from the triazole group have showed inhibition of seedling growth in other studies. With regards to the analysis of photosynthesis-related parameters, seed treatments from the SDHI group contributed to a comparably better tolerance of the imposed drought in spring barley. The above preparations, in addition to protection against abiotic stress, also rendered a higher efficiency of photochemical reactions in the treated plants. These preliminary analysis results indicate the possibility of using substances from the SDHI group to reduce drought stress in plants, however further studies on their activities could improve our knowledge about the mechanisms underlying the beneficial effects observed in the current experiments.

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