

## Article

# Selection of Tomato and Cucumber Accessions for Waterlogging Sensitivity through Morpho-Physiological Assessment at an Early Vegetative Stage

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**Abstract:** Waterlogging anomalies have recently increased, causing a reduction in yield and the loss of billions of dollars. Plant selection for increased tolerance to stress factors requires parameters with high sensitivity, as well as fast and inexpensive measurements. The aim of this study was to select tomato and cucumber accessions that reveal sensitivity and tolerance to waterlogging stress at an early vegetative stage. The selection of effective criteria for assessing plants was also an important issue. A total of 19 cucumber (including four highly homozygous) and 16 tomato accessions were evaluated, and plants with three true leaves were examined. The root zone of stressed plants was waterlogged for 7 days in a deep container. Morphological and physiological characteristics were obtained after 7 days of treatment and used for cluster analysis for discrimination of tolerant and sensitive accessions. Significant decreases in  $F_v/F_0$ ,  $F_v/F_m$ , Area, PI ABS,  $ET_0/ABS$ , and  $ET_0/TR_0$  parameters, as well as increases in  $DI_0/RC$ , were observed in sensitive accessions, with no changes in tolerant plants. The OJIP test parameters ( $F_v/F_0$ , PI ABS,  $DI_0/RC$ , and Area) were more sensitive in selecting for waterlogging stress than  $F_v/F_m$ . The present research can be used in breeding programs. Selected accessions will support a detailed explanation of the physiological differences in response to waterlogging stress in tomato and cucumber plants.

**Keywords:** submergence; OJIP test; selection criteria; hypoxia; tolerance

## 1. Introduction

As a result of climate change, waterlogging events have increased, causing billions of dollars' worth of crop losses [1–3]. The yield loss caused by waterlogging may vary between 15% and 80%, depending on the species (cotton, maize, wheat, rice, and soybean); soil type; and the duration of stress [4,5]. A reduction in the yield of vegetables due to flooding stress has also been observed, in tomato (*Solanum lycopersicum* L.) by 40% and sweet potato (*Ipomea batatas* L. Poir) up to 56% [6]. Understanding the morphological, physiological, and molecular mechanisms that underlie waterlogging (WL) tolerance presents a challenge to research. The establishment of selection criteria for WL-tolerant genotypes and breeding of WL-tolerant cultivars are critical for the expansion of cultivation, particularly in areas with frequent and high rainfall. An ideal WL-tolerant cultivar should not only survive waterlogging, but also rapidly recover to the control level [4].

In agricultural soils, waterlogging often occurs because of heavy rainfall, but can also be due to inadequate soil drainage. Taking into account the height of the water surface produced, flooding could be classified as waterlogging when it covers only the roots, or as submergence when water

completely covers the plant [7]. The saturation of soil with water reduces gas exchange with the atmosphere, causing the oxygen concentration to decrease rapidly and leading to O<sub>2</sub> deficiency (hypoxia) or O<sub>2</sub> absence (anoxia). Oxygen diffuses about 10,000 times slower in water than in the air, and this restricts aerobic respiration by the roots [8]. Limited oxygen availability for plants often occurs in hydroponic cultivation in environments without appropriate aeration [9] and also is induced by improper irrigation [10].

Plants growing in waterlogged soils can tolerate oxygen deficiency by shifting from aerobic to anaerobic respiration, although the latter is less efficient for ATP production. Moreover, this process produces harmful metabolic products that could cause plant death, i.e., acetaldehyde or lactic acid [11,12]. Most crops are WL-sensitive; however, the extent of damage depends on the species, the stage of development, and the climatic conditions, as well as on the duration of exposure to stress [1]. Tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) are classified as sensitive to root hypoxia [13–18], although differences amongst genotypes regarding their tolerance to this stress have been reported [19–21]. The variability of responses to root hypoxia among genotypes suggests that different strategies have evolved to deal with the stress. Tolerance to waterlogging mostly depends on the ability to develop specialized structures, allowing aeration of the tissues, which includes the formation of aerenchyma, adventitious roots, stem hypertrophic lenticels, and stem cracks [22–25]. Waterlogging stress induces senescence, resulting in leaf chlorosis, necrosis, and leaf loss [26]. The root system is strongly affected, as evidenced by the reduction in wheat (*Triticum aestivum* L.) root biomass [27]. Under waterlogging conditions, physiological disturbances are induced in plants, such as stomata closure and reductions in transpiration and photosynthetic rates, leaf water potential and transport of carbohydrates, reduced absorption of nutrients, and hormonal changes [28–30].

High sensitivity parameters are used for the selection of plants with increased tolerance to a stress factor. These also need to be fast and inexpensive because analysis of huge plant populations is required. Visual symptoms (visual assessment) parameters related to agronomic characteristics, such as yield or growth, molecular markers, and physiological parameters, are used to assess plant tolerance to stress factors [31–34]. As early as 1983, researchers used chlorophyll fluorescence to assess the effect of stress on the photosynthetic apparatus of plants, with the authors of the study suggesting their usefulness in plant breeding [35]. Since then, chlorophyll fluorescence has been used in many studies to assess the effect of stress (including waterlogging stress) on green parts of plants, and these have confirmed the usefulness of this method (for example [36–38]). Therefore, in our research, we have applied growth parameters as well as chlorophyll fluorescence parameters for the evaluation of plant tolerance to waterlogging stress.

Plenty of studies have demonstrated that plants at the seedling stage were consistently used for, among others, screening genotypes that displayed tolerance to variety of stresses, such as flooding in barley (*Hordeum vulgare* L.) [37] and hypoxia in cotton (*Gossypium hirsutum* L.) [39]. In the case of the tomato, plants at the seedling stage were used for the selection of plants tolerant to chilling [40], heat [41], and salinity [42,43], whereas cucumber seedlings, according to the literature, were subjected to submergence in order to select ones tolerant and sensitive to a lack of oxygen [44]. As Zou [34] reported, plants of *Brassica napus* L. that reveal tolerance to waterlogging at the seedling stage can demonstrate the same tolerance at later developmental stages, and moreover, assessment of tolerance at the seedling stage can be more efficient.

The aim of this study was to evaluate height, weight, leaf number, and chlorophyll fluorescence parameters of tomato and cucumber accessions and their responses to waterlogging stress at an early vegetative stage. Our research could be useful in indicating accessions that may be exploited as potential parental lines in breeding programs to develop waterlogging-tolerant cultivars. Moreover, an important issue is indicating effective selection criteria for the assessment of plant waterlogging stress tolerance. The hypothesis of this study was that accessions differ with tolerance to waterlogging stress. Furthermore, their morphological and physiological characteristics can be used for discrimination of

the tolerance of tomato and cucumber plants to waterlogging stress at the seedling stage, with cluster analysis being useful for the indication of more tolerant and sensitive accessions. The presented results have the potential to be further used by breeders and scientists for developing cultivars with improved hypoxia tolerance and increased yield production under waterlogging stress.

## 2. Materials and Methods

### 2.1. Plant Materials and Cultivation

Seeds of 19 cucumber and 16 tomato accessions were provided by Polish breeding companies, i.e., KHiNO Polan, PlantiCo, and Spójnia HiNO (Table 1). Seeds were sown in 40-cell multi-pots; each cell had volume of 0.23 dm<sup>3</sup>. Cells were fulfilled with peat substrate Klasmann KTS-2 (Germany). According to the manufacturer, the peat substrate contained, as follows (in mg dm<sup>-3</sup>): 250–500 N, 170–230 P<sub>2</sub>O<sub>5</sub>, 320–500 K<sub>2</sub>O, and 80–120 Mg. The salinity and pH were 2.0 g dm<sup>-3</sup> and 5.5–6.5, respectively. Seeds were cultivated in a greenhouse and, after germination, were lit with supplementary radiation (High-Pressure Sodium HPS lamps) to prolong the day length to 16 h. Minimum photosynthetic photon flux density (PPFD) on plant level during the day was 80 ± 20 µmol m<sup>-2</sup> s<sup>-1</sup> (when only radiation from HPS lamps reached the plants). The ambient temperature during tomato cultivation was 25.1 ± 4.8 °C in the day, and 22.3 ± 6.0 °C in the night. During cucumber cultivation, the daily average temperature was 27.6 ± 6.1 °C, and the night temperature was 24.1 ± 7.1 °C.

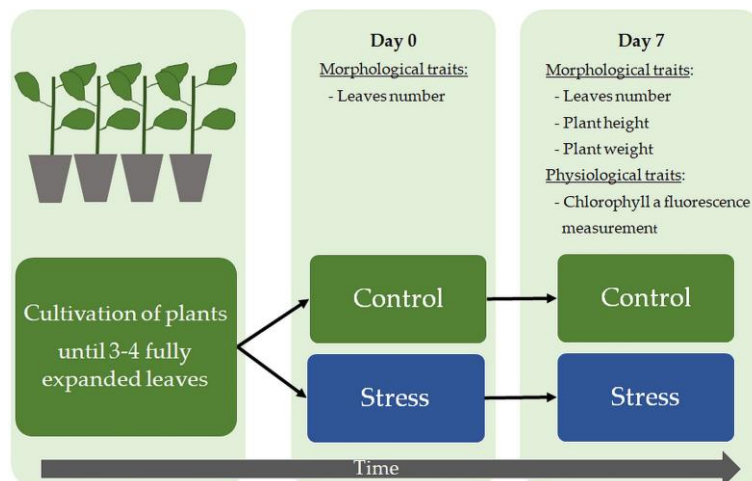
**Table 1.** Description of plant material used in the study.

| <i>Cucumis sativus</i> L. |                         | <i>Solanum lycopersicum</i> L. |                  | Origin          |
|---------------------------|-------------------------|--------------------------------|------------------|-----------------|
| Accession                 | Breeding Status         | Accession                      | Breeding Status  |                 |
| GROT                      | F1 cultivar             | POL 1/15                       | F1 cultivar      | KHiNO Polan, PL |
| MARKUS                    | F1 cultivar             | POL 2/15                       | Breeding line BC |                 |
| TYTUS                     | F1 cultivar             | POL 3/15                       | Breeding line    |                 |
| B1F1                      | Parthenocarpic cultivar | POL 4/15                       | Breeding line    |                 |
| B2F1                      | Parthenocarpic cultivar | POL 5/15                       | Breeding line    |                 |
| DH1                       | Double haploid line     | POL 6/15                       | Breeding line    |                 |
| DH2                       | Double haploid line     | POL 7/15                       | Breeding line BC |                 |
| DH3                       | Double haploid line     | POL 8/15                       | Breeding line    |                 |
| DH4                       | Double haploid line     |                                |                  |                 |
|                           |                         | PZ 115                         | Cultivar         | PlantiCo, PL    |
| PGZ-1                     | Breeding line           | PZ 215                         | Cultivar         |                 |
| GMG-30                    | Breeding line           | PZ 315                         | F1 cultivar      |                 |
| GM-50                     | Breeding line           | PZ 415                         | Cultivar         |                 |
| G404                      | F1 cultivar             | PZ 515                         | F1 cultivar      |                 |
| G598                      | F1 cultivar             | PZ 615                         | Cultivar         |                 |
|                           |                         | PZ 715                         | Cultivar         |                 |
|                           |                         | PZ 815                         | Cultivar         |                 |
| NOE1                      | F1 cultivar             |                                |                  | Spójnia HiNO    |
| NOE2                      | F1 cultivar             |                                |                  |                 |
| NOE3                      | F1 cultivar             |                                |                  |                 |
| NOE4                      | F1 cultivar             |                                |                  |                 |
| NOE5                      | F1 cultivar             |                                |                  |                 |

### 2.2. Stress Treatment

Tomato and cucumber seedlings, at the 3–4 fully expanded mature leaf stage, were divided into 2 equal groups: the Control and Stress groups. Before stress treatment, the percent volumetric water content (VWC) was measured using a Delta-T Devices SM150 soil moisture sensor kit (Delta-T Devices Ltd., Cambridge, United Kingdom) and plants were watered to obtain a soil moisture level up to 30%. The root zone of tomato and cucumber plants from the Stress group were waterlogged for 7 days (Figure 1) in a deep tray containing water. Plants from the Control group were watered as needed. The oxygen level in the air and in the water were monitored during the experiment by

a Dissolved Oxygen (DO) Meter (HI 2040-02 edge, Hanna instruments, Woonsocket, RI, USA). The oxygen concentration in the water reached a value of  $2.6 \text{ mg dm}^{-3}$  (air saturation = 29.2%, temperature =  $20^\circ\text{C}$ ) and that level was maintained to the end of the stress treatment, whereas in the air the oxygen concentration was  $9.20 \text{ mg dm}^{-3}$ .



**Figure 1.** Scheme presenting the parameters measured during the experiment.

### 2.3. Growth Analysis

Before the waterlogging treatment, we labelled 20 random plants from the Control (C) and Stress groups (S) for further analysis. At the 0 time-point and after 7 days of waterlogging, we determined the numbers of leaves on Control and Stress plants. After 7 days of treatment, the following parameters were measured: plant height (only shoots) (cm), measured with a ruler, and stem weight (with leaves) (g), determined by a laboratory scale (Ohaus, Parsippany, NJ, USA) (Figure 1). Plant height and weight were presented as a percentage ratio (%), assuming Control values as 100%.

### 2.4. Chlorophyll a Fluorescence Analysis

Chlorophyll *a* fluorescence was measured on the third leaf from the top of the plant, after 30 min dark adaptation with a special clip. The analyses were made after treatment with  $3500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  light intensity. In the case of each accession, we performed the measurements on 8 plants from the Control or Stress groups. Chlorophyll *a* fluorescence was measured using a HandyPea portable fluorometer (Hansatech, King's Lynn, UK). The fast phase of the fluorescence transient was denoted as OJIP, where the letters indicate characteristic points on the fluorescence induction curve: O is for origin (first measured minimal level), J and I are intermediates, and P is the maximum level of fluorescence curve. For simplicity, the analysis of OJIP fluorescence transient was called the JIP-test [45]. Some of the JIP-test parameters were calculated with formulas from Stirbet and Govindjee [45] and Stirbet et al. [46], as follows:  $F_0$  (minimum chlorophyll *a* fluorescence),  $F_m$  (maximum chlorophyll *a* fluorescence after dark adaptation),  $F_v$  (maximum variable fluorescence),  $F_v/F_0$  (ratio of the photochemical and non-photochemical processes in photosystem II (PSII), the maximum efficiency of the photochemical processes of PSII),  $F_v/F_m$  (the maximum quantum yield of PSII photochemistry),  $T_{fm}$  (time to reach the maximum chlorophyll fluorescence), Area (area above the OJIP transient and  $F_m$  line),  $F_m/F_0$  (the stable parameter in healthy leaves, value between 4–5), PI ABS (performance index on an absorption basis), ABS/RC (absorbed photon flux per PSII reaction center (RC) or apparent antenna size of an active PSII),  $TR_0/RC$  (maximum trapped exciton flux per active PSII),  $ET_0/RC$  (the flux of electrons transferred from the primary electron acceptor (QA) per active PSII reaction center),  $DI_0/RC$  (the flux of energy dissipated in processes other than trapping per active PSII reaction center),  $ET_0/ABS$  (quantum yield of

electron transport from QA), and  $ET_0/TR_0$  (efficiency with which a PSII trapped electron is transferred from QA).

### 2.5. Statistical Analysis

Euclidean distances were computed using Ward's method between samples from Control and Stress groups of each tomato and cucumber accession. Prior to analysis, we standardized the data. Ward's method was applied and observations with high values of measured features were clustered together and the same rule was applied with low values of parameter observations. After cluster analysis was performed, we created a dendrogram with a marked cut-off point dividing the analyzed objects into distinct clusters. The cut-off point was set at a clear clustering point and is marked with a colored line in the graph. Differences between clusters and between Control and Stress groups were determined using Student's *t*-test. The level of significance was established as  $p < 0.05$ . All data analyses were made using STATISTICA 13 (TIBCO Software Inc. (2017) from Statistica (data analysis software system, version 13. <http://statistica.io>)).

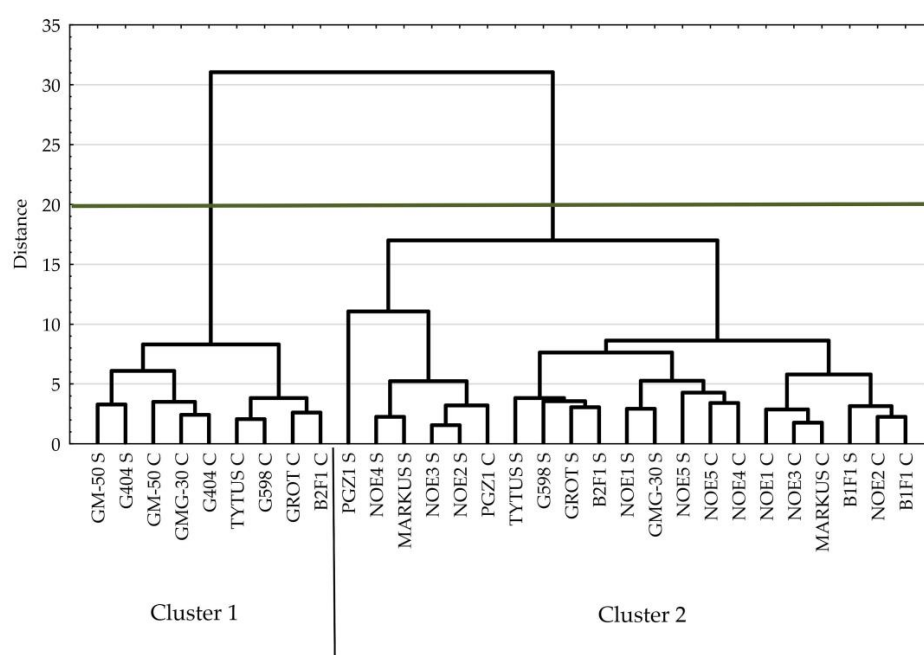
## 3. Results

The presented results compared the response of 15 cucumber and 16 tomato accessions to waterlogging stress. For clear presentation, we divided the results into two subsections. Additional analyses were made with four homozygous accessions of cucumber and are presented in the Supplementary Materials Section.

### 3.1. Cucumber

Cluster analysis based on all tested parameters and 30 treatments (15 cucumber accessions each as Control and Stress) were classified into two discrete groups with an Euclidean distance of 20 (Figure 2). Nine treatments were included in cluster 1 and 21 others were included in cluster 2.

The differences between cluster 1 and 2 are presented in Table 2. Cluster 1 consisted of groups with favorable values of determined parameters, in contrast to cluster 2, where groups with less favorable values were clustered. Interestingly, the mean value of two parameters (increase in leaf number and  $T_{fm}$ ) were similar in both clusters. Other parameters were significantly different between clusters. A significant decrease in growth,  $F_m$ ,  $F_v$ ,  $F_v/F_0$ ,  $F_v/F_m$ , Area,  $F_m/F_0$ , PI ABS,  $ET_0/RC$ ,  $ET_0/ABS$ , and  $ET_0/TR_0$  parameters were observed in cluster 2 as compared to cluster 1. As well as an increase in weight, we also observed increases in  $F_0$ ,  $ABS/RC$ ,  $TR_0/RC$ , and  $DI_0/RC$ .



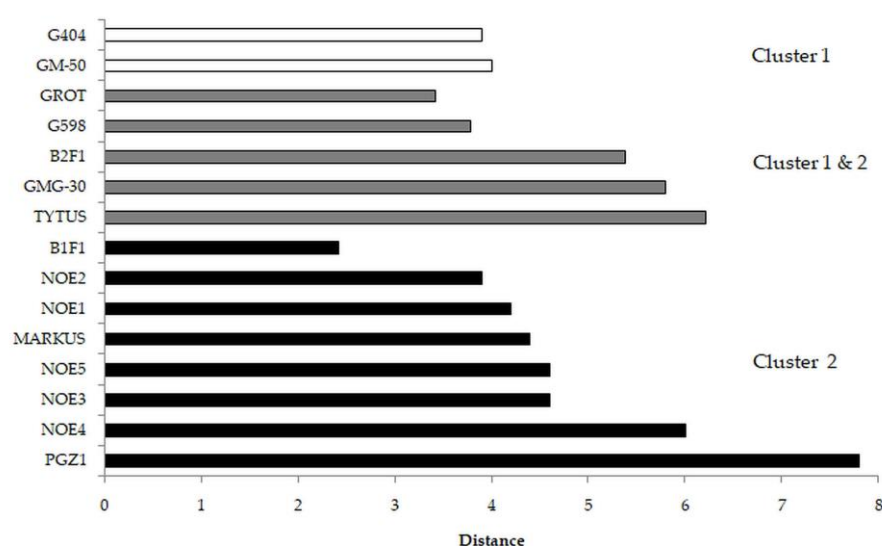
**Figure 2.** Results of cluster analysis for cucumber accessions using the Euclidean distance on the basis of morphological and physiological traits (Ward's hierarchical algorithm); green line indicates the cut-off point.

**Table 2.** Mean value of each parameter determined for both clusters (cucumber plants). Bold *p*-values indicate statistically significant differences between cluster 1 and cluster 2, estimated with Student's *t*-test and  $p < 0.05$ .

| Parameter                        | Cluster 1 | Cluster 2 | <i>p</i> -Value |
|----------------------------------|-----------|-----------|-----------------|
| % weight change                  | 97        | 103       | <b>0.0000</b>   |
| % height change                  | 93        | 88        | <b>0.0136</b>   |
| Relative leaf number             | 1.11      | 1.05      | 0.1955          |
| F <sub>0</sub>                   | 444       | 464       | <b>0.0000</b>   |
| F <sub>m</sub>                   | 2496      | 2397      | <b>0.0000</b>   |
| F <sub>v</sub>                   | 2052      | 1932      | <b>0.0000</b>   |
| F <sub>v</sub> /F <sub>0</sub>   | 4.65      | 4.22      | <b>0.0000</b>   |
| F <sub>v</sub> /F <sub>m</sub>   | 0.82      | 0.80      | <b>0.0000</b>   |
| T <sub>fm</sub>                  | 172       | 171       | 0.8759          |
| Area                             | 27,039    | 22,302    | <b>0.0000</b>   |
| F <sub>m</sub> /F <sub>0</sub>   | 5.65      | 5.22      | <b>0.0000</b>   |
| PI ABS                           | 1.11      | 0.79      | <b>0.0000</b>   |
| ABS/RC                           | 3.29      | 3.54      | <b>0.0000</b>   |
| TR <sub>0</sub> /RC              | 2.71      | 2.84      | <b>0.0000</b>   |
| ET <sub>0</sub> /RC              | 1.15      | 1.07      | <b>0.0000</b>   |
| DI <sub>0</sub> /RC              | 0.59      | 0.70      | <b>0.0000</b>   |
| ET <sub>0</sub> /ABS             | 0.35      | 0.31      | <b>0.0000</b>   |
| ET <sub>0</sub> /TR <sub>0</sub> | 0.43      | 0.38      | <b>0.0000</b>   |

On the basis of Figures 2 and 3, we assigned accessions GM-50 and G404 of both Control and Stress groups in cluster 1, which were close to each other. This meant that their parameters did not change under stress conditions, and thus these accessions could have been considered as tolerant to hypoxia stress. To select one of these, we conducted a comparison of morphological and physiological parameters in Control and Stress groups, followed by statistical analysis (*t*-test,  $p < 0.05$ ) (Table 3). As a result, in GM-50, two morphological parameters were changed between control and stressed

conditions (the decrease of weight change and height change in stress treatment was observed), whereas in G404, four parameters were statistically disparate (the decrease of weight change, height change, and leaf number, as well as increase of Tfm in stress-treated plants were noticed). According to that analysis, we selected plants from cucumber accession GM-50 as they were more tolerant to oxygen deprivation in the root zone.



**Figure 3.** Euclidean distance between Control and Stress groups of each cucumber accession. White bars indicate accessions from control and stress treatments that were classified into cluster 1; light grey bars indicate accessions from control and stress treatments that were classified into different clusters; black bars indicate accessions from control and stress treatments that were classified into cluster 2.

**Table 3.** Mean value of each parameter determined in two cucumber accessions considered as more tolerant to waterlogging. Bold *p*-values indicate statistically significant differences between Control and Stress plants of each accession separately estimated with Student's *t*-test and *p* < 0.05.

| Parameter                        | GM-50 Control | GM-50 Stress | <i>p</i> -Value | G404 Control | G404 Stress | <i>p</i> -Value |
|----------------------------------|---------------|--------------|-----------------|--------------|-------------|-----------------|
| % weight change                  | 100           | 85           | <b>0.0000</b>   | 100          | 93          | <b>0.0244</b>   |
| % height change                  | 100           | 67           | <b>0.0000</b>   | 100          | 65          | <b>0.0000</b>   |
| Relative leaf number             | 1.05          | 1.33         | 0.1529          | 1.30         | 0.78        | <b>0.0010</b>   |
| F <sub>0</sub>                   | 423           | 439          | 0.0907          | 437          | 464         | 0.0696          |
| F <sub>m</sub>                   | 2484          | 2569         | 0.1993          | 2453         | 2521        | 0.2838          |
| F <sub>v</sub>                   | 2060          | 2130         | 0.2541          | 2016         | 2057        | 0.5182          |
| F <sub>v</sub> /F <sub>0</sub>   | 4.88          | 4.85         | 0.8237          | 4.66         | 4.46        | 0.3244          |
| F <sub>v</sub> /F <sub>m</sub>   | 0.83          | 0.83         | 0.7230          | 0.82         | 0.82        | 0.3969          |
| T <sub>fm</sub>                  | 176           | 179          | 0.7788          | 164          | 186         | <b>0.0351</b>   |
| Area                             | 29,868        | 29,264       | 0.6359          | 27,051       | 26,648      | 0.7991          |
| F <sub>m</sub> /F <sub>0</sub>   | 5.88          | 5.85         | 0.8226          | 5.66         | 5.46        | 0.3243          |
| PI ABS                           | 1.13          | 1.21         | 0.3860          | 1.26         | 1.10        | 0.2351          |
| ABS/RC                           | 3.40          | 3.31         | 0.2075          | 3.24         | 3.37        | 0.2383          |
| TR <sub>0</sub> /RC              | 2.82          | 2.74         | 0.1317          | 2.66         | 2.74        | 0.2851          |
| ET <sub>0</sub> /RC              | 1.23          | 1.21         | 0.5859          | 1.20         | 1.21        | 0.7823          |
| DI <sub>0</sub> /RC              | 0.58          | 0.57         | 0.6829          | 0.58         | 0.63        | 0.2378          |
| ET <sub>0</sub> /ABS             | 0.36          | 0.37         | 0.8337          | 0.37         | 0.36        | 0.4029          |
| ET <sub>0</sub> /TR <sub>0</sub> | 0.44          | 0.44         | 0.8227          | 0.45         | 0.44        | 0.4775          |

The selection of sensitive cucumber accession was based on the analysis of distances between accessions and their Control and Stress groups presented in Figures 2 and 3. Accessions GMG-30 and TYTUS were selected as hypothetically sensitive accessions since their Control groups were assigned to cluster 1, whereas the Stress groups were assigned to cluster 2, indicating dissimilarity in parameter

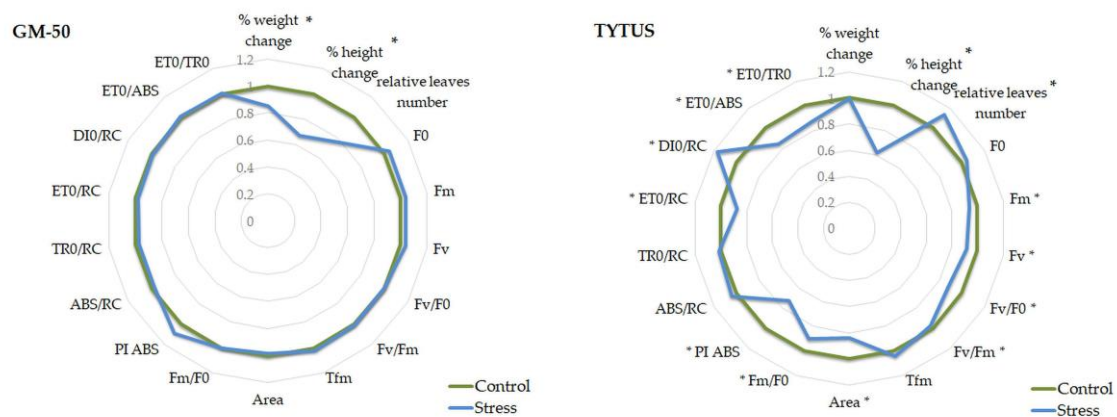
values. TYTUS was chosen as a sensitive cucumber accession due to the number of changed parameters between control and stress conditions, i.e., 13, whereas in GMG-30, only four appeared to be different (Table 4).

**Table 4.** Mean value of each parameter determined in two cucumber accessions considered as more sensitive to waterlogging. Bold *p*-values indicate statistically significant differences between Control and Stress plants of each accession, separately estimated with Student's *t*-test and *p* < 0.05.

| Parameter                        | GMG-30<br>Control | GMG-30<br>Stress | <i>p</i> -Value | TYTUS<br>Control | TYTUS<br>Stress | <i>p</i> -Value |
|----------------------------------|-------------------|------------------|-----------------|------------------|-----------------|-----------------|
| % weight change                  | 100               | 100              | 0.5298          | 100              | 99              | 0.8431          |
| % height change                  | 100               | 72               | <b>0.0000</b>   | 100              | 62              | <b>0.0000</b>   |
| Relative leaf number             | 0.86              | 1.23             | 0.0838          | 1.52             | 1.05            | <b>0.0082</b>   |
| F <sub>0</sub>                   | 413               | 456              | 0.0605          | 450              | 469             | 0.1380          |
| F <sub>m</sub>                   | 2441              | 2405             | 0.6090          | 2527             | 2371            | <b>0.0049</b>   |
| F <sub>v</sub>                   | 2029              | 1949             | 0.3058          | 2077             | 1902            | <b>0.0023</b>   |
| F <sub>v</sub> /F <sub>0</sub>   | 4.93              | 4.42             | 0.0550          | 4.63             | 4.10            | <b>0.0057</b>   |
| F <sub>v</sub> /F <sub>m</sub>   | 0.83              | 0.81             | 0.0820          | 0.82             | 0.80            | <b>0.0035</b>   |
| T <sub>fm</sub>                  | 173               | 159              | 0.2001          | 172              | 179             | 0.4821          |
| Area                             | 26,474            | 24,396           | 0.1341          | 27,277           | 22,771          | <b>0.0044</b>   |
| F <sub>m</sub> /F <sub>0</sub>   | 5.93              | 5.42             | 0.0549          | 5.63             | 5.10            | <b>0.0057</b>   |
| PI ABS                           | 1.30              | 0.99             | <b>0.0357</b>   | 1.04             | 0.75            | <b>0.0323</b>   |
| ABS/RC                           | 3.24              | 3.51             | 0.0599          | 3.28             | 3.41            | 0.0802          |
| TR <sub>0</sub> /RC              | 2.69              | 2.82             | 0.1082          | 2.69             | 2.73            | 0.4889          |
| ET <sub>0</sub> /RC              | 1.21              | 1.17             | 0.1676          | 1.11             | 0.97            | <b>0.0139</b>   |
| DI <sub>0</sub> /RC              | 0.55              | 0.69             | 0.0734          | 0.58             | 0.68            | <b>0.0052</b>   |
| ET <sub>0</sub> /ABS             | 0.38              | 0.34             | <b>0.0247</b>   | 0.34             | 0.29            | <b>0.0161</b>   |
| ET <sub>0</sub> /TR <sub>0</sub> | 0.45              | 0.42             | <b>0.0286</b>   | 0.42             | 0.36            | <b>0.0200</b>   |

The parameters of both the more tolerant (GM-50) and more sensitive (TYTUS) accessions are presented in Figure 4. Control parameters were set as 1 and the parameters of Stress-treated plants were expressed as a percentage of Control. On the presented radar graph, it is easy to notice differences in the response of both genotypes to the given stress.

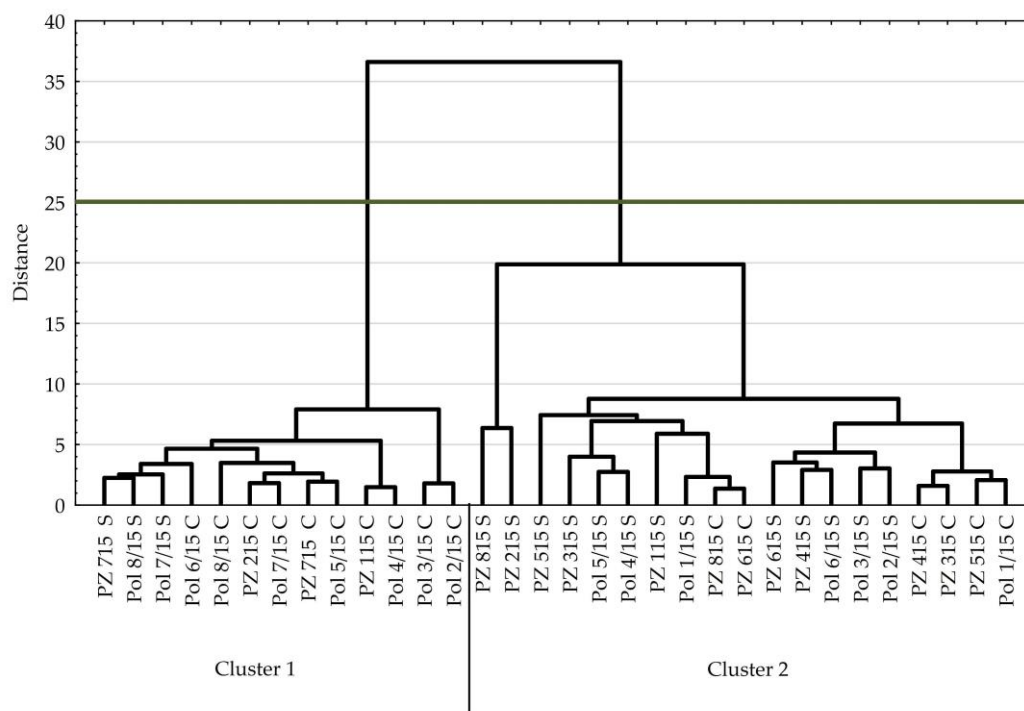
In the case of cucumber accessions provided by the Polish breeding company, we carried out additional experiments with highly homozygous plants (see the Supplementary Materials Section). Homozygous lines of cucumber plants were classified into two groups according to cluster analysis (Figure S1). Five treatments were included in cluster 1, and three others into cluster two. Cluster 1 consisted of groups with better values of determined parameters, in contrast to cluster 2, where groups with worse values were clustered. Differences between cluster 1 and 2 are presented in Table S1; according to Figures S1 and S2, DH2 and DH1 accessions were classified as more tolerant. As a result, DH2 was chosen as a more tolerant form for further investigation. The selection of accessions sensitive to hypoxia stress was based on the assumption that Control and Stress will be in separate clusters. The most sensitive accession was DH4, because the Control plants were included in Cluster 1 and the Stress plants in Cluster 2. This indicated a significant deterioration of parameters after stress treatment.



**Figure 4.** Radar charts comparing 18 traits estimated in Control and Stress plants of two cucumber accessions, GM-50 and TYTUS. Parameters from the Control group were set as 1 and parameters of the Stress-treated plants were expressed in relation to the Control. Asterisks indicate significant differences between Control and Stress according to Student's *t*-test and  $p < 0.05$ , calculated separately for each parameter.

### 3.2. Tomato

The Figure 5 illustrates the relationship among tomato accessions on the basis of differences in morphological and physiological parameters. It was observed that tomato plants were grouped into two main clusters with an Euclidean distance of 25 (Figure 5). In cluster one, we included 13 treatments, whereas 19 were included for cluster two.



**Figure 5.** Results of cluster analysis for tomato accessions using the Euclidean distance on the basis of morphological and physiological traits (Ward's hierarchical algorithm); green line indicates the cut-off point.

The differences between cluster 1 and 2 are presented in Table 5. Cluster 1 consisted of groups with favorable values of determined parameters, in contrast to cluster 2, where groups with worse values

were included. It can be observed that physiological parameters had the main impact on distance calculations, whereas morphological parameters did not influence the hierarchical process.

**Table 5.** Mean value of each parameter determined for both clusters (tomato plants). Bold *p*-values indicate statistically significant differences between cluster 1 and cluster 2, estimated with Student's *t*-test and  $p < 0.05$ .

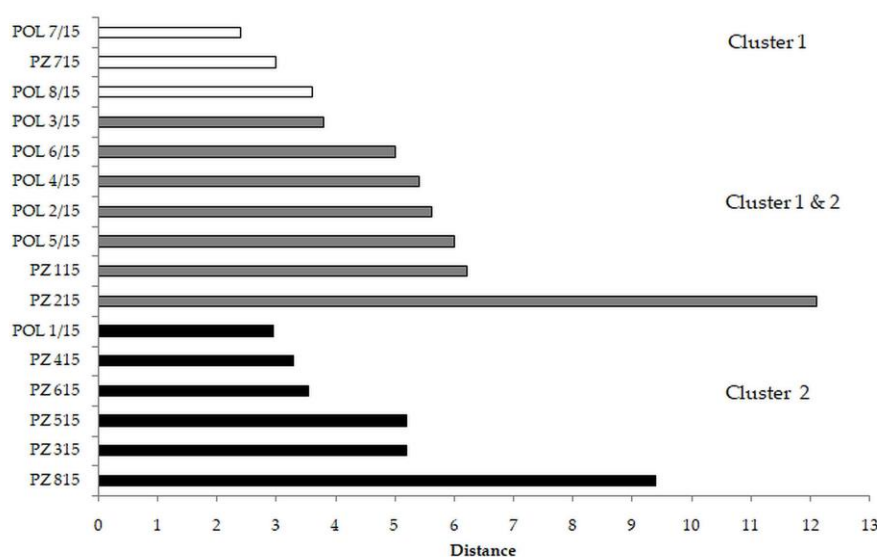
| Parameter                        | Cluster 1 | Cluster 2 | <i>p</i> -Value |
|----------------------------------|-----------|-----------|-----------------|
| % weight change                  | 102       | 102       | 0.798161        |
| % height change                  | 94        | 84        | 0.493144        |
| Relative leaf number             | 1.15      | 1.00      | 0.557965        |
| F <sub>0</sub>                   | 452       | 511       | <b>0.000249</b> |
| F <sub>m</sub>                   | 2374      | 2260      | <b>0.002449</b> |
| F <sub>v</sub>                   | 1922      | 1749      | <b>0.000067</b> |
| F <sub>v</sub> /F <sub>0</sub>   | 4.28      | 3.61      | <b>0.000000</b> |
| F <sub>v</sub> /F <sub>m</sub>   | 0.81      | 0.77      | <b>0.000074</b> |
| T <sub>fm</sub>                  | 188       | 209       | <b>0.015459</b> |
| Area                             | 19,702    | 16,086    | <b>0.000004</b> |
| F <sub>m</sub> /F <sub>0</sub>   | 5.25      | 4.46      | <b>0.000001</b> |
| PI ABS                           | 1.00      | 0.65      | <b>0.000000</b> |
| ABS/RC                           | 3.23      | 3.85      | <b>0.000323</b> |
| TR <sub>0</sub> /RC              | 2.60      | 2.88      | <b>0.000195</b> |
| ET <sub>0</sub> /RC              | 1.07      | 0.99      | <b>0.015152</b> |
| DI <sub>0</sub> /RC              | 0.62      | 0.98      | <b>0.001677</b> |
| ET <sub>0</sub> /ABS             | 0.33      | 0.27      | <b>0.000000</b> |
| ET <sub>0</sub> /TR <sub>0</sub> | 0.41      | 0.35      | <b>0.000000</b> |

Cluster 1 mostly consisted of plants from Control groups of tomato accessions, revealing favorable values of tested parameters, whereas Stress groups were mostly assigned to Cluster 2. However, in Cluster 1, there were control and stress-treated plants from the three accessions (PZ 715, POL 8/15, and POL 7/15); this meant that waterlogging stress did not have negative impact on changes in the parameters. According to this, accessions PZ 715, POL 8/15, and POL 7/15 were considered as more tolerant to oxygen deprivation. Going further in the classification, we conducted a comparison of parameters between Control and Stress groups in the accessions selected above (Table 6). Statistical analysis indicated that in POL 8/15, eight parameters changed under waterlogging stress, whereas in POL 7/15, only two of all estimated parameters were unstable. Therefore, accession POL 7/15 was selected as the most tolerant tomato accession to waterlogging.

When searching for more sensitive accessions, we chose those included in both clusters and with a large Euclidean distance. The Euclidean distance matrix depicts the Control and Stress groups of the PZ 215 accession that were furthest apart and, as a result, were selected as more sensitive (Figure 6). Moreover, we selected PZ 115 Control and Stress groups with large distance. Both selected accessions are compared in Table 7. Controls of both presented accessions were included in cluster 1 and those under Stress treatment in cluster 2. However, more parameters were changed after stress treatment in the case of PZ 215. Figure 6 demonstrates elements of the Euclidean distance matrix of tested accessions and confirms POL 7/15 and PZ 215 as accessions with an opposite response to oxygen deprivation.

**Table 6.** Mean value of each parameter determined in three tomato accessions considered as more tolerant to waterlogging. Bold *p*-values indicate statistically significant differences between Control and Stress plants of each accession separately estimated with Student's *t*-test and  $p < 0.05$ .

| Parameter                        | POL 7/15<br>Control | POL 7/15<br>Stress | <i>p</i> -Value | POL 8/15<br>Control | POL 8/15<br>Stress | <i>p</i> -Value | PZ 715<br>Control | PZ 715<br>Stress | <i>p</i> -Value |
|----------------------------------|---------------------|--------------------|-----------------|---------------------|--------------------|-----------------|-------------------|------------------|-----------------|
| % weight change                  | 100                 | 117                | <b>0.0005</b>   | 100                 | 103                | 0.6168          | 100               | 105              | <b>0.0282</b>   |
| % height change                  | 100                 | 70                 | <b>0.0239</b>   | 100                 | 72                 | <b>0.0255</b>   | 100               | 81               | 0.0537          |
| Relative leaf number             | 1.10                | 1.05               | 0.8010          | 0.95                | 1.00               | 0.7699          | 1.00              | 0.68             | 0.0671          |
| F <sub>0</sub>                   | 441                 | 447                | 0.6030          | 429                 | 453                | 0.2131          | 439               | 496              | <b>0.0002</b>   |
| F <sub>m</sub>                   | 2259                | 2317               | 0.5088          | 2416                | 2324               | 0.1287          | 2292              | 2468             | <b>0.0413</b>   |
| F <sub>v</sub>                   | 1818                | 1869               | 0.5695          | 1987                | 1871               | 0.0507          | 1853              | 1972             | 0.1355          |
| F <sub>v</sub> /F <sub>0</sub>   | 4.17                | 4.20               | 0.9211          | 4.65                | 4.22               | <b>0.0411</b>   | 4.22              | 3.99             | 0.1858          |
| F <sub>v</sub> /F <sub>m</sub>   | 0.80                | 0.81               | 0.5391          | 0.82                | 0.80               | <b>0.0417</b>   | 0.81              | 0.80             | 0.1699          |
| T <sub>fm</sub>                  | 178                 | 191                | 0.3619          | 187                 | 213                | 0.1696          | 177               | 196              | 0.3126          |
| Area                             | 19,874              | 20,181             | 0.8558          | 20,194              | 17,879             | 0.0592          | 19,530            | 19,283           | 0.8493          |
| F <sub>m</sub> /F <sub>0</sub>   | 5.17                | 5.20               | 0.9215          | 5.65                | 5.22               | <b>0.0411</b>   | 5.22              | 4.99             | 0.1862          |
| PI ABS                           | 1.13                | 0.95               | 0.3583          | 1.32                | 0.94               | <b>0.0108</b>   | 0.90              | 0.82             | 0.3596          |
| ABS/RC                           | 3.15                | 3.18               | 0.8164          | 2.97                | 3.37               | <b>0.0003</b>   | 3.49              | 3.35             | 0.1196          |
| TR <sub>0</sub> /RC              | 2.51                | 2.56               | 0.3718          | 2.44                | 2.71               | <b>0.0002</b>   | 2.82              | 2.67             | <b>0.0495</b>   |
| ET <sub>0</sub> /RC              | 1.04                | 1.05               | 0.8469          | 1.08                | 1.11               | 0.4794          | 1.19              | 1.08             | <b>0.0013</b>   |
| DI <sub>0</sub> /RC              | 0.65                | 0.62               | 0.6056          | 0.53                | 0.66               | <b>0.0035</b>   | 0.67              | 0.68             | 0.9076          |
| ET <sub>0</sub> /ABS             | 0.34                | 0.33               | 0.7765          | 0.36                | 0.33               | 0.0726          | 0.34              | 0.32             | 0.1381          |
| ET <sub>0</sub> /TR <sub>0</sub> | 0.42                | 0.41               | 0.7011          | 0.44                | 0.41               | 0.1028          | 0.42              | 0.40             | 0.1901          |

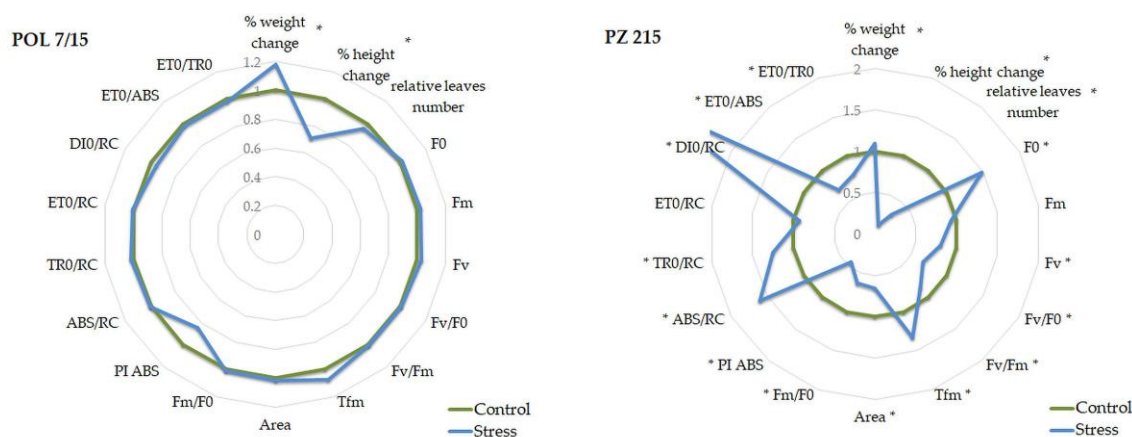


**Figure 6.** Euclidean distance between Control and Stress groups of each tomato accession. White bars indicate accessions from control and stress treatments that were classified into cluster 1; light grey bars indicate accessions from control and stress treatments that were classified into different clusters; black bars indicate accessions from control and stress treatments that were classified into cluster 2.

**Table 7.** Mean value of each parameter determined in two tomato accessions considered as more sensitive to waterlogging. Bold *p*-values indicate statistically significant differences between Control and Stress plants of each accession separately estimated with Student's *t*-test and  $p < 0.05$ .

| Parameter                        | PZ 115 Control | PZ 115 Stress | <i>p</i> -Value | PZ 215 Control | PZ 215 Stress | <i>p</i> -Value |
|----------------------------------|----------------|---------------|-----------------|----------------|---------------|-----------------|
| % weight change                  | 100            | 109           | <b>0.0160</b>   | 100            | 109           | <b>0.0332</b>   |
| % height change                  | 100            | 211           | <b>0.0001</b>   | 100            | 11            | <b>0.0000</b>   |
| Relative leaf number             | 0.00           | 0.20          | 0.0527          | 0.53           | 0.16          | <b>0.0160</b>   |
| F <sub>0</sub>                   | 454            | 491           | 0.1741          | 456            | 677           | <b>0.0069</b>   |
| F <sub>m</sub>                   | 2502           | 2274          | <b>0.0065</b>   | 2371           | 2211          | 0.0645          |
| F <sub>v</sub>                   | 2048           | 1783          | <b>0.0051</b>   | 1915           | 1534          | <b>0.0053</b>   |
| F <sub>v</sub> /F <sub>0</sub>   | 4.54           | 3.80          | <b>0.0149</b>   | 4.22           | 2.82          | <b>0.0014</b>   |
| F <sub>v</sub> /F <sub>m</sub>   | 0.82           | 0.78          | <b>0.0328</b>   | 0.81           | 0.68          | <b>0.0034</b>   |
| T <sub>fm</sub>                  | 210            | 259           | 0.0775          | 183            | 244           | <b>0.0000</b>   |
| Area                             | 19,309         | 15,832        | 0.0864          | 20,171         | 13,332        | <b>0.0010</b>   |
| F <sub>m</sub> /F <sub>0</sub>   | 5.54           | 4.80          | <b>0.0149</b>   | 5.22           | 3.82          | <b>0.0014</b>   |
| PI ABS                           | 1.10           | 0.73          | <b>0.0201</b>   | 0.97           | 0.43          | <b>0.0008</b>   |
| ABS/RC                           | 3.46           | 3.89          | <b>0.0448</b>   | 3.20           | 5.13          | <b>0.0009</b>   |
| TR <sub>0</sub> /RC              | 2.82           | 3.00          | 0.0866          | 2.58           | 3.22          | <b>0.0000</b>   |
| ET <sub>0</sub> /RC              | 1.21           | 1.15          | 0.0975          | 1.04           | 0.97          | 0.3251          |
| DI <sub>0</sub> /RC              | 0.64           | 0.89          | <b>0.0445</b>   | 0.62           | 1.91          | <b>0.0051</b>   |
| ET <sub>0</sub> /ABS             | 0.36           | 0.31          | <b>0.0313</b>   | 0.33           | 0.23          | <b>0.0019</b>   |
| ET <sub>0</sub> /TR <sub>0</sub> | 0.43           | 0.39          | <b>0.0466</b>   | 0.41           | 0.31          | <b>0.0036</b>   |

As a summary, the radar charts were created for POL 7/15 and PZ 215 tomato accessions, defined as more tolerant and more sensitive, respectively (Figure 7). The radar charts strongly highlighted the differences in response to waterlogging stress in selected tomato accessions. There were statistically significant differences in the weight and height of plants between the Control and Stress groups in POL 7/15, and thus only morphological parameters changed. In case of PZ 215, only 2 of 18 parameters were stable: F<sub>m</sub> and ET<sub>0</sub>/RC (Table 7 and Figure 7).



**Figure 7.** Radar charts comparing 18 traits estimated in Control and Stress plants of two tomato accessions POL 7/15 and PZ 215. Parameters from the Control group were set as 1 and parameters of Stress-treated plants were expressed in relation to Control. Asterisks indicate significant differences between Control and Stress plants according to Student's *t*-test and  $p < 0.05$ , calculated separately for each parameter.

#### 4. Discussion

The reaction of tomato or cucumber accessions to waterlogging stress is diversified. As we have shown, accessions can be grouped for those whose parameters significantly worsen after stress and those that do not show a significant deterioration in functioning. In the presented research, we focused on the response of the aerial part to stress present within the root system. During hypoxia of the root system, signals to the aboveground part—often found in optimal oxygen conditions—are transmitted within the plant body [47–49]. The signal that moves from the root to the aboveground part during

hypoxia stress changes the functioning of the shoots. The most important process in the aboveground part of plants is photosynthesis, which generates energy and carbohydrates. Stress conditions affect photosynthesis (see the review in [50]). Chloroplasts, key organelles for photosynthesis, are highly sensitive to many stress factors. The photosynthesis process can be disrupted due to decreases in pigment content, changes in electron transport, or disorders in the activities of enzymes related to CO<sub>2</sub> fixation. Moreover, limitations in gas diffusion (CO<sub>2</sub> and water) can be observed due to stomata closure. Therefore, it is reasonable to study the intensity of photosynthesis or chlorophyll *a* fluorescence during stresses involving the root system, such as hypoxia, salinity, or others that interfere in the functioning of the plant. For example, decreases in net photosynthesis as well as a decline in maximal photochemical efficiency of PSII after hypoxia have been observed in sensitive accessions of cotton [39], while in tolerant forms, the changes were not observed. The JIP-test, widely discussed since 1995, is a good tool for comparing stressed and control samples and allows investigation of the effects of almost any stress factor, although only if the stress affects photosynthesis [51]. For example, Kalaji et al. [52] tested the impact of 14 abiotic stress factors on barley by fast chlorophyll fluorescence kinetics and used this method as a crop phenotyping tool. Chlorophyll fluorescence measurement is a fast and non-destructive method to analyze the photosynthetic apparatus [53], allowing for the detection of stress effects before the visible signs are noticed [52]. Thus, in our research, we chose parameters related to plant growth and chlorophyll *a* fluorescence in the leaves.

After performing statistical analysis, we divided the studied waterlogged and control accessions into clusters. In both species, significant decreases in Fm, Fv, Fv/F<sub>0</sub>, Fv/Fm, Area, Fm/F<sub>0</sub>, PI ABS, ET<sub>0</sub>/RC, ET<sub>0</sub>/ABS, and ET<sub>0</sub>/TR<sub>0</sub> parameters were observed in cluster 2 as compared to cluster 1. In addition, increases in DI<sub>0</sub>/RC was observed. According to this information, we conclude that in cluster 1, plants had parameters with more favorable values, and in cluster 2, plants had worse parameters (Table 2, Table 5, and Table S1). The decrease in Fm or Fv/Fm is connected with a lower ability of PSII to reduce the QA primary acceptor [54]. Furthermore, the decrease in Fv/F<sub>0</sub> during stress could be an indicator of lower efficiency of photochemical processes in PSII [55]. The Area parameter represents the pool size of electron acceptors in PSII, with this pool size being lower during reductions in electron transport in submergence stress [54]. The lower value of ET<sub>0</sub>/RC is also indicator of disturbances in electron transport from QA to other acceptors. Panda et al. [54] state that both the donor and the acceptor side of PSII were damaged because of submergence. The PI ABS index includes information about the probability that the chlorophyll *a* molecule functions as a reactive center in PSII, the efficiency of transfer of the absorbed energy to the reduction of QA, and the probability that an electron moves further than QA. It is a very sensitive parameter that decreases during stress conditions [51]. When the photochemistry of photosynthesis is disrupted by stress factors, the dissipation of absorbed energy increases [56], and this can be observed as increase in the DI<sub>0</sub>/RC value.

Considering the results of the cluster analysis, we chose a more tolerant and more sensitive accession in both species. The parameters of more tolerant accessions did not deteriorate after appropriate stress, and the Control and Stress group of such plants were in cluster 1. In the case of more sensitive accessions, the parameters significantly deteriorated, and the Control group was in cluster 1 and the Stress-treated group in cluster 2. The changes in OJIP test parameters after submergence stress were more pronounced in sensitive rice (*Oryza sativa* L.) cultivars than in tolerant ones [54]. Similarly, in our experiments, more significant differences, indicating the deterioration of the photosynthetic apparatus, between Stress and Control plants could be observed in the case of sensitive accessions compared with that in more tolerant accessions (Figures 4 and 7). For example, the Fv/F<sub>0</sub>, Fv/Fm, Area, Fm/F<sub>0</sub>, PI ABS, and DI<sub>0</sub>/RC parameters remained at the same level after stress treatment in the case of more tolerant accessions, but changed in sensitive accessions after stress. Increases in DI<sub>0</sub>/RC in more sensitive accessions of both species indicated that some of the absorbed energy was dissipated and not used in the photochemistry of photosynthesis. In the case of more tolerant plants, DI<sub>0</sub>/RC was stable. In agreement with our observation of more sensitive samples, we observed increases in DI<sub>0</sub>/RC parameter in cucumber plants after hypoxia stress [56].

The decrease in Fv/Fm was noted in more sensitive tomato and cucumber plants in our experiments. In agreement with our results, a decrease in Fv/Fm after waterlogging was also observed in rice [54,57], cucumber [56,58], tomato [59,60], wild tomato (*Solanum habrochaites* S.Knapp & D.M.Spooner) [61], *Arabidopsis* (*Arabidopsis thaliana* L.) [62], cotton [39], and pepper (*Capsicum annuum* L.) plants [63]. Barik et al. [57] examined the reaction of tolerant and susceptible varieties of rice to submergence and observed that the latter exhibited a greater reduction in Fv/Fm parameters in comparison to tolerant varieties. Similarly, in an experiment with cotton, the Fv/Fm parameter was stable in tolerant varieties and significantly decreased in sensitive varieties under hypoxia [39]. In the case of sorghum after waterlogging stress, the changes in Fv/Fm parameters were not significant, despite the observation of a substantial decrease in the Fv/F<sub>0</sub> parameter [64]. In the present experiment, the decrease in Fv/Fm in more sensitive tomato genotypes was about 16%, and for the Fv/F<sub>0</sub> parameter, this was about 33% after stress treatment; in sensitive cucumber genotypes, these figures were 2.4% and 11.5%, respectively. This suggests that Fv/F<sub>0</sub> is a more sensitive parameter than Fv/Fm, consistent with the findings of Tsimilli-Michael [51]. However, the parameter Fv/Fm is more often described in the literature. Kalaji et al. [52] observed that PI ABS is the most sensitive parameter to different stress conditions. A decrease in this parameter was observed under waterlogging stress in terms of rice [54], cucumber [56], or tomato [59]. In the present experiments, the decrease in PI ABS in the case of sensitive tomato plants was about 56%, and for cucumber plants this was about 28% after stress treatment. This parameter did not change in more tolerant accessions after waterlogging stress. Our observations confirm the high sensitivity and usefulness of this parameter in plant selection to waterlogging stress.

Many studies have presented results of plant morphological observations after waterlogging stress; however, sometimes inconsistent information can be found. Decreases in plant height were observed after waterlogging in cucumber [56] and field bean (*Vicia faba* L. minor) [65]. Six cotton varieties, sensitive and tolerant to hypoxia stress, were tested by Pan et al. [39], and an inhibition in plant growth was observed in more sensitive varieties. However, plant height in stressed tolerant cotton was similar to untreated plants. In the terms of fresh mass of plants, we observed a decrease in terms of cucumber [58], barley [38], and pepper [63] after stress treatment, but no changes were observed by He et al. [56] in cucumber fresh weight after hypoxia. In our results, some accessions indicated an increase in morphological parameters, whereas others decreased. It is worth mentioning that both selected accessions of cucumber and tomato (tolerant and sensitive) demonstrated a decrease in plant height after stress treatment. The appearance of selected accessions is presented in Figure S3. The inhibition of growth does not seem to be correlated with the activity of PSII.

Using physiological parameters and cluster analysis, Barik and co-workers [57] classified seven rice cultivars into two clusters. Cluster one included submergence-tolerant rice varieties whereas susceptible varieties were included in cluster two. These data indicated the usefulness of cluster analysis in stress tolerance classification. Moreover, in our experiment, cluster analysis helped in classification, although the procedure was slightly different. Cluster analysis can be used in stress tolerance plant classification, as also demonstrated by Cao et al. [40], wherein the authors used cluster analysis to divide tomato genotypes into those that are more or less tolerant to chilling stress.

As part of our cooperation with Polish breeders, we also conducted a sensitivity assessment of homozygous cucumber lines for waterlogging stress and selected more tolerant and more sensitive lines (see the Supplementary Materials Section). Selected cucumber homozygous lines can be used for basic research on stress resistance or for breeding new varieties adapted to new breeding programs. The results can be of use not only to Polish breeders, but also to international breeders. According to previous information, choosing more tolerant accessions maintained better PSII activity.

Two tools were used in the present work: chlorophyll *a* fluorescence was applied as the main tool to assess the state of the photosynthetic apparatus of stress-treated plants and statistical analysis was used to select sensitive and tolerant accessions on the basis of the obtained empirical data. The selected objects will be used for further analysis related to understanding the mechanisms of the stress response to hypoxia in tomato and cucumber plants. In future research, we plan to evaluate the effect of

waterlogging of selected tomato and cucumber plants on the photosynthetic rate, chlorophyll and carotenoid accumulation, and other parameters related to the functioning of leaves. We will also investigate if there are differences between selected accessions in terms of yield quality and quantity during stress.

## 5. Conclusions

Chlorophyll *a* fluorescence can be used for the selection of plant accessions sensitive to waterlogging stress.

Not all parameters of the OJIP test seem to have the same sensitivity;  $F_v/F_0$ , PI ABS, as well as  $DI_0/RC$  or Area appear to be better in selecting sensitivity to waterlogging stress than  $F_v/F_m$ .

From the tested accessions, we selected GM-50, POL 7/15, and DH2 as more tolerant, whereas TYTUS, PZ 215, and DH4 were determined to be more sensitive for waterlogging stress.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/10/10/1490/s1>, Figure S1: Results of cluster analysis for cucumber homozygous lines using the Euclidean distance on the basis of morphological and physiological traits (Ward's hierarchical algorithm); green line indicates the cut-off point. Table S1: The mean values of parameters determined for each cluster and statistical comparison between clusters estimated in cucumber homozygous lines (bold p-values mean statistically significant differences between cluster 1 and cluster 2). Figure S2: Euclidean distance between Control and Stress groups of each cucumber homozygous lines. White bars indicate accessions from control and stress treatments that were classified into cluster 1; light grey bars indicate accessions from Control and Stress treatments that were classified into different clusters; black bars indicate accessions from Control and Stress treatments that were classified into cluster 2. Figure S3: Accessions GM-50, POL 7/15, and DH2 selected as more tolerant, and TYTUS, PZ 215, and DH4 determined to be more sensitive for waterlogging stress. C: Control plants, S: Stress-treated plants.

**Author Contributions:** Conceptualization, A.K. and M.C.; methodology, A.K.; formal analysis, A.K. and K.K.; investigation, A.K., M.C., and K.K.; data curation, A.K. and K.K.; writing—original draft preparation, A.K., M.C., and K.K.; writing—review and editing, A.K., M.C., and K.K.; visualization, A.K., K.K., and M.C.; funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.

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