

Article

Evaluation of Spring Wheat (20 Varieties) Adaptation to Soil Drought during Seedlings Growth Stage

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Abstract: The effect of soil drought (10 days) on the growth of plants, the accumulation of water and leakage of electrolytes, gas exchange, the contents of chl *a* + *b* and carotenoids in leaves and photochemical activity of photosystem II was studied at the seedling stage by transient fluorescent analysis in 20 of the popular varieties of polish spring wheat. Drought caused a particularly strong reduction in vigor of growth of seedlings, net photosynthesis rate and triggered an increase in electrolyte leakage from the leaves. Certain varieties during the drought demonstrated relatively intense CO₂ assimilation at low water loss through transpiration. The varieties tested were significantly different in terms of tolerance to drought of the processes of gas exchange and seedlings development. Photochemical processes in PSII showed high tolerance to drought and at the same time low differentiation among varieties. The results obtained suggested that tolerance of growth parameters to drought and CO₂ assimilation at the seedling stage may alleviate consequent depression of final yield of the grain.

Keywords: varieties; drought stress; stress index; wheat; photochemical activity; yield; assimilation CO₂; gas exchange; water management

1. Introduction

Wheat is the most important cereal crop; it is common diet for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crop [1]. Water deficit is the most severe stress and the main cause of significant losses in growth and productivity of crop plants [2,3]. Water deficit is particularly dangerous for spring cereals, which do not use winter reserves of water in the soil. Spring wheat has additionally particularly high water demands. This is caused by the poor development of root system, a shorter growing season, and the fact that these plants are sown in partially dried soil by the spring tillage. What is more, the initial growth of spring wheat is slow, which further increases their vulnerability to drought. This period of high sensitivity to drought occurs in the spring during the tillering phase and later during the shooting and heading stages.

Drought tolerance is not an easily quantifiable plant attribute, because it is a combination of complex physiological, morphological and molecular traits. Physiological traits affected by drought can be correlated with the rate of CO₂ assimilation [4–6], PSII photochemical activity [7–11], leaf water potential (stomatal conductance, transpiration rate, relative water content—RWC) [11–13], plasma membrane integrity [14] and chlorophyll content [15].

The improvement of tolerance to drought has been a long-lasting principal goal of the majority of breeding programs as water deficit in certain stages of wheat growth commonly occurs in many wheat growing regions of the world [16]. In the studies on watering plants, increased emphasis is being placed on understanding reactions to drought, not only in species, but also their particular varieties [17]. Different varieties can display various requirements in terms of environmental conditions [18]. The plant varieties with lower water requirements and/or higher resistance to drought could be useful in the areas with limited access to water, and thus compensate the losses associated with reduced yields [19].

For some genotypes, the seedling response is apparently a good indicator of the plants' reaction to drought stress later in their life cycle. The objective of this research was to verify more reliably some of the physiological traits used for screening the performance on a set of popular Polish wheat genotypes under restricted water conditions in the soil and to correlate the tolerance of seedlings of these varieties with the depression of final grain yield.

2. Results and Discussion

2.1. Results

2.1.1. Water Content and Electrolyte Leakage

The mean values obtained for all varieties tested indicate that soil drought in wheat seedlings resulted in only a low reduction in the water content in leaves (3.1%), and a higher decrease in RWC (20.3%), while simultaneously causing a significant increase in electrolyte leakage (EL) from the cells (38.8%) compared to control (Table 1).

Table 1. The mean, variation coefficient (CV), the maximum and minimum values of the mean and stress index (SI as percentage of control) of physiological parameters of seedlings and the final yield of grain wheat varieties at optimal soil moisture and after the drought stress. Values calculated on the basis of the means for 20 varieties ($N = 20$).

Parameters	Growth Conditions	Mean	CV	Minimum	Maximum	SI
Water Content and Electrolyte Leakage						
water content (%)	control	86.5	1.2	83.6	88.0	
	drought	83.8	1.7	80.7	86.0	96.9
RWC (%)	control	92.2	2.5	88.7	96.1	
	drought	73.5	10.8	59.9	90.0	79.7
EL (%)	control	3.3	29.7	1.8	5.7	
	drought	4.6	18.1	3.2	6.0	138.8
Growth and Accumulation of Biomass						
leaf area (cm ²)	control	30.0	8.8	25.5	34.5	
	drought	21.0	13.0	15.9	25.0	69.9
dry weight of seedling (mg)	control	92.2	11.1	73.5	108.2	
	drought	75.1	13.3	48.2	90.8	81.4
RGRa (m ² m ⁻² day ⁻¹) × 10 ²	control	9.0	11.0	6.9	11.0	
	drought	5.4	27.4	2.4	7.6	59.6
NAR (g m ⁻² day ⁻¹) × 10	control	31.7	11.2	25.4	38.8	
	drought	28.4	16.0	19.4	37.9	89.6
Gas Exchange						
P _N (μmol CO ₂ m ⁻² s ⁻¹)	control	13.1	7.7	11.6	15.9	
	drought	1.5	46.4	0.5	2.6	11.4
WUE (μmol CO ₂ × mmol H ₂ O ⁻¹)	control	5.6	21.6	4.2		
	drought	6.5	59.1	1.7	15.0	115.5
Leaf Pigment Content						
chlorophyll (<i>a</i> + <i>b</i>) (mg m ⁻²)	control	415.5	11.3	348.7	507.2	
	drought	367.0	10.2	324.7	469.5	88.3
carotenoids (mg m ⁻²)	control	32.3	8.4	28.9	40.0	
	drought	29.8	6.0	27.8	34.7	92.2
Photochemical Efficiency						
ABS/CS (ru)	control	1946.6	3.6	1802.9	2085.9	
	drought	1826.8	3.5	1698.7	1919.7	93.8
TRo/CS (ru)	control	1492.1	4.6	1374.4	1642.5	
	drought	1391.1	4.1	1265.5	1472.3	93.2
ETo/CS (ru)	control	699.5	7.6	597.3	801.3	
	drought	654.1	7.1	562.3	737.3	93.5
DIo/CS (ru)	control	454.5	3.4	420.0	481.0	
	drought	435.7	4.3	398.3	471.2	95.9
RC/CS (ru)	control	675.2	7.0	587.0	773.6	
	drought	626.6	5.9	544.5	686.7	92.8
Weight of Seeds						
(g plant ⁻¹)	control	17.4	21.2	13.0	27.6	
	drought	14.3	22.9	10.2	25.9	82.5

ru—relative units.

The diversity of the set of varieties tested in the conditions of optimal watering (control) depended on the analyzed trait/parameter of the plant. Differences in the water content in the leaves and RWC were at extremely low levels (coefficient of variation CV was respectively 1.2% and 2.5%), but the EL was significantly higher (CV *ca.* 30%). Application of drought stress caused a significant increase in differences between particular wheat varieties only with respect to RWC. When water content in the tissues is considered, the increase in the CV value at this growth conditions was low, and the reduction of the EL ratio was also observed, indicating disappearance of the differences between varieties.

Detailed statistical analysis revealed that under drought conditions, none of the varieties tested in this study, except for the cv. Bryza, were able to maintain RWC at the level of controls (Table 2). In contrast, the stability of cell membranes under stress, estimated on the basis of EL measurements, proved to be high for some varieties (“Jasna”, “Katoda”, “Parabola”, “Zadra” and “Żura”), as evidenced by the stress index value, which was close to 100%.

Table 2. Values of stress index (as percentage of control) water content, relative water content (RWC), electrolyte leakage (EL) and growth parameters of seedling (leaf area, dry weight of seedling, RGRa, NAR) of 20 wheat varieties after 10 days of drought stress.

Variety	Water Content	RWC	EL	Leaf Area	Dry Weight of Seedling	RGRa	NAR
Banti	95.0 *	72.4 *	130.6 *	54.5 *	75.5 *	38.5 *	92.6
Bombona	95.8 *	70.3 *	248.0 *	78.1 *	91.8	65.0 *	99.7
Bryza	99.5	93.8	198.9 *	81.8 *	78.7 *	77.7 *	77.2 *
Cytra	98.5	87.9 *	173.8 *	73.5 *	86.4 *	66.8 *	95.3
Hewilla	98.7	89.3 *	246.9 *	67.6 *	79.9 *	64.5 *	92.1
Jasna	97.7	80.3 *	105.0	82.4 *	91.9	75.8 *	98.3
Katoda	96.6	81.9 *	109.8	76.7 *	85.4 *	70.0 *	92.1
Koksa	98.3	80.9 *	142.3 *	74.4 *	81.9 *	67.9 *	88.3 *
Korynta	97.2	91.7 *	228.4 *	82.1 *	80.3 *	78.6 *	80.3 *
Monsun	96.2 *	82.2 *	142.5 *	57.9 *	65.6 *	44.6 *	68.3 *
Nawra	93.3 *	65.5 *	118.6 *	55.1 *	74.9 *	28.7 *	86.3 *
Parabola	95.9 *	81.6 *	107.8	67.1 *	85.1 *	60.1 *	99.6
Radunia	96.5	84.6 *	138.6 *	83.4 *	89.7	80.7 *	95.5
Raweta	100.0	78.0 *	135.5 *	53.9 *	77.4 *	38.4 *	95.0
Torka	96.5	77.3 *	124.2 *	71.5 *	83.1 *	61.0 *	90.7
Vinjet	95.9 *	69.2 *	115.1 *	72.8 *	83.6 *	54.2 *	89.8 *
Waluta	98.7	81.5 *	132.8 *	80.6 *	83.1 *	75.6 *	85.3 *
Zadra	96.9	79.2 *	105.1	60.9 *	72.1 *	45.7 *	79.1 *
Zebra	95.3 *	67.1 *	156.9 *	64.8 *	84.3 *	47.1 *	97.8
Żura	96.4	79.1 *	104.7	65.7 *	77.5 *	52.7 *	86.1 *
<i>F</i>	1.97	9.68	15.06	13.11	12.63	8.31	3.03
<i>p</i>	0.011	0.000	0.000	0.000	0.000	0.000	0.000

The significance of differences in varieties was determined by the analysis of variance using the *F*-test and probability *p*. Asterisks indicate significant differences ($p < 0.05$) between control and drought treated plants according to *F*-test.

2.1.2. Growth and Accumulation of Biomass

Drought stress resulted in significantly lower growth vigor of seedlings (Table 1). Growth measured as leaf area and mass accumulation of whole plants were inhibited. The mean value of relative growth rate of leaf area (RGRa) decreased in the varieties tested during the period of stress by nearly 40%, while the intensity of net assimilation (NAR) by about 10%. Drought caused differences in the varieties tested in all parameters of growth measured. This effect was most pronounced for RGRa, for which the variation coefficient (CV) was more than 27%. None of the varieties were able to prevent the inhibition of the growth process of leaf area under drought conditions (Table 2). However, some of them retained the ability to intensively increase the weight of the seedlings (cv. “Bombona”, “Jasna” and “Radunia”) under stress and to maintain a high net assimilation rate (NAR), because the values of these parameters were similar to controls and did not differ statistically from them. The growth of leaves of cv. “Raweta” and “Banti” was particularly sensitive to drought. This is reflected in the low values of the SI index for RGRa, reaching about 38%. Relatively, net assimilation was inhibited by stress most significantly in varieties “Monsun” (SI = 68.3%) and “Zadra” (SI = 79.1%).

2.1.3. Gas Exchange and Leaf Pigment Content

The growth under drought conditions reduced the rate of photosynthesis (P_N) in all varieties by an average of about 89%, the content of chl $a + b$ by about 12% and carotenoid content of 8%, while WUE index was elevated by almost 16% (Table 1). In relation to the control, soil drought increased variation in varieties in terms of P_N and WUE indices. The coefficient of variation of both parameters was the highest in drought from all the tested plant traits and amounted to more than 46% and 59%, respectively. In contrast, the differences between the varieties in terms of the content of chl $a + b$ and carotenoids slightly narrowed down in drought conditions. None of the varieties tested could maintain P_N at the control level after 10 days of drought (Table 3). Coefficient of water use efficiency was either increased or decreased under stress depending on the variety.

2.1.4. Photochemical Efficiency

Activity of photochemical processes in PSII was subject to slight inhibition in the absence of water in the soil (Table 1). The reduction of energy absorption by the antenna system, the energy flux transported to the center and outside the center of PSII, energy dissipation and the concentration of active reaction centers, did not exceed an average of 8% when compared to the well-watered plants. Diversification of varieties in terms of photochemical activity was also low. At both levels of water content in the soil, CV coefficient did not exceed 8% for the photochemical parameters (Table 3).

2.1.5. Consequent Effect on the Yield of Grain

Drought at the seedling stage caused a reduction in the final yield of wheat varieties by an average of 18% (Table 1). For most varieties, a decrease of grain yield was statistically significant compared to the properly watered controls (Table 3). The varieties “Jasna” and “Radunia” did not show a significant change in the yield compared to control, which indicated their tolerance to stress. Good

tolerance to drought was demonstrated by “Zadra”, “Cytra”, “Bombona” and “Torka” varieties. The highest decrease in yield was recorded for “Koksa”, “Monsun” and “Zebra” varieties.

Table 3. Values of stress index of gas exchange, chlorophyll and carotenoids contents, parameters of photochemical efficiency (PSII) of seedling and final weight of seeds of 20 wheat varieties after 10 days of drought stress.

Odmiana	P _N	WUE	Chl (<i>a</i> + <i>b</i>)	Carotenoids	ABS/CS	TRO/CS	ETo/CS	DIO/CS	RC/CS	Weight of Seeds
Banti	13.3 *	79.2 *	77.3 *	85.7 *	93.9 *	93.9 *	94.9	93.7 *	96.7	73.3 *
Bombona	4.8 *	54.8 *	89.0 *	93.6	94.8 *	95.8	98.6	91.8 *	95.4	87.3 *
Bryza	15.8 *	180.6 *	92.0	95.3	93.7 *	92.8 *	91.0 *	96.9	89.3 *	75.5 *
Cytra	11.7 *	192.3 *	97.3	98.3	97.8	96.1	92.0 *	103.4	92.5 *	89.7 *
Hewillia	21.3 *	121.3 *	91.9	94.9	95.3	94.6 *	97.9	97.3	92.7 *	81.2 *
Jasna	13.3 *	76.4 *	97.1	98.4	96.4	96.0	99.1	97.6	96.3	98.8
Katoda	15.7 *	266.9 *	80.8 *	88.3 *	97.6	98.1	101.6	95.7 *	96.7	93.8 *
Koksa	7.3 *	260.4 *	83.7 *	89.5 *	87.1 *	84.8 *	82.7 *	95.2	83.1 *	71.0 *
Korynta	10.4 *	85.8 *	85.2 *	90.9 *	88.6 *	86.3 *	78.7 *	96.3	83.2 *	74.8 *
Monsun	4.3 *	48.7 *	86.9 *	91.2	92.7 *	92.1 *	92.8	94.5 *	91.8 *	72.3 *
Nawra	6.3 *	92.1 *	93.7	86.8 *	92.0 *	92.0 *	92.7	92.0 *	92.2 *	78.5 *
Parabola	13.6 *	124.6 *	97.7	98.7	99.1	97.8	99.9	103.4	97.8	84.8 *
Radunia	12.5 *	240.9 *	89.3 *	93.4	89.9 *	88.0 *	84.3 *	96.2 *	86.1 *	96.2
Raweta	11.5 *	48.8 *	93.2	95.6	98.6	98.8	100.7	98.1	102.6	85.3 *
Torka	19.5 *	118.1 *	93.1	95.9	95.1 *	94.8 *	98.4	96.1	93.5	86.3 *
Vinjet	4.5 *	97.3 *	88.8 *	93.0	90.0 *	90.4 *	94.7	88.5 *	92.4 *	85.3 *
Waluta	4.3 *	35.5 *	81.6 *	88.4 *	93.2 *	92.5 *	92.1	95.6 *	92.1 *	78.8 *
Zadra	19.5 *	125.0 *	79.8 *	87.1 *	93.5 *	93.0 *	94.9	95.1 *	91.0 *	91.5 *
Zebra	4.1 *	512 *	81.6 *	88.1 *	91.9 *	91.7 *	94.1	92.5 *	92.8 *	71.3 *
Żura	15.4 *	79.6 *	93.1	96.2	97.1	96.9	93.9	98.0	102.2	80.9 *
<i>F</i>	14.91	13.62	1.94	1.85	2.94	3.07	3.18	4.78	3.52	11.02
<i>p</i>	0.000	0.000	0.013	0.019	0.000	0.000	0.000	0.000	0.000	0.000

The significance of differences in varieties was determined by the analysis of variance using the *F*-test and probability *p*. Asterisks indicate significant differences ($p < 0.05$) between control and drought treated plants – according to *F*-test.

2.1.6. Correlation between Seedlings Tolerance, Final Yield and Drought

There was a relatively small number of statistically significant linear correlation coefficients between the relative tolerance to drought of plant traits studied (Table 4). SI index of the final yield of grains was rather poorly related and showed correlation only with the weight of seedlings and NAR. The weight of seedlings was correlated with the leaves area and RGRa. Since the correlation coefficients between the single traits of the seedlings and the final yield were low, multiple correlations were calculated, providing an opportunity to measure the collective impact of seedlings' traits on yield (Table 5). Calculations carried out stepwise, revealed a steep increase of the determination coefficient (R^2) when fourth trait was introduced to the regression equation. SI values of the yield calculated on the basis of this equation (theoretical yield) were compared with the respective data of the actual yield (Figure 1). The equation indicated seven most tolerant wheat varieties, which

stayed in agreement with experimental observations. An analysis of discrimination carried out for the same data also confirmed the possibility of predicting the tolerance of wheat varieties to drought based on SI of selected traits of seedlings (Table 6).

2.2. Discussion

2.2.1. Water Management and Growth of Seedlings

Growth processes are generally very vulnerable to water deficit [10]. Keeping a high value of water content provides an opportunity not only to better protect physiological processes during drought, but also to improve recovery of plants after re-watering. Maintaining a high value of this parameter during drought provides an opportunity not only to better protect physiological processes, but also to improve recovery of plants after re-watering. In our work, variation observed in varieties with respect to RWC under stress, measured by the coefficient of variation, was about four-fold higher than the variation observed in well-watered plants (Table 1). It probably reflects considerable variation resources among varieties of wheat tested. The water shortage in the soil causes a reduction in the growth of leaf area by an average of 30%, and a reduction in the dry weight of seedlings by 19%, the restriction in RGRa and NAR. Under the adopted criterion, the majority of plant's growth features were highly diversified between the varieties (Table 2).

Soil drought has caused a significant increase in electrolyte leakage from leaves, similarly as in the study by Bajji *et al.* [20]. Unfortunately, in our work, the drought pressure has contributed to the reduction of differences between varieties, which might indicate their inability to more effectively protect the membranes against dehydration.

Figure 1. The values of drought stress index for the final empirical grain yield and yield calculated based on multiple regression equation—theoretical yield (Table 4) for the 20 varieties of spring wheat. Horizontal line defines seven most tolerant varieties to drought in empirical and theoretical terms.

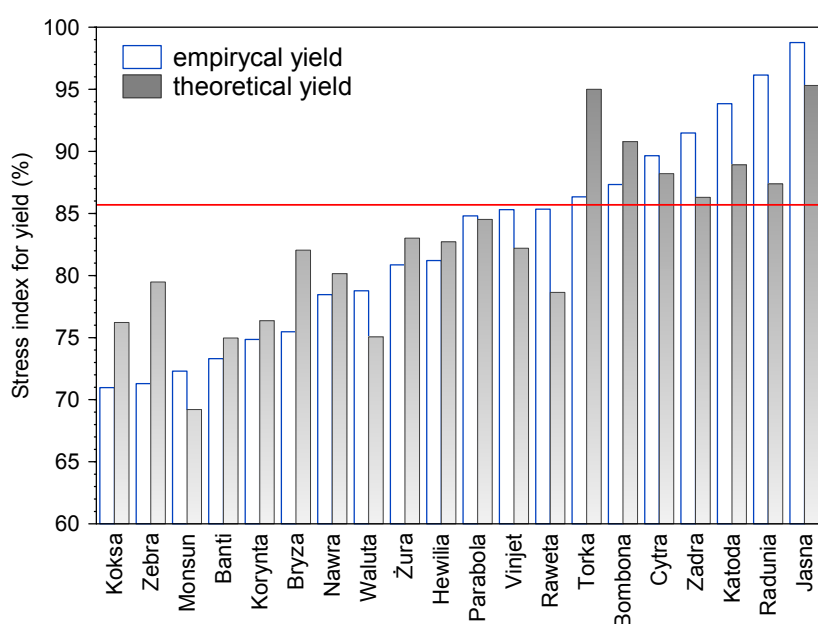


Table 4. Values of stress index of gas exchange, pigment contents, parameters of photochemical efficiency (PSII) of seedling and final weight of seeds after drought stress.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.701	ns	ns	ns	0.489	ns	ns	ns	ns	ns	ns	ns	ns	0.471	ns	ns
2	1.000	ns	0.476	ns	0.671	ns	0.475	ns	ns	ns	ns	ns	ns	0.631	ns	ns
3		1.000	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4			1.000	0.705	0.958	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.454	ns
5				1.000	0.627	0.783	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.546
6					1.000	ns	ns	0.444	ns	ns	ns	ns	ns	ns	ns	ns
7						1.000	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.444
8							1.000	ns	ns	ns	ns	ns	ns	0.478	ns	ns
9								1.000	ns	ns	ns	ns	ns	ns	ns	ns
10									1.000	0.868	0.459	ns	ns	0.521	ns	ns
11										1.000	0.519	ns	ns	0.628	ns	ns
12											1.000	0.975	0.801	0.598	0.839	ns
13												1.000	0.890	ns	0.902	ns
14													1.000	ns	0.833	ns
15														1.000	ns	ns
16															1.000	ns

Only statistically significant values ($p < 0.05$) are shown; ns, not significant ($N = 20$); 1–water content; 2–RWC; 3–EL; 4–leaf area; 5–dry weight of seedling; 6–RGRa; 7–NAR; 8–PN; 9–WUE; 10–chl ($a + b$); 11–carotenoid; 12–ABS/CS; 13–TRo/CS; 14–ETo/CS; 15–DIO/CS; 16–RC/CS; 17–weight of seeds.

Table 5. Results of stepwise multiple regression for SI indexes of the final yield of grains and seedlings parameters after drought stress ($N = 20$).

Step	Trait	R^2	F	Standard Deviation
1	dry weight of seedling	0.546	7.63	7.28
2	photosynthesis (P_N)	0.682	7.40	6.53
3	leaf area	0.688	7.38	6.42
4	NAR	0.813	7.33	5.54

Table 6. Summary of discriminatory function analysis and squares of Mahalanobis distance from the centroids for SI index of selected traits.

	Wilkins's λ	Partial λ	F Removal	p
dry weight of seedling	0.686	0.433	19.60	0.000
leaf area	0.602	0.494	15.38	0.001
NAR	0.628	0.474	16.67	0.001
photosynthesis (P_N)	0.656	0.454	18.07	0.001

$$F(4,15) = 8.86, p < 0.0007.$$

2.2.2. Gas Exchange

Reducing the intensity of net photosynthesis by drought nearly by 90% proved to be stronger than the NAR (Table 1). This is understandable, since the NAR index is the average of the initial and final (after 10 days of drought) state of plant's growth and P_N measurements recorded only consequences of stress at the end of the period. Some varieties of wheat (Table 3) had a relatively good ability to maintain high intensity of net assimilation and/or net photosynthesis ("Hewilla", "Torka", "Zadra"). It is known that soil drought occurring over several days can cause both stomatal and non-stomatal inhibition of photosynthesis. With a sudden decrease in water supply to plants, the stomata closed resulting in rapid reduction of water losses in the transpiration process and increased diffusion resistances to CO_2 [21]. The process of photosynthesis is very sensitive to changes in water supply to leaves and responds quickly to water deficit [4,22]. This depends not only on the increase in the diffusion resistance towards CO_2 , but also on damaging the structures in the chloroplasts, mainly PSII [8]. As a result, the rate of CO_2 assimilation decreases markedly during drought conditions. In C3 plants, closing of stomata is considered as the most important mechanism for protecting against dehydration of tissues, but at the same time it results in reduced CO_2 assimilation. The rate of decrease of transpiration and P_N in drought was not uniform in the varieties studied in this work. The effect of this phenomenon was both a reduction and an increase of the WUE value in some varieties under drought conditions. The diversity of wheat varieties in this regard has already been observed, but on a very limited number of varieties [13]. The ability to assimilate CO_2 at relatively low transpiration losses was observed in this study in the varieties "Katoda", "Koksa" and "Radunia". High value of WUE is desirable because of the possibility of higher biomass accumulation without large losses of water compared to other varieties. Considering the high drought tolerance of photosynthetic electron transport process [23], the high intensity of the absorption of CO_2 prevents the transfer of electrons to O_2 molecules, which, in turn inhibits the formation of reactive oxygen species (ROS) [24]. Generation of ROS has adverse consequences, causing lethal damage to the tissues [25]. The reduction of chlorophyll and carotenoid

pigments was rather low, which may be indicative of a moderate intensity of drought stress. On the other hand, chlorophyll content in the wheat leaves during drought is dependent on the type of N ions present in the nutrient solution [26]. An initial increase of the chlorophyll content during drought took place in the presence of NH_4^+ , which was followed by a decrease in severity of the drought. By contrast in the presence of NO_3^- , the chlorophyll content in leaves decreased immediately at the initiation of the drought until the end of the experiment.

2.2.3. PSII Photochemical Activity and the Final Yield

The analysis of photochemical efficiency of PSII showed that the described changes in wheat are not very sensitive to moderate soil drought (Table 1). This observation is consistent with the opinions of other authors [27,28]. Our study confirmed a slight decrease in the absorption of energy by the antenna system and reduction of the size of energy streams reaching PSII photochemical reaction center and beyond the center. An undesirable effect of stress detected was also a low diversification of responses to drought of studied varieties. Therefore, it seems that the attempts to select wheat genotypes resistant to drought based on the analysis of photochemical activity are likely to be unsuccessful.

The inhibition of the rate of photosynthesis causes a drop in productivity and agricultural yield [29,30]. The rates of biomass growth do not increase to the level observed prior to the stress, which usually results in a reduced yield. After the stress is overcome, the resulting agricultural yield represents the balance between damage, regeneration and post-stress compensation [31]. In our work, due to the application of drought at the seedling stage, the final reduction in grain yield varied depending on the variety, because some of the genotypes were distinctly tolerant to this stress (Table 3).

2.2.4. The Relationship between Seedlings Traits under Drought and the Final Yield

The regression equation derived on the basis of an index value of stress demonstrated a correlation of some traits of seedlings and the final yield of grain (Table 5). Traits of seedlings were related to the tolerance of the trait of growth vigor (weight and size of seedling leaves) to drought and the intensity of assimilation throughout the whole period of drought (NAR) and its transient value at the end of the drought period (P_N). Repeating the calculations with the discrimination analysis (Table 6), confirmed the accuracy of the selection of these traits of seedlings for predicting the tolerance of varieties. The presented value of Wilks' lambda test statistics can range from 0 (perfect discrimination) to 1 (no discrimination), while the value of the partial statistics of Wilks' lambda test is related to the individual contribution of the variable to the discriminatory power of resulting model. F and p removals are statistics related to the corresponding value of the partial lambda test. A good illustration of the use of data to predict the tolerance is squared values of Mahalanobis distances from the centroids (Table 7).

The greater the distance, the farther apart the respective groups of varieties (tolerant/sensitive), resulting in higher power of the model applied. Data obtained in this work indicate a relatively high probability of correct classification of varieties to the appropriate group of sensitivity to drought stress.

Measurements carried out in this experiment revealed differences of wheat varieties studied in terms of consequent effect of soil drought at seedling stage on the final grain yield. Tolerant to stress in this regard were varieties "Jasna" and "Radunia", as well as "Zadra", "Cytra", "Bombona" and

“Torka”. Drought caused a particularly strong reduction in the growth vigor of the seedlings, net photosynthesis rate and caused an increase in the leakage of electrolytes from the leaves. Varieties tested were highly variable in terms of the stress response of processes of gas exchange and growth of seedlings, which may indicate potential variation resources to drought. Photochemical processes in PSII showed high tolerance to drought and simultaneously low variation between varieties.

Table 7. Squares of Mahalanobis distance from centroids.

Variety *	Sensitivity to Stress	$p = 0.65$ Sensitive	$p = 0.35$ Tolerant
Koksa	Sensitive	148	1512
Zebra	Sensitive	286	1255
Monsun	Sensitive	883	2752
Banti	Sensitive	547	2492
Korynta	Sensitive	371	1532
Bryza	Sensitive	618	763
Nawra	Sensitive	492	980
Waluta	Sensitive	360	1745
Żura	Sensitive	205	460
Hewilla	Sensitive	504	1234
Parabola	Sensitive	293	683
Vinjet	Sensitive	343	519
Raweta	Sensitive	314	1583
Torka	Tolerant	2026	295
Bombona	Tolerant	1458	360
Cytra	Tolerant	596	068
Zadra	Tolerant	1065	570
Katoda	Tolerant	702	061
Radunia	Tolerant	607	277
Jasna	Tolerant	1921	205

* variations ordered by increasing stress index value calculated for grain yield.

3. Experimental Section

3.1. Plant Material

The study was performed on 20 varieties of spring wheat (*Triticum aestivum*). Seeds were obtained from following Polish breeding companies: DANKO Plant Breeders Ltd. (cv. “Bombona”, “Katoda” “Vinjet”, “Waluta”, “Zebra”), Strzelce Plant Breeders Ltd. (cv. “Cytra”, “Koksa”, “Korynta”, “Nawra”, “Torka”, “Zadra”), Nasiona Kobierzyc Crop Breeding Station Ltd. (cv. “Banti”, “Hewilla”, “Jasna”, “Parabola”, “Radunia”, “Żura”), Institute for Plant Breeding Radzików (cv. “Raweta”) and Lochow Petkus Bergen (cv. “Bryza”, “Monsun”). In our experiment, we have used the most popular varieties currently grown in Poland.

3.2. Plant Growth Condition

Plants were grown in a growth chamber in nine dm³ pots (six pots per genotype with 12 plants each) filled with mixture of sieved soil and sand (1:1, v/v) [32]. Vegetation was held at a 15 h photoperiod, an irradiance of 400 $\mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$, temperature of 20/17 °C (day/night) and in 50% air humidity. Plants were watered and fertilized with Hoagland nutrient solution as required [33]. Up to the third leaf stage (18 days after emerging), soil water content was kept at 75% maximum water capacity (MWC) by adding an appropriate amount of water each day. The drought treatment started by discontinuing the watering of plant, and reaching 30% of MWC (four days). This level of soil humidity was maintained during next 10 days. After this time, symptoms of visual drought as turgor loss of leaves became apparent. At this time, the control plants were grown at 75% of MWC.

Physiological measurements were performed (a) after reaching 30% of MWC (only the leaves' area and seedlings' mass associated with the RGRa and NAR measurements at time t_1); and (b) after 10 days of drought stress (all other measurements at time t_2).

3.3. Plant Measurements

For every variety, the measurements were taken on plants subjected to drought and control plants. According to Bouslama and Schapaugh [34], stress index (SI) was calculated for some of the parameters measured where:

$$\text{SI (\%)} = (X_2/X_1) \times 100\% \quad (1)$$

and X_2 and X_1 represent the mean values of the parameters measured under drought stress and control.

3.3.1. The Rates of Growth and Accumulation of Biomass

The analysis of growth included measurements of mass (seedlings and leaves) and area of leaves. The following different growth indices were used: relative growth rate—area (RGRa) and net assimilation rate (NAR). The surface of leaves was measured using a ScanMaker3880 (Microtek, Hsinchu, Taiwan), and Delta-T Skan 2.03 software (Delta-T Devices, Cambridge, UK). The plant material was dried for 48 h at 65 °C. The following formulas were used [35]:

$$\text{RGR}_a = (\ln A_2 - \ln A_1) / 10 \quad (2)$$

$$\text{NAR} = [(W_2 - W_1) / (t_2 - t_1)] \times [(A_2^{\alpha-1} - A_1^{\alpha-1}) / (A_2^{\alpha} - A_1^{\alpha})] \times [\alpha / (\alpha - 1)] \quad (3)$$

where

10—number of days of drought (30% MWC)

W—dry mass of plant, W_1 ; W_2 at the time t_1 and t_2 , respectively

$$\alpha = \text{RGR}_w / \text{RGR}_a \quad (4)$$

$$\text{RGR}_w = (\ln W_2 - \ln W_1) / (t_2 - t_1) \quad (5)$$

A—leaves area, A_1 ; A_2 at the time t_1 and t_2 , respectively.

The measurements were taken in 12 replicates.

3.3.2. The Integrity of Plasma Membrane and Water Balance

Plasma membrane integrity was determined by means of an electrolyte leakage (EL) test [36,37]. For each genotype, 12 segments (three per leaf) 1 cm in length were cut from the third leaf. Samples were washed in deionized water and immersed in 5 cm³ of deionized water. After 24 h (EL₁) of shaking at room temperature, samples were frozen at −40 °C for 24 h, then heated and shaken again (24 h, room temperature, EL₂). EL was calculated as follows:

$$EL = (EL_1/EL_2) \times 100\% \quad (6)$$

Measurements of electrical conductance were performed by means of a microcomputer conductivity meter CC-317 (Elmetron, Warsaw, Poland) with a platinum electrode at a frequency of 3 kHz.

The water balance in seedlings was determined by measuring the water contents of all leaves and the relative water content (RWC) on the third leaves. RWC was determined according to Barrs [38]:

$$RWC = [(FW-DW)/(TW-DW)] \times 100\% \quad (7)$$

Where FW is fresh weight, DW is dry weight and TW is turgid weight. To measure TW, leaves were placed in darkness for 24 h in vials containing water, which permitted complete rehydration. All these measurements were performed in 12 replicates (plants).

3.3.3. Gas Exchange

Net photosynthetic (P_N) and transpiration (E) rates were measured using an infrared gas analyser (Ciras-1, PP Systems, Hitchin, UK) with a Parkinson leaf chamber (PLC6, PP Systems, Hitchin, UK). The flow rate of air with constant CO₂ concentration (400 μmol (CO₂) mol^{−1} (air)) through the assimilation chamber was 350–400 cm³ min^{−1}. The measurements were made in the middle part of the second leaf at 22 °C (the leaf temperature), where irradiance was equal to 500 μmol (quanta) m^{−2} s^{−1} and RH equal to 30%. The measurements were performed in 12 replicates. WUE was calculated as (P_N/E).

3.3.4. Photochemical Efficiency

Photochemical efficiency was measured in the middle part of the second leaf by a Plant Efficiency Analyzer PEA (Hansatech, Kings Lynn, UK). Before measurements, the LED-light source of the fluorimeter was calibrated using an SQS light meter (Hansatech Ltd, Kings Lynn, UK). The excitation irradiance had an intensity of 3000 μmol (quanta) m^{−2} s^{−1} (peak at 650 nm). Measurements were taken after 30 min of leaves adaptation to darkness. Changes in fluorescence were registered during irradiation between 10 μs and 1 s. During the initial 2 ms, data were collected every 10 μs with 12 bit resolution. After this period, the frequency of measurements was reduced automatically. The following equations were used for the quantification of PSII [39]:

(a) The energy fluxes (per active cross section of leaf, CS) for absorption, ABS (ABS/CS), trapping (TRo/CS), electron transport (ETo/CS) and dissipation (Dlo/CS):

In this work, we used the proportionality $ABS/CS \approx F_m$,

$$TRo/CS = \phi_{P_0} \times (ABS/CS) \quad (8)$$

$$TRo/CS = \phi_{P_0} \times (ABS/CS) \quad (9)$$

$$ET_o/CS = \phi_{P_o} \times \psi_o \times (ABS/CS) \quad (10)$$

$$DIO/CS = ABS/CS - TR_o/CS \quad (11)$$

where:

$$\phi_{P_o} = 1 - (F_o/F_m) \quad (12)$$

$$\psi_o = 1 - V_J \quad (13)$$

F_o is the fluorescence intensity at 50 μ s, F_m is the maximum fluorescence intensity,

$$V_J = (F_{2ms} - F_o)/(F_m - F_o) \quad (14)$$

F_{2ms} is the fluorescence intensity at 2 ms.

(b) The amount of active PSII reaction centers per CS (RC/CS):

$$RC/CS = ABS/CS \times \phi_{P_o} \times (V_J/M_o) \quad (15)$$

where:

$$M_o = 4 \times (F_{300\mu s} - F_o)/(F_m - F_o) \quad (16)$$

$F_{300\mu s}$ is the fluorescence intensity at 300 μ s.

The measurements were performed in 20 replicates.

3.3.5. Leaf Pigment Content

For the extraction of chlorophyll and carotenoids, 0.25 g of the second leaf was homogenized with 80% (v/v) acetone then crude extract was centrifuged at 3000 g for 5 min, at 4 °C. The absorbance of supernatant was measured spectrophotometrically at 450, 645 and 663 nm. The formulas of Arnon [40] and Jaspars [41] were used to calculate chlorophyll and carotenoids levels, respectively. The measurements were performed in 12 replicates on the second leaf.

4. Conclusions

Drought caused a particularly strong reduction in vigor of growth of seedlings, net photosynthesis rate and triggered an increase in electrolyte leakage from the leaves. In some varieties, drought induced ability to assimilate CO₂ at a relatively low loss of water through transpiration.

Varieties tested showed high variations in terms of the stress response of processes of gas exchange and growth of seedlings, which may indicate the existence of potential variability resources to drought. Photochemical processes in PSII showed high tolerance to drought and at the same time low differentiation among varieties.

The results obtained suggest that tolerance of seedlings to drought with respect to certain growth parameters and CO₂ assimilation may alleviate subsequent depression of the final yield of grains.

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Author Contributions

Jolanta Biesaga-Kościelniak, Agnieszka Ostrowska, Maria Filek and Janusz Kościelniak designed the research; Jolanta Biesaga-Kościelniak, Agnieszka Ostrowska, Maria Filek, Michał Dziurka, Piotr Waligórski, Magdalena Mirek and Janusz Kościelniak conducted the research; Jolanta Biesaga-Kościelniak, Agnieszka Ostrowska and Michał Dziurka analyzed the data; Jolanta Biesaga-Kościelniak, Agnieszka Ostrowska and Janusz Kościelniak wrote the paper; Jolanta Biesaga-Kościelniak has primary responsibility for the final content. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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