Uniwersytet Rolniczy im. H. Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr inż. Jakub Pastuszak
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Tolerancja wybranych form pszenicy twardej (*Triticum durum* Desf.) na zasolenie gleby, jony kadmu oraz porażenie *Fusarium culmorum*

Rozprawa doktorska

Praca wykonana pod kierunkiem
Prof. dr hab. inż. Agnieszki Płażek
w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa
Wydziału Rolniczo-Ekonomicznego

Promotor pomocniczy:
Dr inż. Przemysław Kopeć
Zakład Biologii Komórki
Instytut Fizjologii Roślin *im. Franciszka Górskiego*
Polska Akademia Nauk

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Wzór karty dyplomowej

mgr inż. Jakub Pastuszak
/ Imię i nazwisko autora pracy /

prof. dr hab. inż. Agnieszka Płażek
/ Imię i nazwisko promotora pracy /

Wydział Rolniczo-Ekonomiczny
/ Wydział - kierunek studiów /

Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
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Tytuł pracy w języku polskim

Tolerancja wybranych form pszenicy twardej (*Triticum durum* Desf.) na zasolenie gleby, jony kadmu oraz porażenie *Fusarium culmorum*

Słowa kluczowe

Fusarium culmorum, pszenica twarda, tolerancja, stres abiotyczny, stres biotyczny

Streszczenie pracy

Ze względu na zmieniające się w ostatnich latach warunki klimatyczne, polegające na coraz wyższych temperaturach letnich oraz wydłużający się okres wegetacji w Polsce, wzrasta zainteresowanie uprawą pszenicy twardej (*Triticum durum* Desf.). Pszenica ta wyróżnia się korzystnym składem chemicznym ziarna, przydatnym w przemyśle spożywczym, zwłaszcza do produkcji makaronów. W odróżnieniu od pszenicy zwyczajnej, pszenica twarda posiada predyspozycję do zwiększonej akumulacji jonów kadmu, jest wrażliwa na zasolenie gleby oraz podatna na infekcje wywołane przez *Fusarium culmorum*. Czynniki te ograniczają plonowanie roślin, zmniejszają jakość technologiczną ziarna, a po przekroczeniu norm zawartości toksyn grzybowych lub zawartości kadmu, ziarno jest całkowicie dyskwalifikowane z dalszego obrotu. W ostatnich latach w Polsce wzrasta niebezpieczeństwo zasalania gleb w wyniku wyraźnego wzrostu temperatur w sezonie wegetacyjnym i koniecznością nawadniania upraw. Również zwiększająca się intensyfikacja rolnictwa zwiększa ilość metali ciężkich w środowisku. Dodatkowo, zaniedbania ze strony agrotechnicznej tj. brak zmianowania czy stosowanie monokultur powodują, że grzyby z rodzaju *Fusarium* coraz częściej stają się główną przyczyną pogorszenia jakości ziarna zbóż.

Celem niniejszej rozprawy doktorskiej, obejmującej cztery publikacje, było określenie zależności pomiędzy badanymi fizjologicznymi i biochemicznymi parametrami wybranych trzech form pszenicy twardej, a ich tolerancją na zasolenie gleby chlorkiem sodu, skażenie jonami kadmu i infekcję grzybową. Hipotezy badawcze postawione w pracy zakładają, że: 1) stopień odporności pszenicy twardej na zasolenie gleby jest powiązany z tolerancją na zanieczyszczenie gleby jonami kadmu, 2) stopień akumulacji jonów kadmu w ziarnach pszenicy twardej zależy od wydajności aparatu fotosyntetycznego oraz aktywności enzymów antyoksydacyjnych, wchodzących w skład cyklu glutationowo-askorbinianowego, 3) poziom akumulacji mykotoksyn w ziarnie zależy od stopnia porażenia kłosów pszenicy twardej *Fusarium culmorum*, 4) zawartość związków organicznych takich, jak pigmenty fotosyntetyczne, związki fenolowe, cukry rozpuszczalne i aktywność antyoksydacyjna decydują o stopniu tolerancji pszenicy twardej na *F. culmorum*. Do weryfikacji postawionych hipotez

wykorzystano trzy formy pszenicy twardej: krajową linię SMH87 i dwa australijskie genotypy różniące się stopniem tolerancji na zasolenie gleby: odmianę Tamaroi (wrażliwą) i linie BC_5Nax_2 (odporną).

W badaniach przeprowadzono szereg analiz fizjologicznych i biochemicznych, których celem było określenie stopnia odporności wybranych form pszenicy twardej na wymienione powyżej czynniki stresowe oraz wytypować markery odporności, mogące posłużyć do selekcji roślin pod kątem ich tolerancji na stres. W doświadczeniach mierzono fluorescencję chlorofilu *a* i wydajność wymiany gazowej, dokonano wizualnej oceny uszkodzeń roślin wywołanych przez stres, analizowano zawartości związków fenolowych, ogólnej puli cukrów, barwników fotosyntetycznych i nadtlenku wodoru, mierzono aktywność enzymów antyoksydacyjnych oraz stopień akumulacji jonów kadmu i mykotoksyn w ziarnie.

Przeprowadzone analizy wykazały, że stopień odporności badanych genotypów pszenicy na zanieczyszczenie kadmem koreluje ze stopniem tolerancji na zasolenie jonami NaCl. Również istotne korelacje wykazano pomiędzy parametrami fluorescencji chlorofilu *a,* a zawartością zakumulowanych jonów Cd w ziarnie. Stosunek zredukowanej i utlenionej formy glutationu może być uznawany za marker zdolności akumulowania jonów Cd w ziarnie. Wysoki poziom zanieczyszczenia gleby kadmem zaburza i ogranicza transport jonów tego metalu z korzeni do pędów, co powoduje, że w wyższych stężeniach tego metalu w glebie mniej jego jonów akumulowanych jest w ziarnie. W przypadku zasolenia gleby, parametry takie jak procent kiełkujących nasion i wigor kiełkowania najbardziej różnicują stopień tolerancji na ten stres badanych form pszenicy twardej. Z kolei, takie parametry fluorescencji chlorofilu, jak ET_o/CS_m – maksymalna wydajność reakcji rozszczepiania wody po stronie donorowej fotosystemu II (PSII) oraz DI_o/CS_m – rozpraszanie energii z PSII mogą być stosowane jako wskaźniki tolerancji na zasolenie pszenicy w fazie generatywnej. Odporna na zasolenie linia BC_5Nax_2 przy podwyższonym stężeniu NaCl w podłożu wyróżnia się najwyższym procentem kiełkujących nasion i wigorem kiełkowania oraz najwyższą zawartością chlorofilu *a, b* i karotenoidów w liściach flagowych, a także posiada najwyższą masę tysiąca nasion. Wrażliwa na zasolenie odmiana Tamaroi, w porównaniu do pozostałych genotypów, charakteryzuje się znacznie wyższym stężeniem nadtlenku wodoru w liściach przy zasoleniu gleby na poziomie 125 i 150 mM NaCl, co dowodzi o oddziaływaniu intensywnego stresu oksydacyjnego. Wyniki uzyskane w stresie wywołanym infekcją *F. culmorum* wskazują, że porażenie tym patogenem zmniejsza zawartość pigmentów fotosyntetycznych oraz redukuje masę liści i korzeni siewek. Australijskie genotypy pszenicy twardej są bardziej odporne na infekcje *F. culmorum* niż krajowa linia SMH87. Niwalenol i deoksyniwalenol zmniejszają plon ziarna w badanych roślinach. Wczesna ocena stopnia infekcji na kłosach pozwala przewidzieć stopień akumulacji toksyn w ziarnie.

Tytuł pracy w języku angielskim

Tolerance of selected forms of durum wheat (*Triticum durum* Desf.) to soil salinity, cadmium ions and *Fusarium culmorum* infection

Słowa kluczowe

Fusarium culmorum, durum wheat, tolerance, abiotic stress, biotic stress

Streszczenie pracy

In recent years, in Poland, an interest in durum wheat (*Triticum durum* Desf.) cultivation is increasing due to the changing climatic conditions, in term of higher temperatures in summer and longer vegetation season. This wheat species is distinguished by favorable grain chemical composition, useful in the food industry, especially for pasta production. Durum wheat comparing with common wheat has a predisposition to increased accumulation of cadmium ions, is more sensitive to soil salinity, and is susceptible to *Fusarium culmorum* infections. These factors limit the yield, reduce the technological grain quality, and when the norm for the content of fungal toxins or cadmium ions is exceeded, the grain is completely disqualified from further turnover. In recent years, in Poland, the risk of soil salinity increases as a result of high temperatures during the vegetation season and an irrigation of crop plants. Also, the higher intensification of agriculture increases the amount of heavy metals in the environment. In addition, negligence in the agrotechnical part, *i.e.* no plant rotation or cultivation in monoculture, make the fungi from *Fusarium* species the main cause of the deterioration of the quality of cereal grains.

The aim of this doctoral dissertation, including a series of four publications, was to determine the relationship between the studied physiological and biochemical parameters of three chosen durum wheat forms and their tolerance to the soil salinity with NaCl, cadmium ions contamination, and fungal infection. The research hypotheses are: 1) the resistance degree of durum wheat to soil salinity is related to the tolerance of soil contamination with cadmium ions, 2) the degree of cadmium ions accumulation in durum wheat grains depends on the photosynthetic apparatus efficiency and the activity of antioxidant enzymes, which are part of the ascorbate-glutathione cycle, 3) the level of mycotoxin accumulation in durum wheat grain depends on the degree of spike infestation by *Fusarium culmorum*, 4) the content of organic compounds such as photosynthetic pigments, phenolic compounds, soluble carbohydrates and antioxidant activity determine durum wheat tolerance degree to *F. culmorum*. To verify the hypotheses three durum wheat genotypes were studied: Polish line: SMH87 and two Australian genotypes differing in the tolerance degree to soil salinity: cultivar Tamaroi (sensitive) and the BC_5Nax_2 line (resistant). In the study a series of physiological and biochemical analyzes were carried out to investigate if indicated markers of resistance degree to the above-mentioned stress factors can be used to durum wheat plants selection. In the experiments following analyses were carried out: visual symptoms of plant damage caused by stress, fluorescence of chlorophyll *a*, gas exchange efficiency, the content of photosynthetic pigments, phenolic compounds, total soluble carbohydrates, hydrogen peroxide, the activity of antioxidant enzymes and the accumulation of cadmium ions and mycotoxins in the grain.

The conducted analyses revealed that the degree of resistance of the studied durum wheat genotypes to the cadmium ions contamination correlates with the degree of tolerance to salinity with NaCl. Significant correlation was also found between the parameters of chlorophyll *a* fluorescence and the content of Cd ions accumulated in the grain. The ratio of the reduced and oxidized forms of glutathione can be considered as a marker of the ability to Cd ions accumulation in the grain. The high level of the cadmium soil contamination disturbs and limits the transport of this metal ions from roots to the shoots, which suggests that in the higher concentration of this metal in the soil, less of its ions are accumulated in the grain. In the case of the soil salinity, parameters such as the percentage of germinating seeds and the germination vigour, differ

most the tolerance degree to this stress in studied durum wheat genotypes. Moreover, the parameters of chlorophyll fluorescence such as ET_o/CS_m – the maximum efficiency of water-splitting reaction on the donor side of photosystem II (PSII) and DI_o/CS_m – energy dissipation from PSII can be used as salinity tolerance indicators for wheat in the generative phase. The salinity-resistant BC_5Nax_2 line with an increased concentration of NaCl is characterized by the highest percentage of germinating seeds and germination vigour, the highest content of chlorophyll *a, b* and carotenoids in flag leaves, and the highest mass of a thousand seeds. The salt sensitive cultivar Tamaroi compared with the other genotypes is characterized by a much higher concentration of hydrogen peroxide in the leaves at 125 and 150 mM NaCl, which is an effect of intense oxidative stress.

The results obtained in the stress conditions caused by *F. culmorum* indicate that this infection reduces the content of photosynthetic pigments and the mass of seedlings' leaves and roots. Australian durum wheat genotypes are more resistant to *F. culmorum* infections than the domestic line SMH87. Nivalenol and deoxynivalenol reduce the grain yield in the studied plants. Early assessment of the degree of an infection on the spikes allows for the prediction of the degree of toxin accumulation in the grain.

/ Podpis promotora pracy/

Oświadczenie autora pracy *Załącznik nr 2*

Ja, niżej podpisany/-a:

Jakub Pastuszak

/ Imię i nazwisko /

977

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autor pracy dyplomowej pt.:

Tolerancja wybranych form pszenicy twardej (*Triticum durum* Desf.) na zasolenie gleby, jony kadmu oraz porażenie *Fusarium culmorum*

/ Tytuł pracy /

Student/-ka Uniwersytetu Rolniczego im. Hugona Kołłątaja w Krakowie

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a Studentem: Jakubem Pastuszakiem

Wydział Rolniczo-Ekonomiczny
/Wydział - kierunek studiów/

autorem pracy dyplomowej pt. Tolerancja wybranych form pszenicy twardej (*Triticum durum* Desf.) na zasolenie gleby, jony kadmu oraz porażenie *Fusarium culmorum*

zrealizowanej w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa

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a Studentem: Jakubem Pastuszakiem

Wydział Rolniczo-Ekonomiczny
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zrealizowanej w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa

pod kierunkiem prof. dr hab. inż. Agnieszki Płażek
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Spis treści

Wykaz stosowanych skrótów i terminów

***A* lub P_n** (ang. *net photosynthesis rate*) – wydajność fotosyntezy netto

AsA–GSH (ang. *ascorbate-glutathione cycle*) – cykl glutationowo-askorbinianowy (Halliwell-Asada)

ABS/CS_m (ang. *energy absorbed by antennas*) – energia zaabsorbowana przez anteny

APX (ang. *ascorbate peroxidase*) – peroksydaza askorbinianowa

AsA (ang. *ascorbate*) – kwas askorbinianowy

Car (ang. *carotenoid*) – karotenoidy

CAT (ang. *catalase*) **–** katalaza

Chl *a* (ang. *chlorophyll a*) – chlorofil *a*

Chl *b* (ang. *chlorophyll b*) – chlorofil *b*

CL (ang. *coleoptile length*) – długość koleoptyla

ChlF (ang. *chlorophyl a fluorescence*) – fluorescencja chlorofilu *a*

***C*i** (ang. *intercellular CO_2 concentration*) – międzykomórkowe stężenie CO_2

CS_m (ang. *excited cross section of a leaf*) – wzbudzana powierzchnia fotosyntetyzująca

CWP (ang. *cell wall-bound phenolic content)* – zawartość fenoli związanych ze ścianą komórkową

DAI (ang. *day after inoculation*) – liczba dni po inokulacji (zainfekowaniu)

DHA (ang. *dehydroascorbate*) – utleniona forma kwasu askorbinianowego

DHAR (ang. *dehydroascorbate reductase*) – reduktaza dehydroaskorbinianowa

DI_o/CS_m *(*ang. *energy dissipation from PSII*) – rozpraszanie energii z fotosystemu II

DM lub DW (ang. *dry matter or dry weight*) – sucha masa

DON (ang. *deoxynivalenol*) – deoksyniwalenol

DON^1 (ang. *total amount of deoxynivalenol forms*) – całkowita zawartość pochodnych form deoksyniwalenolu

DR (ang. *disease rating*) – stopień porażenia

E (ang. *transpiration rate*) – współczynnik transpiracji

EL (ang. *electrolyte leakage*) – wypływ elektrolitów

EL_1 (ang. *initial electrolyte leakage*) – przewodność początkowa elektrolitów

EL_2 (ang. *final conductivity*) – przewodność końcowa elektrolitów

ET_o/CS_m (ang. *energy used for electron transport*) – energia wykorzystywana do transportu elektronów poza Q_A (plastochinon)

FHB (ang. *fusarium head blight*) – fuzarioza kłosów

FHBi (ang. *fusarium head bligh index*) – indeks fuzariozy kłosów

FSB (ang. *fusarium seedling blight*) – fuzaryjna zaraza siewek

F_v/F_0 (ang. *maximum efficiency of water-splitting reaction of the donor side of PSII)* – maksymalna efektywność reakcji rozkładu wody po donorowej stronie fotosystemu II

FW (ang. *fresh weight*) – świeża masa

GSH (ang. *glutathione*) – glutation

GSSG (ang. *oxidized glutathione*) – utleniona forma glutationu

g_s (ang. *stomatal conductance*) – przewodnictwo szparkowe

GR (ang. *glutathione reductase*) – reduktaza glutationowa

H_2O_2 (ang. *hydrogen peroxide*) – nadtlenek wodoru

MDHAR (ang. *monodehydroascorbate reductase*) – reduktaza monodehydroaskorbinianowa

MTS (ang. *mass of one thousand seeds*) – masa tysiąca nasion

NADH (ang. *nicotinamide adenine dinucleotide, reduced form*) – dinukleotyd nikotynoamidoadeninowy, forma zredukowana

NADPH (ang. *nicotinamide adenine dinucleotide phosphate, reduced form*) – fosforanu dinukleotydu nikotynoamidoadeninowego, forma zredukowana

NIV (ang. *nivalenol*) – niwalenol

TR_0/CS_m (ang. *excitation energy trapped in PSII*) – energia związana w PSII

RC/CS_m (ang. *number of active reaction centers*) – liczba aktywnych centrów reakcji

PI (ang. *performance index of PSII photochemistry*) – wskaźnik wydajności PSII

PGS (ang. *percentage of germinated seeds*) – procent kiełkujących nasion

POX (ang. *peroxidase*) – peroksydaza

ROS (ang. *reactive oxygene species*) – reaktywne formy tlenu

PS (ang. *photosystem*) – fotosystem

PSII (ang. *photosystem*) – fotosystem II

SOD (ang. *superoxide dismutase*) – dysmutaza ponadtlenkowa

SSI (ang. *salt susceptibility index*) – indeks podatności na stres zasolenia

TPC (ang. *total phenolic conten*) – ogólna zawartość fenoli

TSC (ang. *total soluble carbohydrate content*) – ogólna zawartość cukrów rozpuszczalnych

Vi (ang. *germination vigor index*) – wigor kiełkowania nasion

ZEN (ang. *zearalenone*) – zearalenol

ZEN^2 (ang. *total amount of zearalenone forms*) – całkowita zawartość pochodnych form zearalenolu

1. Wykaz publikacji stanowiących rozprawę doktorską

Publikacja nr 1:

Pastuszak J., Kopeć P.*, Płażek A., Gondek K., Szczerba A., Hornyák M., Dubert F. (2020). Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree. *Plant Soil Environment*, 66(6), 257-263.
https://doi.org/10.17221/61/2020-PSE

IF_{2020}: 1,799
5-letni IF: 2,169
Punktacja MEiN: 70

Publikacja nr 2:

Pastuszak J.*, Kopeć P., Płażek A., Gondek K., Szczerba A., Hornyák M., Dubert, F. (2020). Antioxidant activity as a response to cadmium pollution in three durum wheat genotypes differing in salt-tolerance. *Open Chemistry*, 18, 1230–1241.
https://doi.org/10.1515/chem-2020-0113

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Punktacja MEiN: 70

Publikacja nr 3:

Pastuszak J.*, Dziurka M., Hornyák M., Szczerba A., Kopeć P., Płażek A.* (2022). Physiological and biochemical parameters of salinity resistance of three durum wheat genotypes. *International Journal of Molecular Sciences,* 2022; 23(15):8397.
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Publikacja nr 4:

Pastuszak J.*, Szczerba A., Dziurka M., Hornyák M., Kopeć P., Szklarczyk M., Płażek A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, 22(14), 7433
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Punktacja MEiN: 140

Sumaryczny IF: 15,769
Sumaryczny 5-letni IF: 17,225
Suma punktów MEiN: 420

* - autor korespondencyjny

2. Streszczenie

Ze względu na zmieniające się w ostatnich latach warunki klimatyczne, polegające na coraz wyższych temperaturach letnich oraz wydłużający się okres wegetacji w Polsce, wzrasta zainteresowanie uprawą pszenicy twardej (*Triticum durum* Desf.). Pszenica ta wyróżnia się korzystnym składem chemicznym ziarna, przydatnym w przemyśle spożywczym, zwłaszcza do produkcji makaronów. W odróżnieniu od pszenicy zwyczajnej, pszenica twarda posiada predyspozycję do zwiększonej akumulacji jonów kadmu, jest wrażliwa na zasolenie gleby oraz podatna na infekcje wywołane przez *Fusarium culmorum.* Czynniki te ograniczają plonowanie roślin, zmniejszają jakość technologiczną ziarna, a po przekroczeniu norm zawartości toksyn grzybowych lub zawartości kadmu, ziarno jest całkowicie dyskwalifikowane z dalszego obrotu. W ostatnich latach w Polsce wzrasta niebezpieczeństwo zasalania gleb w wyniku wyraźnego wzrostu temperatur w sezonie wegetacyjnym i koniecznością nawadniania upraw. Również zwiększająca się intensyfikacja rolnictwa zwiększa ilość metali ciężkich w środowisku. Dodatkowo, zaniedbania ze strony agrotechnicznej tj. brak zmianowania czy stosowanie monokultur powodują, że grzyby z rodzaju *Fusarium* coraz częściej stają się główną przyczyną pogorszenia jakości ziarna zbóż.

Celem niniejszej rozprawy doktorskiej, obejmującej cztery publikacje, było określenie zależności pomiędzy badanymi fizjologicznymi i biochemicznymi parametrami wybranych trzech form pszenicy twardej, a ich tolerancją na zasolenie gleby chlorkiem sodu, skażenie jonami kadmu i infekcję grzybową. Hipotezy badawcze postawione w pracy zakładają, że: 1) stopień odporności pszenicy twardej na zasolenie gleby jest powiązany z tolerancją na zanieczyszczenie gleby jonami kadmu, 2) stopień akumulacji jonów kadmu w ziarnach pszenicy twardej zależy od wydajności aparatu fotosyntetycznego oraz aktywności enzymów antyoksydacyjnych, wchodzących w skład cyklu glutationowo-askorbinianowego, 3) poziom akumulacji mykotoksyn w ziarnie zależy od stopnia porażenia kłosów pszenicy twardej *Fusarium culmorum*, 4) zawartość związków organicznych takich, jak pigmenty fotosyntetyczne, związki fenolowe, cukry rozpuszczalne i aktywność antyoksydacyjna decydują o stopniu tolerancji pszenicy twardej na *F. culmorum*. Do weryfikacji postawionych hipotez wykorzystano trzy formy pszenicy twardej: krajową linię SMH87 i dwa australijskie genotypy różniące się stopniem tolerancji na zasolenie gleby: odmianę Tamaroi (wrażliwą) i linie BC_5Nax_2 (odporną).

W badaniach przeprowadzono szereg analiz fizjologicznych i biochemicznych, których celem było określenie stopnia odporności wybranych form pszenicy twardej na wymienione

powyżej czynniki stresowe oraz wytypować markery odporności, mogące posłużyć do selekcji roślin pod kątem ich tolerancji na stres. W doświadczeniach mierzono fluorescencję chlorofilu *a* i wydajność wymiany gazowej, dokonano wizualnej oceny uszkodzeń roślin wywołanych przez stres, analizowano zawartości związków fenolowych, ogólnej puli cukrów, barwników fotosyntetycznych i nadtlenku wodoru, mierzono aktywność enzymów antyoksydacyjnych oraz stopień akumulacji jonów kadmu i mykotoksyn w ziarnie.

Przeprowadzone analizy wykazały, że stopień odporności badanych genotypów pszenicy na zanieczyszczenie kadmem koreluje ze stopniem tolerancji na zasolenie jonami NaCl. Również istotne korelacje wykazano pomiędzy parametrami fluorescencji chlorofilu *a,* a zawartością zakumulowanych jonów Cd w ziarnie. Stosunek zredukowanej i utlenionej formy glutationu może być uznawany za marker zdolności akumulowania jonów Cd w ziarnie. Wysoki poziom zanieczyszczenia gleby kadmem zaburza i ogranicza transport jonów tego metalu z korzeni do pędów, co powoduje, że w wyższych stężeniach tego metalu w glebie mniej jego jonów akumulowanych jest w ziarnie.

W przypadku zasolenia gleby, parametry takie jak procent kiełkujących nasion i wigor kiełkowania najbardziej różnicują stopień tolerancji na ten stres badanych form pszenicy twardej. Z kolei, takie parametry fluorescencji chlorofilu, jak ET_o/CS_m – maksymalna wydajność reakcji rozszczepiania wody po stronie donorowej fotosystemu II (PSII) oraz DI_o/CS_m – rozpraszanie energii z PSII mogą być stosowane jako wskaźniki tolerancji na zasolenie pszenicy w fazie generatywnej. Odporna na zasolenie linia BC_5Nax_2 przy podwyższonym stężeniu NaCl w podłożu wyróżnia się najwyższym procentem kiełkujących nasion i wigorem kiełkowania oraz najwyższą zawartością chlorofilu *a, b* i karotenoidów w liściach flagowych, a także posiada najwyższą masę tysiąca nasion. Wrażliwa na zasolenie odmiana Tamaroi, w porównaniu do pozostałych genotypów, charakteryzuje się znacznie wyższym stężeniem nadtlenku wodoru w liściach przy zasoleniu gleby na poziomie 125 i 150 mM NaCl, co dowodzi o oddziaływaniu intensywnego stresu oksydacyjnego.

Wyniki uzyskane w stresie wywołanym infekcją *F. culmorum* wskazują, że porażenie tym patogenem zmniejsza zawartość pigmentów fotosyntetycznych oraz redukuje masę liści i korzeni siewek. Australijskie genotypy pszenicy twardej są bardziej odporne na infekcje *F. culmorum* niż krajowa linia SMH87. Niwalenol i deoksyniwalenol zmniejszają plon ziarna w badanych roślinach. Wczesna ocena stopnia infekcji na kłosach pozwala przewidzieć stopień akumulacji toksyn w ziarnie.

3. Summary

In recent years, in Poland, an interest in durum wheat (*Triticum durum* Desf.) cultivation is increasing due to the changing climatic conditions, in term of higher temperatures in summer and longer vegetation season. This wheat species is distinguished by favorable grain chemical composition, useful in the food industry, especially for pasta production. Durum wheat comparing with common wheat has a predisposition to increased accumulation of cadmium ions, is more sensitive to soil salinity, and is susceptible to *Fusarium culmorum* infections. These factors limit the yield, reduce the technological grain quality, and when the norm for the content of fungal toxins or cadmium ions is exceeded, the grain is completely disqualified from further turnover. In recent years, in Poland, the risk of soil salinity increases as a result of high temperatures during the vegetation season and an irrigation of crop plants. Also, the higher intensification of agriculture increases the amount of heavy metals in the environment. In addition, negligence in the agrotechnical part, *i.e.* no plant rotation or cultivation in monoculture, make the fungi from *Fusarium* species the main cause of the deterioration of the quality of cereal grains.

The aim of this doctoral dissertation, including a series of four publications, was to determine the relationship between the studied physiological and biochemical parameters of three chosen durum wheat forms and their tolerance to the soil salinity with NaCl, cadmium ions contamination, and fungal infection. The research hypotheses are: 1) the resistance degree of durum wheat to soil salinity is related to the tolerance of soil contamination with cadmium ions, 2) the degree of cadmium ions accumulation in durum wheat grains depends on the photosynthetic apparatus efficiency and the activity of antioxidant enzymes, which are part of the ascorbate-glutathione cycle, 3) the level of mycotoxin accumulation in durum wheat grain depends on the degree of spike infestation by *Fusarium culmorum*, 4) the content of organic compounds such as photosynthetic pigments, phenolic compounds, soluble carbohydrates and antioxidant activity determine durum wheat tolerance degree to *F. culmorum.* To verify the hypotheses three durum wheat genotypes were studied: Polish line: SMH87 and two Australian genotypes differing in the tolerance degree to soil salinity: cultivar Tamaroi (sensitive) and the BC_5Nax_2 line (resistant). In the study a series of physiological and biochemical analyzes were carried out to investigate if indicated markers of resistance degree to the above-mentioned stress factors can be used to durum wheat plants selection. In the experiments following analyses were carried out: visual symptoms of plant damage caused by stress, fluorescence of chlorophyll *a*, gas exchange efficiency, the content of photosynthetic pigments, phenolic compounds, total

soluble carbohydrates, hydrogen peroxide, the activity of antioxidant enzymes and the accumulation of cadmium ions and mycotoxins in the grain.

The conducted analyses revealed that the degree of resistance of the studied durum wheat genotypes to the cadmium ions contamination correlates with the degree of tolerance to salinity with NaCl. Significant correlation was also found between the parameters of chlorophyll *a* fluorescence and the content of Cd ions accumulated in the grain. The ratio of the reduced and oxidized forms of glutathione can be considered as a marker of the ability to Cd ions accumulation in the grain. The high level of the cadmium soil contamination disturbs and limits the transport of this metal ions from roots to the shoots, which suggests that in the higher concentration of this metal in the soil, less of its ions are accumulated in the grain. In the case of the soil salinity, parameters such as the percentage of germinating seeds and the germination vigour, differ most the tolerance degree to this stress in studied durum wheat genotypes. Moreover, the parameters of chlorophyll fluorescence such as ET_o/CS_m – the maximum efficiency of water-splitting reaction on the donor side of photosystem II (PSII) and DI_o/CS_m – energy dissipation from PSII can be used as salinity tolerance indicators for wheat in the generative phase. The salinity-resistant BC_5Nax_2 line with an increased concentration of NaCl is characterized by the highest percentage of germinating seeds and germination vigour, the highest content of chlorophyll *a*, *b* and carotenoids in flag leaves, and the highest mass of a thousand seeds. The salt sensitive cultivar Tamaroi compared with the other genotypes is characterized by a much higher concentration of hydrogen peroxide in the leaves at 125 and 150 mM NaCl, which is an effect of intense oxidative stress.

The results obtained in the stress conditions caused by *F. culmorum* indicate that this infection reduces the content of photosynthetic pigments and the mass of seedlings' leaves and roots. Australian durum wheat genotypes are more resistant to *F. culmorum* infections than the domestic line SMH87. Nivalenol and deoxynivalenol reduce the grain yield in the studied plants. Early assessment of the degree of an infection on the spikes allows for the prediction of the degree of toxin accumulation in the grain.

4. Wprowadzenie

Ze względu na zmieniające się warunki termiczne klimatu Polski zaistniała możliwość uprawy pszenicy twardej, której dotychczasowe rejony uprawy obejmowały cieplejsze rejony Europy. Pszenica twarda w porównaniu do pszenicy zwyczajnej cechuje się lepszymi parametrami jakościowymi mąki, wykorzystywanej głównie w przemyśle spożywczym. Odmiany tego gatunku, dopuszczone do uprawy muszą spełniać wymagania Unii Europejskiej dotyczące określonych norm zawartości toksyn grzybowych produkowanych przez grzyby z rodzaju *Fusarium*, jak też zawartości jonów kadmu. Wymagania te zostały wyznaczone ze względu na fakt, że pszenica twarda charakteryzuje się dużą podatnością na infekcje fuzaryjne, jak też większą zdolnością akumulowania jonów tego ciężkiego metalu.

Nawiązano kontakt z australijskim ośrodkiem naukowym Commonwealth Scientific and Industrial Research Organisation (CSIRO) Plant Industry zajmującym się hodowlą pszenicy twardej. Ośrodek ten udostępnił materiał badawczy w postaci ziarniaków dwóch genotypów różniących się odpornością na zasolenie: wrażliwej odmiany Tamaroi i odpornej linii BC_5Nax_2. W naszym kraju w wyniku prac hodowlanych prowadzonych przez dr Jarosława Bojarczuka z Hodowli Roślin w Smolicach wyprowadzono pierwszą jarą linię pszenicy twardej, która posłużyła w badaniach do porównania z australijskimi genotypami pod kątem tolerancji na zanieczyszczenie jonami kadmu, zasolenie gleby oraz infekcje wywołana przez *Fusarium culmorum.* Głównym celem rozprawy doktorskiej było wyznaczenie parametrów różnicujących badane genotypy pod kątem tolerancji na powyższe stresy środowiskowe.

5. Przegląd literatury

5.1. Charakterystyka pszenicy twardej

Pszenica twarda (*Triticum durum* Desf.) nazywana potocznie pszenicą makaronową lub pszenicą durum, podobnie jak pozostałe zboża z rodzaju *Triticum*, zaliczana jest do klasy roślin jednoliściennych (*Monocotyledatae*), rzędu wiechlinowate (*Poales*), rodziny wiechlinowatych (*Poaceae*) (Podbiełkowski, 1989). Gatunek ten powstał w wyniku mutacji genowej (jest tetraploidem posiadającym 28 chromosomów), której dzikim przodkiem jest pszenica płaskurka (*Triticum turgidum* ssp. *dicoccoides*) (Peng et al. 2011), choć pojawiają się spekulacje o rzekomej hybrydyzacji i poliploidyzacji międzygatunkowej pomiędzy dwoma gatunkami traw: *Aegilops speltoides* i *Triiticum urartu* (Prażak, 2001; Budzyński, 2012).

Pszenica twarda jest roślina jednoroczną. Cechuje ją znacznie krótszy okres wegetacji (90–100 dni) w porównaniu do pszenicy zwyczajnej, jednak okres wegetacji w dużym stopniu

zależny jest od warunków klimatycznych. Rośliny osiągającą wysokość 1–1,8 m, lecz w światowych zasobach tego gatunku dostępne są też formy półkarłowe, liczące 80–85 cm. System korzeniowy pszenicy twardej zbudowany jest z korzeni nasiennych wytwarzanych podczas kiełkowania i korzeni przybyszowych, które wraz ze wzrostem rośliny tworzą mocny wiązkowy system korzeniowy. W optymalnych warunkach uprawy, pszenica twarda może wytworzyć dodatkowe trzy źdźbła, z których nie wszystkie zawiążą nasiona (Bozzini, 1988). Roślina posiada płaskie i ostro zakończone blaszki liściowe, koloru żółto-, niebiesko-ciemnozielonego, o długości 16–35 cm i szerokości 0,7–1,1 cm. Na przyczepie pochwy liściowej znajduje się przezroczysta błonka zwana języczkiem wraz dwoma małymi bocznymi wyrostkami nazywanymi uszkami. Źdźbło wyprostowane o cylindrycznym kształcie podzielone jest międzywęźlami, natomiast wnętrze źdźbła zazwyczaj jest puste. Na całej długości źdźbła nie obserwuje się włosków, lecz mogą pojawiać się sporadycznie (Wyzińska and Różewicz, 2021). Zakończenie źdźbła stanowi kwiatostan właściwy, który tworzy gruby, zwarty i ościsty czworokanciasty lub niekiedy spłaszczony kłos długości 5–16 cm. Ości długie (7–23 cm), wyprostowane, koloru od białego do czarnego, niekiedy mogą przybierać bladoczerwone zabarwienie (Wyzińska and Różewicz, 2021). Kłos stanowią osadzone naprzemianlegle kłoski, otoczone plewkami, Na każdym kłosie znajduje się pięciokwiatowe kłoski, które związują 3–4 ziarniaków. W odróżnieniu do pszenicy zwyczajnej, ziarniaki pszenicy twardej po osiągnieciu pełniej dojrzałości są zasłonięte przez ości, co zmniejsza prawdopodobieństwo osypywania się nasion przed ich zbiorem. Ziarniaki nieoplewione, wypukłe z wyraźnym garbem, cechują się duża twardością i szklistością. Kolor ziaren od bursztynowego złota poprzez różne odcienie żółtego do lekko antocyjanowego wynika z czynników fenotypowych (warunki wzrostu, agrotechnika) i genetycznych (Ficco i in. 2014).

Historyczne początki uprawy pszenicy twardej sięgają 8 tys. lat p.n.e., kiedy to w rejonach historycznej krainy Żyznego Półksiężyca rolnicy w wyniku udomawiania dzikich form pszenicy płaskurki, wyodrębnili osobny genotyp, który cechował się większymi ziarnami i znacznie większą produktywnością (Zohary i in., 2012; Kabbaj et al. 2017). W wyniku licznych migracji i rozwijającego się handlu, pszenica twarda została wprowadzona do upraw europejskich i azjatyckich. W czasach nowożytnych pierwsze doniesienia na temat tego gatunku pojawiają się w XVI w. Podaje się, że w 1800 roku Desfontaines botanik francuskiego pochodzenia po raz pierwszy do określenia tego gatunku użył łacińskiej nazwy *Triticum durum* (Gąsiorowski i Obuchowski, 1978). W Polsce uprawę pszenicy twardej rozpoczęto tuż po I wojnie światowej, a zapoczątkował ją prof. Stefan Lewicki, który wyprowadził pierwszą krajową odmianę o nazwie Puławska Twarda. Po II wojnie światowej pojawiła się kolejna

odmiana Hela, która pod kątem cech morfologiczno-agronomicznych była bardzo podobna do pierwszej odmiany. Niestety, uprawa ich nie przetrwała z powodu słabego plonowana. Od 1976 roku prowadzono badania z uwzględnieniem odmian pochodzących z całego świata, których celem było krzyżowane odmian i wyprowadzenie krajowych odmian tego gatunku spełniających wysokie wymagania jakościowe i technologiczne. Badania te jednak zostały przerwane, że względu na brak pożądanych cech. Po wielu latach wznowiono badania nad tym gatunkiem. Podjęli się hodowcy z Hodowli Roślin w Smolicach sp. z o.o., Grupa IHAR. Owocem ich pracy są zarówno formy jare i ozime pszenicy twardej, o których informacje zostały zebrane w Tabeli 1.

Tabela 1. Lista krajowych odmian pszenicy twardej (dane COBORU).

Nazwa w KR	**Nazwa hodowlana**	**Forma**	**Data wpisu do KR**	**Data wygaśnięcia wpisu w KR**	**Hodowca**
Ceres	SMH 166	ozima	14.03.2017	31.12.2027	HR Smolice
SM Eris	SMH 282	ozima	03.04.2020	31.12.2030	HR Smolice
SM Metis	SMH 257	ozima	15.03.2019	31.12.2029	HR Smolice
SM Tetyda	SMH 281	ozima	03.04.2020	31.12.2030	HR Smolice
Komnata	SMH 103	ozima	06.03.2009	02.06.2014	HR Smolice
SMH87	SMH 87	jara	02.02.2011	31.12.2021	HR Smolice

5.2. Wymagania klimatyczno-glebowe

Pierwotnie tereny uprawy pszenicy twardej obejmują regiony, w których panuje klimat typowo kontynentalny i/lub stepowy. Cechami charakterystycznymi tego klimatu jest duża ilość promieniowania słonecznego, wysokie temperatury powietrza oraz ograniczona ilość opadów. Międzynarodowa literatura podaje, że tradycyjne (regionalne) odmiany pszenicy twardej cechują się większymi możliwościami adaptacyjnymi do panujących w danym regionie warunków środowiskowych. W związku z tym uprawa tego gatunku obejmuje głównie rejony suche i ciepłe z ograniczoną liczbą opadów atmosferycznych, gdzie najwyższe temperatury powietrza przypadają na okres wypełniania ziarna, a stanowisko uprawy może obejmować tereny o zwiększonym zasoleniu i odczynie alkalicznym (Rharrabti i in. 2003).

Wymagania temperaturowe pszenicy twardej są znacznie większe niż pszenicy zwyczajnej. Dla odmian ozimych okres wegetacji jesiennej jest decydującym czynnikiem wpływającym na krzewienie roślin, hartowanie i odporność na pojawiające się przymrozki. W tym czasie najodpowiedniejszy zakres temperatur wynosi 6–9ºC. Optymalna temperaturą

dla fazy kiełkowania nasion to 2–4°C. Wschody trwające 12 dni najlepiej przebiegają w temperaturze dobowej wynoszącej 10°C, a za najkorzystniejszy zakres temperatury uznaje się 15–20°C. W fazie strzelania w źdźbło temperatura 15°C jest najkorzystniejsza, co ma powiązanie z prawidłowym gospodarowaniem wody podczas tej fazy. Ilość opadów w okresie kwiecień–maj jest największym czynnikiem determinującym plonowanie, natomiast często pojawiające się majowe susze powodują skrócenie fazy strzelania w źdźbło. W trakcie kłoszenia, za optimum temperaturowe uznaje się 16°C, natomiast w początkowej fazie kwitnienia za najkorzystniejsze uznaje się temperaturę powyżej 16°C. Wysokie temperatury przyspieszają dojrzewanie nasion. Czynnik ten skraca okres wegetacji roślin i pozwala na szybszy zbiór ziarna. Formy jare pszenicy twardej, podobnie jak formy ozime, również cechują się wysoką wrażliwością na zmiany temperatur w całym okresie swojej wegetacji. Formy te bardzo źle reagują na zwiększoną liczbę opadów, natomiast w warunkach okresowych susz obserwuje się u nich znacznie mniejsze straty w plonie. Jara forma jest odporna na przymrozki sięgające nawet –7°C. Dla fazy krzewienia zakres temperaturowy jest podobny do form ozimych (6–8°C). Optymalną temperaturą w fazie strzelania w źdźbło jest temperatura poniżej 14–15°C. Jare formy pszenicy twardej w okresie dojrzewania potrzebują znacznie dłuższego okresu nasłonecznienia, a preferowany zakres temperatur wynosi 18–20°C. Sprzyjające warunki pogodowe w ostatnich fazach dojrzewania ziarna przyczyniają się do znacznie lepszego wypełnienia ziarna poprzez tworzenie znacznie twardszego i szklistego bielma, niż obserwuje się u pszenicy zwyczajnej (Rachoń, 2001; Deryło, 2011; Budzyński, 2012).

Obecny stan wiedzy, który prezentuje dostępna literatura krajowa, pozwala na wstępne określenie wymagań glebowych, na których polskie odmiany pszenicy twardej będą osiągały zadowalający plony. Poza układem czynników pogodowych, które determinują plon ziarna, również wymagania glebowe odgrywają kluczowy czynnik plonotwórczy. Większość dotychczasowych badań przeprowadzona była w stacjach doświadczalnych uczelni wyższych lub stacjach hodowli roślin, dlatego też wyniki oparte są o stanowiska glebowe wymienionych instytucji. Na podstawie przeprowadzonych doświadczeń wyciągnięto spostrzeżenia, które wskazują, że najlepsze gleby pod uprawę pszenicy twardej to czarnoziemy, gleby brunatne, lessy, mady oraz rędziny. Gleby te cechują się dużą żyznością, bogatą zawartością próchnicy i składników pokarmowych, a także zwięzłością i zdolnością do zatrzymywania (retencji) wody. Według klas bonitacyjnych najlepiej sprawdzać się będą gleby klasy I i II, ale uprawa jest też możliwa na glebach kompleksu III a – IV b, przy czym pH gleby powinno mieścić się w przedziale 5,6–7,2. Gleby kompleksu pszennego dobrego i żytniego bardzo dobrego również nadają się pod uprawę pszenicy twardej, przy czym należy zadbać o obojętny odczyn gleby.

Z przeprowadzonych doświadczeń wynika, że najlepsze rejony uprawy obejmują zatem Kujawy, część Lubelszczyzny, część województwa Świętokrzyskiego, a także rejony Kotliny Sandomierskiej (Woźniak, 2006; Jarecki i in. 2013).

5.3. Agrotechnika pszenicy twardej

Wraz z bardzo dużymi wymaganiami klimatyczno-glebowymi, uprawa pszenicy twardej pociąga za sobą konieczność stosowania wysokiej kultury agrotechnicznej (Deryło, 2011). Dotyczy to wszystkich elementów wchodzących w skład zabiegów agrotechnicznych. Zarówno zalecenia hodowców, jak i dostępna literatura wskazują, że uprawa pszenicy twardej powinna odbywać się zgodnie z zasadami uprawy pszenicy zwyczajnej. Siew jest istotnym elementem wpływającym na plonowanie. Ze względu na fakt, że pszenica twarda ma dłuższy okres kiełkowania (3–4 dni) niż pszenica zwyczajna, zaleca się by odmiany ozime wysiewać już 2–3 dekadzie września tak, aby zdążyły wykształcić 7–9 liści jeszcze przed zakończeniem jesiennej wegetacji. Ozime formy cechuje bardzo duża wrażliwość względem opóźnionego terminu siewu. Z kolei zbyt wczesny siew naraża rośliny na choroby podsuszkowe i atak insektów (mszyc i skoczków). Formę jarą należy wysiewać jak najwcześniej wiosną, najlepiej z końcem marca/początkiem kwietnia (Budzyński, 2012). Przed siewem należy wykonać uprawki przedsiewne i zastosować nawożenie azotowe. Siew należy wykonać na głębokość ok. 3 cm, natomiast rozstawa między rzędami powinna wynosić 11–15 cm. Ze względu na słabą zdolność krzewienia optymalna gęstość siewu wynosi 450–650 ziaren/m^2, co przekłada się na wysiew około 250–300 kg/ha^{-2} nasion (Woźniak, 2007; Rachoń i Wożniak, 2020). Nawożenia azotowe jest kluczowym elementem agrotechnicznym w uprawie pszenicy twardej, gdyż determinuje nie tylko wzrost i rozwój roślin, ale odpowiada finalnie za plon ziarna, a także za jego cechy jakościowe i technologiczne (Rachoń i Woźniak, 2020). Wielkość dawki i termin stosowania nawożenia azotowego uzależniony jest od stanowiska uprawy, formy (jara lub ozima) oraz systemu uprawy. Wielkość dawki nawożenia azotowego szacuje się między 140 a 160 kg/ha^{-2}. Dla nawożenia w trzech dawkach zaleca się nawożenie I dawka – 50% przedsiewnie i po 25% w fazie strzelania w źdźbło (II dawka) i fazie kłoszenia (III dawka). System dwóch dawek nawożenia azotowego w uprawach jarych form zakłada wysiew 40–60% dawki przedsiewnie, a pozostałą cześć w fazie strzelania w źdźbło. W przypadku nawożenia fosforowego (P_2O_5) i potasowego (K_2O) dawki mieszczą się w przedziale 30–80 kg/ha^2 dla P_2O_5 i 40–100 kg/ha^2 dla K_2O. Pielęgnacja uprawy poprzez usuwanie zachwaszczenia również musi być utrzymywana na wysokim poziomie, dlatego zaleca się by użycie herbicydów było

dobierane na podstawie dominujących w plantacji gatunków chwastów (Budzyński, 2012; Gorczyca i in. 2017; Rachoń i Woźniak, 2020).

5.4. Znaczenie gospodarcze pszenicy twardej

Pszenica twarda jest dziesiątym najważniejszym i najczęściej uprawianym zbożem na świecie. Światowy areał uprawy tego gatunku w sezonie 2020/2021 sięgał 13,5 mln ha, a produkcja wynosiła 33,8 mln ton ziarna, co stanowi 6,2% całkowitej produkcji zbóż (Beres i in. 2020; Martínez-Moreno i in. 2022). Uprawa tego gatunku w Polsce nabiera coraz większego znaczenia gospodarczego, ze względu na jej szczególne wykorzystanie w przemyśle spożywczym (Rachoń i in. 2022). Główne przeznaczenie ziaren pszenicy twardej to produkcja makaronu. Po przemiale ziarna uzyskuję się mąkę o kaszkowatej strukturze - semolinę. Właśnie ona stanowi jedyny i niezastąpiony składnik kluczowy w produkcji najwyższej jakości makaronów (Sissons 2008). Mąka stanowi także dodatek mający na celu poprawę smaku różnego rodzaju placków, ciast i chleba (Wondołowska-Grabowska, 2015). Ziarno pszenicy twardej cechuje się bardzo dobrymi parametrami jakościowymi i technologicznymi, które są szczególne ważne w przemyśle spożywczym. Do najważniejszych cech jakościowych ziarna, które odróżniają pszenicę twardą od pszenicy zwyczajnej to przede wszystkim większa szklistość i twardość bielma, wysoka zawartość luteiny oraz znacznie większa zawartość białka i glutenu. Do innych cech, które mają szczególne znaczenie w ocenie towaroznawczej ziarna jest wyrównanie, gęstość usypana i masa tysiąca ziaren (MTZ) (Rachoń i Szumiło, 2002; Ficco i in. 2014; Rachoń i in. 2021). Według Fu i in. (2018) oraz Bobryk-Mamczarz i in. (2021) wymienione cechy jakościowe podnoszą wartość technologiczną ziarna, co ma istotny wpływ na jakość wytwarzanego makaronu. Warto zaznaczyć, że w niektórych krajach Unii Europejskiej wewnętrze ustawodawstwa zobowiązują producentów makaronów do wytwarzania ich tylko i wyłącznie z pszenicy twardej cechującej się wysoką jakością technologiczną ziarna (Mefleh i in. 2019).

Ziarno pszenicy twardej stanowi źródło wielu ważnych składników odżywczych. Zawiera ono znacznie większe ilości błonnika pokarmowego, luteinę oraz takich witamin, jak niacyna (B_1), ryboflawina (B_2), kwas pantotenowy (B_5), pirydoksyna (B_6), kwas foliowy (B_9), niacyna (PP). Semolina zawiera bardzo dużą ilość cynku (Zn), fosforu (P), potasu (K), magnezu (Mg), miedzi (Cu), żelaza (Fe) i selenu (Se). Zaleca się spożywanie produktów zwierających mąkę pszenicy twardej przez osoby cierpiące na cukrzycę insulino-oporną, gdyż jej spożycie powoduje znacznie mniejszy wyrzut cukru i insuliny do krwi niż produkty zawierające tradycyjną mąkę (Schulthess i in. 2013; Sciacca i in. 2018). Pszenica twarda przeciwdziała

chorobom układu krążenia i nowotworom dzięki zawartym w ziarnie antyoksydantom takim, jak karotenoidy, fenole i tokoferole (Di Loreto i in. 2018).

Innym czynnikiem decydującym o zwiększającym się znaczeniu gospodarczym pszenicy twardej w Polsce jest ekonomiczny aspekt jej uprawy. Według Rocznika Statystycznego Głównego Urzędu Statystycznego w 2020 roku przeciętny Polak spożył w ciągu roku około 5,04 kg makaronu, a sama jego produkcja wynosiła 208,4 tys. ton (GUS, 2020). Na chwilę obecną w Polsce produkcja makaronów opiera się wyłącznie na ziarnie importowanym z zagranicy. Sytuacja ta wpływa znacząco na koszty, jakie ponosi Państwo w związku z zakupem ziarna, posiadającego najlepsze parametry jakościowe i technologiczne (Szumiło i Rachoń, 2009; Rachoń i in. 2021).

5.5. Wpływ badanych czynników stresowych na fizjologiczne i biochemiczne procesy pszenicy twardej

Pszenica twarda należy do gatunku zbóż, które cechuje się dużą specyfiką względem pozostałych gatunków zbóż. Jej specyfikę określają genetyczne predyspozycje zwiększające wrażliwość na czynniki stresowe. Pszenica twarda posiada genetyczną zdolność do akumulacji jonów kadmu, a także jest wysoce podatna na infekcje wywoływane przez grzyby z rodzaju *Fusarium*. W przypadku obu czynników stresowych, wrażliwość na nie warunkowana jest przez określone geny. Za zwiększoną akumulację jonów kadmu odpowiada gen w *locus Cdu1,* natomiast gen w *locus Fhb1* zwiększa odporność na porażenie przez grzyby fuzaryjne (Clarke i in. 2002; Poppenberger i in. 2003; Lemmens i in. 2005; Wiebe i in. 2010). Równie istotnym czynnikiem wskazującym na specyfikę tego gatunku jest zwiększona wrażliwość na stres solny w porównaniu do pszenicy zwyczajnej. Wysoka tolerancja na zasolenie warunkowana jest genami *TmHKT 1;4* i *TmHKT1;5* odpowiedzialnymi za usuwanie toksycznych jonów Na^+ z komórek ksylemu (James i in. 2008; James i in. 2012).

5.5.1. Wpływ jonów kadmu

Jednym z objawów toksycznego działania kadmu jest zaburzenie w pobieraniu składników mineralnych. Dodatkowo, łatwość pobierania jonów tego metalu przez pszenicę twardą przyczynia się do jego zwiększonej akumulacji w różnych częściach rośliny, a szczególnie w ziarnie (Hasan i in. 2009). Jony kadmu wywołują również stres osmotyczny objawiający się m. in. zmniejszeniem uwodnienia tkanek, ograniczeniem przewodnictwa szparkowego i transpiracji (Rizwan i in. 2016). Co więcej, jony kadmu hamują kiełkowanie

nasion oraz ograniczają prawidłowy rozwój poprzez zmniejszenie długości liści i korzeni oraz redukcję biomasy (Rascio i in. 1993; Hermans i in. 2011). Do innych negatywnych skutków wywołanych obecnością jonów kadmu można zaliczyć zwijanie liści, chlorozy i nekrozy (Jali i in. 2016), zmniejszenie zawartości chlorofilu *a*, ograniczenie fotosyntezy czy zamykanie się aparatów szparkowych (Clemens 2006). Obserwuje się również uszkodzenia błon komórkowych, peroksydację lipidów, uszkodzenia struktur białkowych i kwasów nukleinowych oraz zmniejszenie aktywności enzymów. Jony kadmu powodują także nadprodukcję reaktywnych form tlenu (ROS), wywołującą stres oksydacyjny (Abbas i in. 2017).

5.5.2. Wpływ zasolenia

Podobnie jak inne stresy abiotyczne, stres zasolenia podłoża powoduje wiele trwałych zaburzeń fizjologicznych i biochemicznych, które można obserwować na każdym etapie rozwoju rośliny. Wysokie stężenie soli obniża zdolność kiełkowania nasion (Khān i in. 2006), hamuje prawidłowy wzrost korzeni i pędów oraz powoduje powstawanie chloroz i nekroz części nadziemnych (Zhu i in. 2016). Pod kątem plonotwórczym, stres ten przyczynia się do zmniejszenia plonowania (Yadav i in. 2020). Zasolenie gleby wywołuje także stres osmotyczny (zaburzenie w pobieraniu wody) i stres jonowy (ograniczenie pobierania ważnych dla wzrostu i rozwoju składników pokarmowych) (Salim i Raza, 2020; Hafeez i in. 2021). Do innych szkodliwych czynników spowodowanych stresem zasolenia zalicza się uszkadzenie frakcji chlorofilowych, ograniczenie tempa fotosyntezy, zwiększone przymykanie aparatów szparkowych rzutujące na zmiany w parametrach wymiany gazowej (Karmous i in. 2013). Co więcej, stres solny podobnie jak stres spowodowany toksycznymi jonami kadmu, przyczynia się do powstawania stresu oksydacyjnego i wzmożonej produkcji ROS. Nadprodukcja ROS doprowadza m. in. do peroksydacji lipidów, denaturacji DNA, zmian w profilu hormonalnym i inaktywacji enzymów (Ahmad i in. 2010; Ryu i Cho, 2015).

5.5.3. Infekcje wywołane przez grzyby z rodzaju *Fusarium*

Do porażenia roślin pszenicy grzybami z rodzaju *Fusarium* może dochodzić na każdym etapie rozwoju rośliny. Infekcje te powodują takie choroby, jak zgorzele siewek, zgorzel podstawy źdźbła i korzeni oraz fuzariozę kłosów. Oprócz wizualnych objawów chorobowych takich, jak zmniejszona zdolność kiełkowania nasion, żółkniecie liści, zahamowanie wzrostu siewek, zamieranie korzeni czy karłowacenie roślin, obserwuje się mniejszy plon (mniejszą

liczbę ziarniaków, zmniejszoną ich wielkość masę). Szczególnie niebezpieczne dla zdrowia ludzi i zwierząt jest porażenie ziarna, a w związku z tym, zwiększone stężenie mykotoksyn produkowanych przez *Fusarium culmorum,* do których należą deoksyniwalenol (DON), niwalenol (NIV), zearalenon (ZEN) i toksyna T-2, należące do rodziny trichiotecenów (Wang i in. 2020; Kochman i in. 2021). Infekcje fuzaryjne mogą powodować wiele zaburzeń fizjologicznych i biochemicznych roślin. Do takich zaburzeń można zaliczyć m in.: zmniejszenie zawartości barwników fotosyntetycznych (Laguette i in. 2004), uszkodzenia aparatu fotosyntetycznego i procesu fotosyntezy (Yang i in. 2016), zwiększoną produkcja związków fenolowych (Nicholson i Hammerschmidt, 1992), nadprodukcję ROS określaną jako wybuch oksydacyjny (*ang. oxidative burst*) (Low i Merida, 1996), zmiany pH ściany komórkowej (Wojtasik i in. 2016), zmiany struktur błon wewnątrzkomórkowych i ekspresji genów (Wojtasik i in. 2020), zaburzenia szlaków metabolicznych np. węglowodanów czy hormonów (Bari i Jones, 2009; Iwaniuk i in. 2018).

6. Hipotezy badawcze i cel pracy

Na podstawie aktualnego stanu wiedzy dotyczącego pszenicy twardej i jej tolerancji/wrażliwości na wybrane czynniki stresowe postawiono następujące hipotezy badawcze:

1. Stopień odporności pszenicy twardej na zasolenie gleby jest związany z tolerancją na zanieczyszczenie gleby jonami kadmu.
2. Stopień akumulacji jonów kadmu w ziarnach pszenicy twardej zależy od wydajności aparatu fotosyntetycznego roślin uprawianych w glebie skażonej metalem ciężkim oraz aktywnością enzymów antyoksydacyjnych wchodzących w skład cyklu glutationowo-askorbinianowego.
3. Zastosowanie poszczególnych fizjologicznych i biochemicznych pomiarów posłuży do wytypowania markerów przydatnych do selekcji roślin pod kątem tolerancji/wrażliwości na zasolenie gleby.
4. Na podstawie oceny stopnia porażenia kłosów pszenicy twardej przez *Fusarium culorum* można przewidzieć stopień akumulacji mykotoksyn w ziarnie produkowanych przez tego grzyba.
5. Zawartość takich związków organicznych, jak pigmenty fotosyntetyczne, związki fenolowe, cukry rozpuszczalne i aktywność antyoksydacyjna decydują o stopniu tolerancji pszenicy twardej na *F. culmorum.*

Aby zweryfikować przedstawione powyżej hipotezy badawcze zrealizowano poszczególne cele, polegające na określeniu zależności pomiędzy badanymi fizjologicznymi i biochemicznymi parametrami, a tolerancją na zasolenie gleby chlorkiem sodu, jonami kadmu i infekcję grzybową.

7. Materiał i metody

Badania wykonane w ramach przygotowania rozprawy doktorskiej zostały przeprowadzone w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa, Uniwersytetu Rolniczego im. Hugona Kołłątaja w Krakowie. Poszczególne doświadczenia wykonano w kontrolowanych warunkach szklarniowych (zasolenie gleby i akumulację jonów kadmu) oraz w tunelu foliowym (infekcja roślin zarodnikami *Fusarium culmorum*). W przypadku stresu zasolenia i porażenia roślin przez patogen, przeprowadzono także dodatkowe doświadczenia laboratoryjne: test szalkowy związany z odpornością na zasolenie w fazie kiełkowania i siewek oraz test w kulturach *in vitro* określający stopień porażenia siewek i korzeni przez *F. culmorum*. Doświadczenie dotyczące akumulacji jonów kadmu zostało przeprowadzone w kontrolowanych warunkach szklarniowych**.**

7.1. Materiał roślinny

Materiał badawczy stanowiły trzy genotypy pszenicy twardej, różniące się między sobą stopniem wrażliwości na zasolenie:

- krajowa linia SMH87 – pierwsza w Polsce jara forma pszenicy twardej wyhodowana przez HR Smolice Sp. z o.o. Grupa IHAR, udostępniona przez dr Jarosława Bojarczuka,
- australijska odmiana Tamaroi cechująca się wrażliwością na zasolenie gleby,
- australijska linia BC_5Nax_2 wykazująca wysoką tolerancję na zasolenie gleby. Oba australijskie genotypy przekazane zostały przez dr Richard A. Jamesa z Commonwealth Scientific and Industrial Research Organisation (CSIRO) Plant Industry.

7.2. Schemat doświadczeń

Poglądowy schemat doświadczeń prowadzonych w ramach pracy doktorskiej został przedstawiony w Tabeli 2. Pełne schematy doświadczeń zostały szczegółowo opisane w rozdziale Materials and Methods w każdej z publikacji wchodzącej w skład niniejszej dysertacji.

Tabela 2. Schemat wykonanych doświadczeń

	Doświadczenie I – stres wywołany jonami kadmu (**Publikacja nr 1 i 2)**	**Doświadczenie II** – stres zasolenia gleby **(Publikacja nr 3**)	**Doświadczenie III** – infekcja *F. culmorum* **(Publikacja nr 4)**
Doświadczenia laboratoryjne			
miejsce przeprowadzenia doświadczenia	-	komora wzrostowa	komora wzrostowa
warunki doświadczenia	-	szalki Petriego + sterylne bibuły filtracyjne	kultury *in vitro* w magentach na pożywce MS
czynnik stresowy	-	roztwory NaCl o stężeniu: 50; 100; 125; 150 mM	izolat *F. culmorum* IPO348–01
warunki świetlne	-	ciemność	oświetlenie LED o natężeniu 150 µmol m^{-2} s^{-1} PPFD, fotoperiod 12/12 h (dzień/noc)
temperatura	-	25°C (dzień/noc)	22/20°C (dzień/noc)
czas trwania doświadczenia	-	2-6 dni (w zależności od badanego parametru)	14 dni
Doświadczenia w warunkach kontrolowanych			
miejsce przeprowadzenia doświadczenia	szklarnia	szklarnia	tunel foliowy
warunki i podłoże uprawowe	doniczki wypełnione ziemią czarnoziem zdegradowany	doniczki wypełnione uniwersalnym podłożem ogrodniczym	doniczki wypełnione uniwersalnym podłożem ogrodniczym
czynnik stresowy	roztwory $CdSO_4$ o stężeniu 3 i 5 mg kg^{-1} gleby	roztwory NaCl o stężeniu: 100; 125; 150 mM	izolat *F. culmorum* IPO348–01, zawiesina zarodników w stężeniu 5×10^5 cm^{-3}
warunki świetlne	światło dzienne (marzec-maj) uzupełniane do 16-godzinnego dnia lampami sodowymi 400 µmol $m^{-2}s^{-1}$ PPFD	światło dzienne (maj-sierpień)	światło dzienne (lipiec-wrzesień)
temperatura	22/18 °C (dzień/noc)	22/20 °C (dzień/noc)	temperatura otoczenia

7.3. Metody wykorzystane do wyznaczenia fizjologicznych i biochemicznych parametrów tolerancji/wrażliwości na wybrane czynniki stresowe

Do określenia stopnia tolerancji/wrażliwości na zastosowane w doświadczeniach czynniki stresowe użyto wybranych metod pomiarowych opartych zarówno o analizy fizjologiczne i biochemiczne, które zastosowano osobno dla każdego zastosowanego stresu.

7.3.1. Doświadczenie I – wpływ jonów kadmu

Publikacja nr 1:

- Wybrane parametry fluorescencji chlorofilu (ChlF) mierzono w liściach flagowych pszenicy twardej przy użyciu przenośnego urządzenia Handy PEA (Hansatech Ltd.,)
- Parametry wymiany gazowej w liściach flagowych mierzono analizatorem dwutlenku węgla w podczerwieni CIRAS-1 (Hansatech Ltd.,)
- Zawartość zakumulowanego kadmu w ziarnach pszenicy twardej mierzono techniką atomowej spektrometrii emisyjnej ze wzbudzeniem w plazmie indukowanej (ICP-OES) wg. metodyki opisanej przez Ostrowską i in. (1991).
- Plon oceniano na podstawie liczby ziarniaków w kłosie, ich masy i masy tysiąca nasion

Publikacja nr 2:

- Zawartość nadtlenku wodoru (H_2O_2) w liściach flagowych oznaczono spektrofotometrycznie wg. protokołu producenta (ThermoFisher Protocol of Amplex®Red Hydrogen Peroxide/Peroxidase Assay Kit),
- Aktywność enzymów i związków antyoksydacyjnych wykonano według następujących metod:
  - zawartość askorbinianu (AsA) oznaczano wg. Foyer i in. (1983),
  - zawartość utlenionej formy kwasu askorbinianowego (DHA) obliczano wg. Harrach i in. (2007),
  - zawartość glutationu (GSH) i glutationu ultenionego (GSSG) analizowano wg. Law i in. (1983),
  - zawartość reduktazy dehydroaskorbinianowej (DHAR) i reduktazy glutationowej (GR) oznaczano wg. Klapheck i in. (1990),
  - aktywność peroksydazy askorbinianowej (APX) oznaczano wg. Nakano i Asada (1981).

- Zawartość zakumulowanego kadmu w ziarnach pszenicy twardej analizowano jak w Publikacji nr 1.

7.3.2. Doświadczenie II – zasolenie gleby wywołane przez NaCl

Publikacja nr 3:

- Określano zdolność kiełkowania nasion poprzez obliczenie procentu kiełkujących nasion (PGS), wigoru kiełkowania (*Vi*) oraz na podstawie długości koleoptyli (CL); obliczano wskaźnik podatności na zasolenie (SSI) w stresie zasolenia wg. metody opisanej przez Płażek i in. (2013).
- Przepuszczalność membran cytoplazmatycznych oceniano metodą konduktometryczną, analizując wypływ elektrolitów (EL) nasion kiełkujących w roztworach NaCl.
- Wybrane parametry fluorescencji chlorofilu (ChlF) mierzono w liściach flagowych pszenicy twardej przy użyciu przenośnego urządzenia Handy PEA (Hansatech Ltd.,).
- Parametry wymiany gazowej w liściach flagowych analizowano przy użyciu przenośnego analizatorem dwutlenku węgla w podczerwieni CIRAS-3 (Hansatech Ltd.,).
- Zawartość barwników fotosyntetycznych: chlorofilu *a* i *b* (Chl *a*, Chl *b*) oraz karotenoidów (*Car*) w liściach oznaczono zgodnie z metodyką Czyczyło-Mysza i in. (2013).
- Ogólną pulę cukrów (TSC) rozpuszczalnych w liściach i korzeniach analizowano wg. metodyki opisanej przez Dubois i in. (1951) z modyfikacją wg. Bach i in. (2015).
- Zawartość ogólnej puli fenoli (TPC) w liściach i korzeniach mierzono zgodnie z protokołem Bach i in. (2015).
- Zawartość fenoli związanych ze ścianą komórkową (CWP) w liściach i korzeniach oznaczano wg. metody opisanej przez Hurę i in. (2016),
- Aktywność enzymów antyoksydacyjnych w liściach i korzeniach oznaczano według następujących metod:
  - aktywność katalazy (CAT) wg. Aebi (1984),
  - aktywność peroksydazy (POX) wg. Lück (1962),
  - aktywność dysmutazy ponadtlenkowej (SOD) wg. McCord i Fridovic (1969),

- Zawartość nadtlenku wodoru (H_2O_2) w liściach i korzeniach mierzono wg. protokołu producenta (ThermoFisher Protocol of Amplex®Red Hydrogen Peroxide/Peroxidase Assay Kit.)

7.3.3. Doświadczenie III – infekcja wywołana patogenem *F. culmorum*.

Publikacja nr 4:

- Grzybnię *F. culmorum* hodowano na pożywkach wg. metodyki opisanej przez Wiśniewską i in. (2014) i Warzechę i in. (2015),
- Wizualną ocenę stopnia porażenia (DR) liści i korzeni oraz pomiary wagowe siewek i korzeni wykonano zgodnie z metodyką opisaną przez Warzechę i in. (2019).
- Analizy biochemiczne takie jak zawartość barwników fotosyntetycznych (chlorofilu *a* i *b* (Chl *a*, Chl *b*) oraz karotenoidów (*Car*), zawartość ogólnej puli cukrów (TSC), fenoli (TPC), fenoli związanych ze ścianą komórkową (CWP), ocenę aktywności enzymów: katalazy (CAT), peroksydazy (POX) i dysmutazy ponadtlenkowej (SOD) oraz pomiar zawartości nadtlenku wodoru (H_2O_2) wykonano jak w Publikacji nr 3.
- Zawiesinę zarodników do infekowania kłosów otrzymano wg. metody opisanej przez Górala i in. (2018), porażenia kłosów przeprowadzono wg. metody Warzechy i in. (2010), natomiast indeks fuzariozy kłosów obliczano wg. metody Górala i in. (2018).
- Oznaczanie zawartości mykotoksyn: deoksyniwalenolu (DON), niwalenolu (NIV), zearalenonu (ZEN) i toksyny T-2 (oraz ich pochodnych) w ziarnie pszenicy twardej wykonano przy użyciu ultrawysokosprawnej chromatografii cieczowej sprężonej z tandemem spektrometrii mas (UHPLC–MS/MS) wykonanej wg. metodyki Dziurki i in. (2020).
- Określenie parametrów plonu jak w Publikacji 1.

Szczegółowy opis materiału roślinnego i zastosowanych metod pomiarowych wykorzystanych w poszczególnych doświadczeniach zamieszczono w rozdziałach Materials and Methods każdej z publikacji wchodzącej w skład rozprawy doktorskiej.

8. Najważniejsze wyniki z przeprowadzonych badań

8.1. Akumulacja kadmu w ziarnie pszenicy twardej w zależności od stopnia odporności na zasolenie oraz odpowiedź systemu antyoksydacyjnego na zanieczyszczenie kadmem.

Publikacja nr 1: Pastuszak J., Kopeć P.*, Płażek A., Gondek K., Szczerba A., Hornyák M., Dubert F. (2020). Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree. *Plant Soil Environment*, 66(6), 257-263.

Pszenica twarda, w porównaniu do pszenicy zwyczajnej posiada większą zdolność do akumulacji kadmu. Jak podają źródła literaturowe, różnice dotyczące akumulacji jonów Cd w poszczególnych częściach rośliny wynikają nie tylko z genetycznych różnic gatunkowych i odmianowych, ale mogą być determinowane przez czynniki fizjologiczne, jak np. pobieranie jonów Cd z roztworu glebowego, transport z korzenia do pędu, sekwestracja Cd w przedziałach subkomórkowych oraz przemieszczaniu Cd do ziarna podczas rozwoju nasion (Hart i in. 1998). W badaniach dotyczących stresu wywołanego przez jony kadmu skupiono się na fizjologicznych i biochemicznych parametrach, które pomogą w określeniu stopnia tolerancji na ten stres. Należy zaznaczyć, że sole kadmu zostały wprowadzone do gleby przed wysiewem nasion, a więc jego oddziaływanie obserwowane było we wszystkich fazach wzrostu i rozwoju roślin. Pomiary fizjologiczne i biochemiczne zostały przeprowadzone na liściach flagowych oraz ziarnie uzyskanym z roślin po zakończeniu wegetacji. Taki układ doświadczenia miał dostarczyć informacji, jak kondycja roślin uprawianych w glebie skażonej kadmem decyduje o ilości i jakości plonu.

Najważniejsze wyniki:

1. Wszystkie badane parametry kinetyki fluorescencji chlorofilu *a* z wyjątkiem rozproszenia energii (DI_o/CS_m) zmniejszały się u wszystkich genotypów pod wpływem obu zastosowanych dawek jonów Cd w glebie. Największe spadki wartości obserwowano dla wskaźnika funkcjonowania PSII (PI), zwłaszcza u odmiany Tamaroi.
2. Na obniżenie parametrów wymiany gazowej u badanych odmian istotnie wpływała wyższa dawka jonów Cd (5 mg/kg DW gleby). Najsilniejszy efekt w tym wypadku odnotowano w dla polskiej linii SMH87.
3. W liniach bardziej odpornych na zasolenie tj. SMH87 i BC_5Nax_2 stężenie 3 mg Cd/kg DW gleby spowodowało znaczny wzrost liczby ziaren i ich masy w kłosie

w porównaniu do roślin kontrolnych (uprawianych w glebie bez dodatku Cd) i uprawianych w glebie skażonej wyższą dawką, czyli 5 mg Cd/kg DW gleby. Wynik ten, dość zaskakujący, może sugerować wystąpienie zjawiska nazywanego „hormesis". Jest to zjawisko polegające na tym, że czynnik występujący w przyrodzie, szkodliwy dla organizmu w większych dawkach, w małych dawkach działa na niego korzystnie.

4. Najwięcej zakumulowanego kadmu odnotowano w ziarnie najbardziej wrażliwej na zasolenie odmiany Tamaroi.
5. Pomiędzy wszystkimi badanymi parametrami kinetyki fluorescencji chlorofilu *a* i zawartością Cd w ziarniakach znaleziono istotne korelacje.

Publikacja nr 2: Pastuszak J.*, Kopeć P., Płażek A., Gondek K., Szczerba A., Hornyák M., Dubert, F. (2020). Antioxidant activity as a response to cadmium pollution in three durum wheat genotypes differing in salt-tolerance. *Open Chemistry*, 18, 1230–1241.

Tolerancja roślin na jony kadmu jest zależna od zdolności antyoksydacyjnych względem reaktywnych form tlenu (ROS), których zwiększoną ilość obserwuje się u roślin poddanych temu stresowi. Za proces usuwania ROS odpowiadają m.in. takie związki, jak glutation (GSH) i askorbinian (AsA), należące do nieenzymatycznych antyoksydantów wchodzących w skład cyklu glutationowo-askorbinianowego (cyklu AsA-GSH) Hasanuzzaman i in. (2018). Badania opisane w Publikacji nr 2 omawiają reakcję systemu antyoksydacyjnego pod wpływem jonów kadmu.

Najważniejsze wyniki:

1. Wykazano duże zróżnicowanie badanych genotypów pod względem zawartości nadtlenku wodoru w roślinach rosnących w glebie skażonej jonami Cd. Największa zawartość tego związku wykazano w linii SMH87 i BC_5Nax_2, średnio i bardzo odpornej na zasolenie. Wynik ten wskazuje na istotną rolę nadtlenku wodoru jako cząsteczki sygnałowej niezbędnej w aktywacji procesów obronnych uruchamianych w roślinie podczas stresu.
2. Najniższe aktywności badanych enzymów: peroksydazy askorbinianowej, reduktazy glutationowej, reduktazy monodehydroaskorbinianowej i reduktazy dehydroaskorbinianowej odnotowano u linii SMH87. Najwyższe aktywności tych enzymów pod wpływem kadmu odnotowano dla najbardziej wrażliwej na zasolenie

odmiany Tamaroi, co wiązało się z obniżaniem poziomu nadtlenku wodoru. Wynik ten potwierdza poprzedni rezultat, świadczący o tym, że wysoka zawartość nadtlenku wodoru jest istotna dla procesów obronnych.

3. Stosunek glutationu do jego utlenionej formy (GSH:GSSG) był specyficzny dla badanych genotypów. Stosunek ten u SMH87 i BC_5Nax_2 wzrastał w przypadku obu zastosowanych dawek Cd, natomiast u odmiany Tamaroi obserwowano drastyczny spadek tego parametru.
4. Stosunek ilości kwasu askorbinianowego do jego utlenionej formy (AsA:DHA) nie różnicował reakcji badanych odmian na zastosowane dawki kadmu.
5. Podobnie jak w publikacji nr 1, najwięcej zawartości zakumulowanego kadmu obserwowano u wrażliwej na zasolenie odmianie Tamaroi, przy czym u wszystkich badanych odmian, więcej zakumulowanego Cd w ziarnie notowano u roślin rosnących w glebie skażonej 3 mg Cd/kg DW gleby.

8.2. Parametry odporności pszenicy twardej na zasolenie gleby

Publikacja nr 3: Pastuszak J.*, Dziurka M., Hornyák M., Szczerba A., Kopeć P., Płażek A.* (2022). Physiological and biochemical parameters of salinity resistance of three durum wheat genotypes. *International Journal of Molecular Sciences,* 2022; 23(15):8397.

Pszenica twarda wykazuje znacznie większą zdolność do akumulacji jonów soli w porównaniu do pszenicy zwyczajnej. Zwiększona wrażliwość na stres zasolenia spowodowany jest przez znacznie gorzej rozwinięty mechanizm usuwaniu jonów soli z cytozolu (Munns i Tester, 2008). Za prawidłowe działanie tego mechanizmu odpowiadają dwa *loci – Nax1* odpowiadający za usuwanie jonów Na^+ z ksylemu korzeni, dolnych częściach blaszki liściowej oraz pochew liściowych, oraz *Nax2* usuwający jony Na^+ tylko z ksylemu korzeni (Lindsay i in. 2004; James i in. 2012).

Najważniejsze wyniki:

Eksperyment 1: Wpływ zasolenia na proces kiełkowania oceniano na podstawie procentu skiełkowanych nasion (PGS), wigoru kiełkowania (*Vi*), długości koleoptyli (CL), indeksu podatności na zasolenie (SSI) oraz wypływu elektrolitów (EL) z kiełkujących nasion.

1. Najlepiej kiełkowały nasiona odpornej na zasolenie linii BC_5Nax_2 we wszystkich zastosowanych stężeniach soli w porównaniu do pozostałych genotypów. Również *Vi* dla tej linii był najwyższy w stężeniach od 50-150 mM NaCl. Zasolenie redukowało

CL u wszystkich genotypów, ale w najmniejszym stopniu u BC_5Nax_2. Z kolei SSI istotnie zwiększał się pod wpływem wzrastającego zasolenia u wszystkich badanych genotypów, ale w najmniejszym stopniu u BC_5Nax_2.

2. Wypływ elektrolitów (EL) z kiełkujących nasion nie różnicował badanych genotypów pod kątem ich wrażliwości na zasolenie.

Eksperyment 2: Ta część doświadczenia ukierunkowana była na odnalezienie fizjologicznych i biochemicznych parametrów, które potencjalnie mogą zostać wykorzystywane do oceny stopnia tolerancji pszenicy twardej na zasolenie gleby.

1. Wszystkie badane parametry fluorescencji chlorofilu zmieniały się specyficznie dla każdej badanej odmiany. Jedynymi parametrami różnicującymi wrażliwość badanych genotypów na zasolenie gleby były rozproszenie energii z fotosystemu II (DI_o/CS_m) i maksymalna efektywność reakcji rozkładu wody po donorowej stronie PSII (F_v/F_0). Nie zanotowano zmian w stopniu rozproszonej energii dla wrażliwej na zasolenie odmiany Tamaroi i odpornej BC_5Nax_2 pod wpływem rosnącego stężenia NaCl, natomiast linia SMH87 w 100 i 125 mM NaCl wykazała mniejszy stopień rozproszenia energii niż rośliny kontrolne, ale w 150 mM NaCl nastąpił drastyczny wzrost wartości tego parametru. Również ta linia wykazała istotny spadek efektywności rozkładu wody w 150 mM NaCl, natomiast u pozostałych odmian spadek ten był stosunkowo niewielki.
2. Spośród badanych parametrów wymiany gazowej najbardziej różnicującym reakcje genotypów na zasolenie była wydajność fotosyntezy netto (*A*). U linii SMH87 wydajność ta istotnie wzrosła w zasoleniu 100–150 mM NaCl. U wrażliwej na zasolenie odmiany Tamaroi obserwowano drastyczny spadek wartości tego parametru w 100 mM NaCl, a następnie jego wzrost, bowiem największą wydajność *A* zaobserwowano u tej odmiany w 150 mM NaCl. Ta wydajność fotosyntetyczna była jednocześnie najwyższą efektywnością dla wszystkich trzech badanych genotypów. Linia BC_5Nax_2 zareagowała istotnym spadkiem efektywności fotosyntetycznej w 100 mM, a w wyższych stężeniach zasolenia jej wzrostem.
3. Największą zawartość chlorofilu *a*, *b* i karotenoidów obserwowano u odpornej na zasolenie BC_5Nax_2 w 150 mM NaCl.

4. Zawartość rozpuszczalnych węglowodanów (TSC) wzrastała u wszystkich badanych genotypów wraz ze wzrostem zasolenia, ale największą ich zawartość obserwowano u wrażliwej na zasolenie odmianie Tamaroi.
5. Ogólna zawartość związków fenolowych (TPC) wraz ze wzrostem stężenia chlorku sodu w glebie zmniejszała się u wszystkich badanych genotypów, natomiast zawartość związków fenolowych związanych ze ścianą komórkową (CWP) zmieniała się specyficznie u każdego genotypu.
6. Aktywność enzymów antyoksydacyjnych pod wpływem zasolenia gleby zmieniała się specyficznie dla każdego genotypu. Zmiany w poziomie nadtlenku wodoru zachodziły podobnie u wszystkich genotypów, jednakże w stresie solnym zawartość tego związku była najwyższa w roślinach wrażliwej na zasolenie odmiany Tamaroi.
7. Wszystkie parametry plonu: liczba nasion, ich masa oraz masa tysiąca nasion (MTS) zmniejszały się pod wpływem zasolenia u wszystkich badanych genotypów. W 150 mM NaCl najmniejszą liczbę nasion, ich masę i MTS odnotowano w przypadku wrażliwej na zasolenie odmiany Tamaroi. U odpornej na zasolenie linii BC_5Nax_2 najwyższą MTS obserwowano w stężeniu 125 i 150 mM NaCl.

8.3. Fizjologiczna i biochemiczna reakcja pszenicy twardej na infekcję *F. culmorum*

Publikacja nr 4: Pastuszak J.*, Szczerba A., Dziurka M., Hornyák M., Kopeć P., Szklarczyk M., Płażek A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, 22(14), 7433

Fusarium culmorum powoduje choroby zbóż, które mogą pojawić się na każdym etapie rozwoju rośliny. Infekcje te stanowią poważny problem dla rolnictwa, gdyż powodują zmniejszenie plonu i pogarszają jego jakość technologiczną. Grzyb ten produkuje toksyny, których zwiększone ilości są szkodliwe dla ludzi i zwierząt. Podobnie, jak w przypadku zwiększonej wrażliwości na zasolenie i zdolności do akumulacji jonów kadmu, również w przypadku infekcji fuzaryjnych pszenica twarda charakteryzuje się znacznie większą na nie podatnością, w porównaniu do pszenicy zwyczajnej. Oprócz predyspozycji morfologicznych i różnic genetycznych, czynnikiem decydującym o większej podatności na zakażenie fuzariozami stanowi brak genu odporności w *locum Fhb1* (Miedaner i Longin, 2014; Wang i in. 2020).

W tym eksperymencie, głównym celem było znalezienie parametrów najbardziej różnicujących stopień odporności genotypów pszenicy twardej na zakażenie *Fusarium culmorum.* Badania przeprowadzono na siewkach i roślinach w fazie kłoszenia.

Najważniejsze wyniki:

Eksperyment 1. Stopień uszkodzeń spowodowanych infekcją oceniano na liściach i korzeniach siewek badanych genotypów w warunkach *in vitro*.

1. Najsilniej porażone były liście linii SMH87, a najsłabiej porażone były liście linii BC_5Nax_2 odpornej na zasolenie. Objawy porażenia korzeni były słabsze i również w tym przypadku najmniejsze porażenie obserwowano na korzeniach linii BC_5Nax_2.
2. Stopień infekcji oceniany na podstawie utraty świeżej masy liści i korzeni był najsilniejszy w przypadku linii SMH87 i odmiany Tamaroi, a u linii BC_5Nax_2 porażone liście wykazywały taką samą masę, jak liście roślin kontrolnych. Korzenie silniej reagowały na porażenie spadkiem masy; najsilniejszy ubytek masy po infekcji w stosunku do kontroli obserwowano u linii SMH87, zaś najmniejszy u BC_5Nax_2. W wyniku infekcji najmniejszą zawartość chlorofilu *a* wykazały liście linii SMH87, a największą zawartość tego barwnika notowano u odmiany Tamaroi. Zawartość chlorofilu *b* i karotenoidów była najmniejsza w infekowanych liściach SMH87, natomiast pozostałe odmiany po infekcji wykazały taką sama zawartość tych barwników.
3. Ogólna zawartość cukrów rozpuszczalnych (TSC) w infekowanych liściach zmniejszyła się w przypadku linii SMH87, natomiast u pozostałych genotypów obserwowano wzrost zawartości TSC w porównaniu do liści roślin kontrolnych. Odwrotną zależność zaobserwowano w korzeniach, gdzie w infekowanych korzeniach SMH87 zawartość TSC wzrosła, a u odmiany Tamaroi i linii BC_5Nax_2 zmniejszyła się.
4. Linia SMH87 wykazała również w infekowanych liściach istotne zmniejszenie się zawartości fenoli (TSP), podczas gdy u pozostałych genotypów zawartość ta się nie zmieniła lub była niewiele mniejsza.
5. Zmiany zawartości fenoli związanych ze ścianą komórkową pod wpływem infekcji nie różnicowały znacząco badanych genotypów pod względem reakcji na infekcję.
6. Aktywność enzymów antyoksydacyjnych (katalazy, peroksydazy niespecyficznej i dysmutazy anionorodnika ponadtlenkowego) oraz poziom nadtlenku wodoru nie różnicowały badanych genotypów pod względem odpowiedzi na infekcję.

7. Analiza statystyczna wykazała wysoce istotną ujemną korelację pomiędzy stopniem porażenia (DR) przez *F. culmorum,* a zawartością barwników fotosyntetycznie czynnych oraz świeżą masą liści i korzeni.

Eksperyment 2: W tej części doświadczenia analizowano porażenie skalą FHBi (*Fusarium head blight index*) wywołane przez *F. culmorum* po 7 i 14 dniach od inokulacji kłosów zarodnikami. Oceniano liczbę ziarniaków w kłosie i ich masę oraz masę tysiąca ziarniaków (MTS). Analizowano również zawartość toksyn produkowanych przez *F. culmorum* w ziarnie.

1. Stopień porażenia roślin zarodnikami *F. culmorum* oceniany po czternastu dniach był dużo wyższy niż po siedmiu dniach. W obu terminach najbardziej porażona była linia SMH87. Linia SMH87 była najbardziej wrażliwa na infekcję, co stwierdzono na podstawie analizowanych parametrów plonu.

2. Najwięcej mykotoksyn: niwalenolu (NIV), deoksyniwalenolu (DON), toksyny T-2 oraz zearalenonu (ZEN) w ziarnie zakumulowała wrażliwa na zasolenie odmiana Tamaroi. W przypadku krajowej linii SMH87, zaobserwowano znacznie mniejszą akumulację w ziarnie DON, ZEN i T-2 w porównaniu do pozostałych genotypów.

3. Analiza statystyczna wykazała istotny wpływ toksyn na parametry plonu, dodatnią korelację pomiędzy stopniem porażenia kłosów i zawartością NIV i DON w ziarniakach, oraz ujemną korelację pomiędzy stopniem porażenia kłosów i parametrami plonu.

4. W przypadku linii SMH87 nie wykazano zależności pomiędzy indeksem porażenia kłosów (FHBi), a zawartością mykotoksyn w ziarnie. Mimo licznych objawów infekcji linia ta zakumulowała najmniej mykotoksyn (głównie ZEN i T-2).

9. Wnioski

W ramach prezentowanego cyklu czterech publikacji wchodzących w skład niniejszej rozprawy doktorskiej wykazano następujące wnioski:

1. Wydajność aparatu fotosyntetycznego odgrywa istotną rolę w ilości zakumulowanych jonów kadmu w ziarniakach pszenicy twardej. (Publikacja 1)
2. Tolerancja na toksyczny wpływ jonów kadmu i na zasolenie NaCl prawdopodobnie ma wspólne podłoże fizjologiczne. Sugestia ta wymaga przeprowadzenia dalszych badań,

mających na celu stwierdzenie, które procesy decydują o odporności na oba te stresy. (Publikacja 1)

3. Stosunek GSH:GSSG może być uznany za marker zdolności akumulowania jonów Cd w ziarnie pszenicy. (Publikacja 2) Wyniki dotyczące większej akumulacji kadmu w ziarniakach roślin rosnących przy 3 mg Cd/kg DW gleby niż przy 5 mg Cd/kg DW gleby potwierdzają doniesienia literaturowe, że przy wyższych dawkach tego metalu może nastąpić zahamowanie transportu jonów kadmu z korzeni do pędów poprzez łączenie ich ze związkami wchodzącymi w skład ściany komórkowej oraz ich sekwestracją w wakuolach komórek korzeniowych. (Publikacja 2)
4. Procent kiełkujących nasion (PGS) i wigor kiełkowania (*Vi*) są parametrami różnicującymi stopień tolerancji na zasolenie genotypów pszenicy twardej w fazie kiełkowania. (Publikacja 3)
5. Parametry fluorescencji chlorofilu takie jak maksymalna wydajność reakcji rozszczepiania wody po stronie donorowej fotosystemu II (PSII) – F_v/F_0 oraz rozpraszanie energii z PSII – DI_o/CS_m mogą być stosowane jako parametry różnicujące genotypy pszenicy twardej pod względem tolerancji na zasolenie. (Publikacja 3)
6. Masa tysiąca nasion (MTS) jest może posłużyć jako fizjologiczny wskaźnik tolerancji na zasolenie pszenicy twardej. (Publikacja 3)
7. Odporna na zasolenie linia BC_5Nax_2 charakteryzuje się najwyższym procentem kiełkujących nasion (PGS) i wigorem kiełkowania (*Vi*) w fazie kiełkowania oraz najwyższą zawartością chlorofilu *a, b* i karotenoidów oraz MTS w fazie kłoszenia. (Publikacja 3)
8. Wrażliwa na zasolenie odmiana Tamaroi wykazuje znacznie wyższy poziom nadtlenku wodoru w 125 i 150 mM NaCl, co dowodzi znacznych uszkodzeń oksydacyjnych spowodowanych stresem zasolenia. (Publikacja 3)
9. Infekcja wywołana przez *Fusarium culmorum* zmniejsza zawartość aktywnych barwników fotosyntetycznych oraz masę liści i korzeni badanych siewek. (Publikacja 4)
10. Odmiana Tamaroi i linia BC_5Nax_2 są bardziej odporne na *F. culmorum* niż linia SMH87. Genotypy te gromadzą większe ilości cukru w liściach, co świadczy o większej reakcji obronnej. (Publikacja 4)
11. Zawartość niwalenolu i deoksyniwalenolu wydzielanych przez *F. cumlorum* ujemnie koreluje z plonem pszenicy twardej. (Publikacja 4)

12. Wczesna ocena infekcji kłosów pszenicy twardej przeprowadzona już po siedmiu dniach od inokulacji zarodnikami *F. culmorum* pozwala przewidzieć stopień akumulacji DON i NIV w ziarnie. (Publikacja 4)

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Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree

Jakub Pastuszak[1], Przemysław Kopeć[2], Agnieszka Płażek[1], Krzysztof Gondek[3], Anna Szczerba[1], Marta Hornyák[1], Franciszek Dubert[2]*

[1]Department of Plant Physiology, University of Agriculture, Kraków, Poland
[2]The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland
[3]Department of Agriculture and Environmental Chemistry, University of Agriculture, Kraków, Poland

**Corresponding author: przemyslawkopec@gmail.com*

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Abstract: A serious problem in durum wheat cultivation is its genetic capacity to accumulate cadmium (Cd) in the grain. The aim of the study was to verify if the degree of durum wheat resistance to NaCl salinity is related to its tolerance to Cd contamination, and to search for physiological markers of Cd accumulation in the grain. The experiment involved a salt sensitive cv. Tamaroi and a salt resistant line BC_5Nax_2, as well as a moderately salt tolerant line SMH87. The plants grew in the soil supplemented with 3 mg or 5 mg Cd/kg dry weight. The plant response to Cd was evaluated based on chlorophyll fluorescence (ChlF) and Cd content in the grains. Toxic effects of both Cd levels on photosynthetic performance index were the strongest in salt sensitive cv. Tamaroi, which showed the highest Cd content in the seeds. We therefore assumed that tolerance to salinity and Cd has a common physiological background, and that ChlF parameters may be used as the markers of Cd tolerance.

Keywords: *Triticum durum* Desf.; heavy metal; salinity resistance; grain yield

Durum wheat is one of the cereals most commonly used by food industry and cultivated around the world (Oleson 1994). Durum wheat is grown due to its grain protein and fibre content, low glycemic index, and high level of vitamins (Olmos et al. 2003). Tolerance to salt stress is crucial in durum wheat cultivation. The mechanisms of plant tolerance to salinity entail mainly excretion of salt ions (Munns and Tester 2008). Two loci: *Nax1* and *Nax2* carrying genes that control excretion of sodium ions from xylem were transferred from *Triticum monococcum* L. to salt sensitive cultivar Tamaroi of *T. turgidum* L. ssp. *durum* (Desf.) to produce a new line resistant to high salinity (James et al. 2012). A serious problem in durum wheat cultivation is its genetic capacity to accumulate cadmium (Cd) ions in the grain (Cheli et al. 2010). The EU has proposed a limit of Cd concentration in food (0.2 mg/kg fresh weight of a product) (CEC 2006). Heavy metal pollution of soil seriously limits crop yield and quality, and affects animals, humans and the natural environment (Paradiso et al. 2008). Crop plants growing at high levels of Cd show many disorders, such as inhibition of seed germination and plant growth (Rascio et al. 1993), leaf rolling, chlorosis and necrosis (Greger et al. 1991), nutrient distribution disorder (Moral et al. 1994), reduction of chlorophyll content and photosynthesis efficiency, and stomatal closure (Clemens 2006). Jalil et al. (1994) identified durum wheat plants as more effective accumulators of Cd in the grain than bread wheat. Zhang et al. (2012) found differences between species and cultivars in the amount of absorbed Cd and resistance to its toxic effects. Cadmium increases lipid peroxidation, and protein and nucleic acid oxidation. The element inactivates several enzymes by binding with their

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sulfhydryl groups (Dorta et al. 2003). The measurement of chlorophyll *a* fluorescence (ChlF) is a widely used, non-invasive method of determining photosynthesis efficiency and can also be used to predict yields of crop plants under various environmental conditions (Kalaji and Pietkiewicz 2004). The aim of the presented study was to investigate whether: (1) the degree of durum wheat resistance to soil NaCl salinity is related to the species tolerance to soil Cd contamination and (2) to search for physiological markers of the ability to accumulate Cd in the grain. We compared plant response to Cd in three durum wheat genotypes: NaCl-resistant line BC_5Nax_2, NaCl-sensitive cv. Tamaroi, and SMH87 line moderately tolerant to salinity. The defence response of the studied durum genotypes included evaluation of chlorophyll *a* fluorescence, photosynthesis efficiency, as well as yield and Cd content in the grains.

MATERIAL AND METHODS

Plant material. The line SMH87, moderately tolerant to salinity, was obtained from the Plant Breeding Centre in Smolice (Poland). The salt resistant line BC_5Nax_2, involving Nax_2 locus, and the sensitive cv. Tamaroi were obtained from Dr. Richard A. James from Commonwealth Scientific and Industrial Research Organisation (CSIRO) Plant Industry (Canberra, Australia).

Experimental treatments. Seeds sterilised with 70% ethanol for 1 min were germinated in the dark at 4 °C for 21 days on filter paper wetted with distilled water. Next, they were sown into pots (4 L) filled with soil classified as degraded chernozem. Cadmium was added as 3 $CdSO_4 \cdot 8\ H_2O$ salt. Cd dose of 3 mg/kg DW (dry weight) was used in the experiment as average soil pollution with Cd ions, while 5 mg/kg DW corresponded to the maximum Cd content (Tóth et al. 2016). After adding Cd at both concentrations, soil salinity did not exceed 0.2 mS. Each pot harboured five plants, while each treatment (genotype/Cd dose) involved 10 pots. The plants were grown until full seed ripening in an air-conditioned glasshouse at 22 °C/18 °C day/night, in daylight (March–May), supplemented with light intensity by AGRO Philips sodium lamps (Philips, Aache, Germany) of 400 µmol/m^2/s PPFD (photosynthetic photon flux density), up to a 16 h photoperiod. Relative humidity was 65%. The plants were fertilised with Hoagland medium (Hoagland and Arnon 1938) once a week.

Chlorophyll *a* fluorescence. Measurements involved the middle part of the flag leaf and the plant efficiency analyser (PEA) (Hansatech Ltd., King's Lynn, UK) was used. Excitation irradiance was 3 000 µmol/m^2/s (peak at 650 nm). The measurements were taken after 30 min of the leaf adaptation to darkness. The ChlF parameters were calculated based on the theory of energy flow in photosystem II (PSII) and JIP test as described by Strasser et al. (2000).

Gas exchange. The gas exchange was measured in the flag leaf. Net photosynthesis rate (P_n), transpiration rate (E), stomatal conductance (g_s) and internal CO_2 concentration (C_i) were analysed with an infrared gas analyser (CIRAS-1, Hansatech Ltd., King's Lynn, UK), with a Parkinson leaf chamber (PLC6). The irradiation system consisted of halogen lamps. The flow rate of air with constant CO_2 concentration (400 µmol CO_2/mol air) through the assimilation chamber was 300 cm^3/min. The temperature of the leaves was 25 °C, the air humidity was 40%, and the irradiance 800 µmol photon/m^2/s. The measurements involved 10 plants for each genotype/treatment.

Grain yield. Mature seeds were harvested and their number and dry weight per spike were calculated. Analyses were done in 25 replicates for each genotype/treatment.

Cd content in grains. The Cd content was determined as described by Ostrowska et al. (1991). The grains were dried in an air flow dryer at 65 °C for 48 h, weighed, and powdered. The milled samples were dried at 105 °C for determination of hygroscopic water and then they were mineralised at 450 °C for 12 h. The residue was dissolved in diluted nitric acid (acid:water ratio of 1:2; *v*/*v*). Cd content was determined using the ICP-OES method in Optima 7300DV apparatus (Perkin Elmer, Norwalk, USA). Reference material NCS DC73348 (China National Analysis Center for Iron & Steel, Beijing, China) was applied to each analytical series (Fuentes et al. 2004). Analyses were done in five replicates for each genotype/treatment.

Statistical analysis. The experiments were arranged in a fully randomised design. Two-way analysis of variance (ANOVA) and Duncan's multiple range test (at $P < 0.05$) were performed using the statistical package Statistica 13.0 (Stat-Soft, Inc., Tulsa, USA). Data were presented as means ± SE (standard error). Pearson's correlation coefficients were assumed as statistically significant at $P < 0.05$.

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RESULTS AND DISCUSSION

Cadmium affected all investigated ChlF parameters. The most evident changes in ChlF were noted in cv. Tamaroi the most sensitive to salinity. Both Cd levels significantly declined energy absorption (ABS/CS) in cv. Tamaroi and BC_5Nax_2, while in SMH87 this only occurred for 3 mg/kg variant (Figure 1A).

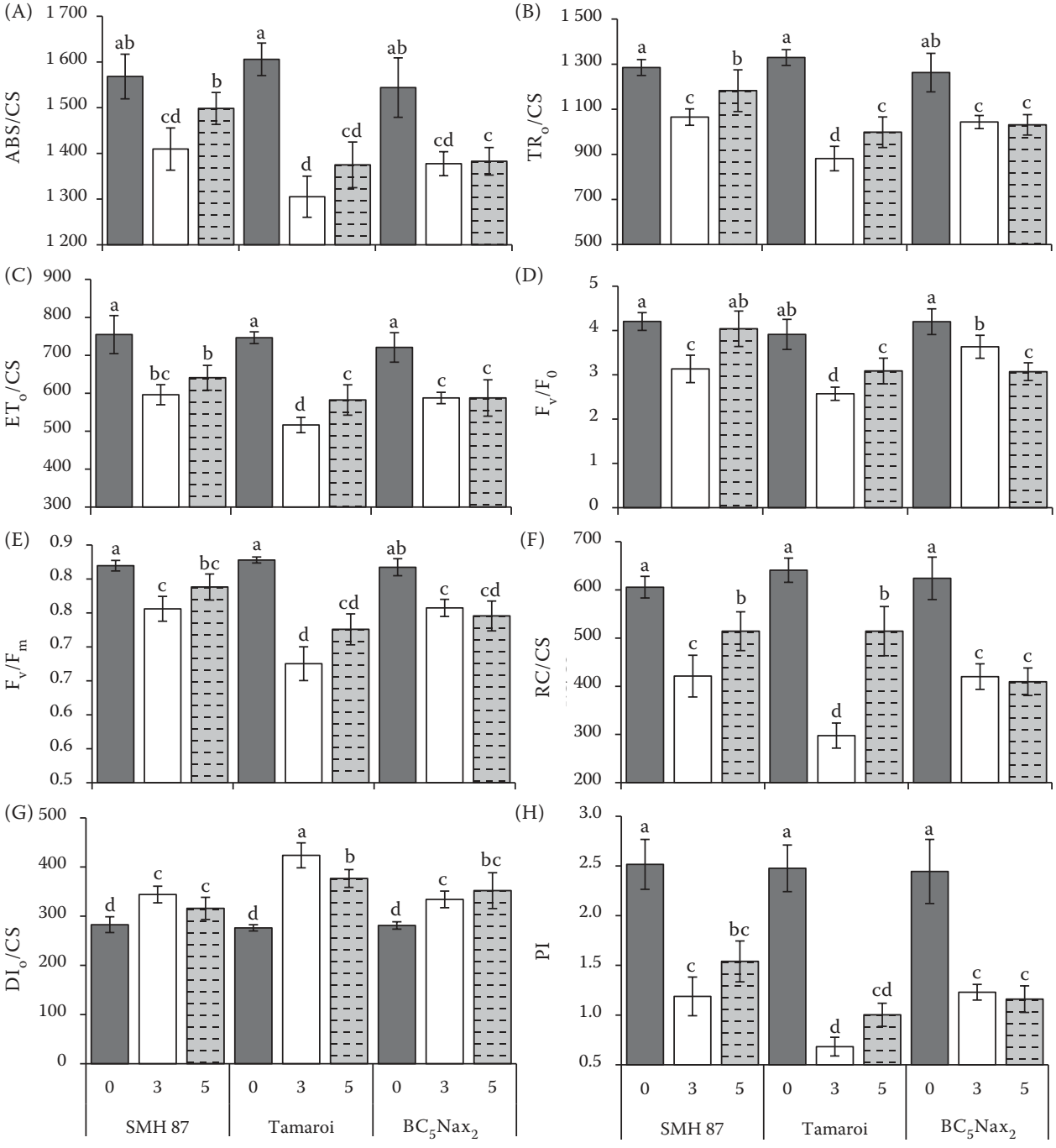

Figure 1. Changes in chlorophyll *a* fluorescence in three durum wheat genotypes grown in the soil with cadmium (Cd) at 0 mg (control), 3 mg/kg DW (dry weight) and 5 mg/kg DW. Values represent means (n =10) ± standard error. Different superscript letters indicate significant differences between means (Duncan's multiple range test; $P < 0.05$). (A) light energy absorbed by leaf cross-section (ABS/CS); (B) trapped energy flux (TR$_o$/CS); (C) quantum yield of photosynthetic electron transport chain (ET$_o$/CS); (D) maximum efficiency of water photodissociation (F$_v$/F$_0$); (E) potential photochemical PSII efficiency (F$_v$/F$_m$); (F) reaction centres (RC/CS); (G) dissipation energy flux (DI$_o$/CS), and (H) performance index (PI)

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A stream of energy resulting from the reduction of plastoquinone (TR_o/CS) thinned after Cd treatment in all plants (Figure 1B). This decrease may indicate partial inactivation of the active reaction centres (RC). Energy flux declined in all plants (Figure 1C), which is typical under stress and may be related to inactivation of RC and oxygen evolving complex. This effect was confirmed by changes in F_v/F_o, describing maximum efficiency of water photodissociation (Figure 1D). Potential photochemical efficiency (F_v/F_m) dropped in all plants at 3 Cd mg/kg DW, whereas in cv. Tamaroi and BC_5Nax_2 this effect occurred also at higher Cd dose (Figure 1E). The density of RC decreased under stressful conditions (Figure 1F). The strongest Cd-induced decline in RC/CS was observed in cv. Tamaroi. An increase in energy dissipation (DI_o/CS) noticed in all plants indicated a decrease in the energy necessary for photochemical transformations (Figure 1G). This effect was particularly pronounced in cv. Tamaroi plants. The strongest reduction of the photosynthetic performance index (PI) at both Cd doses was visible in cv. Tamaroi (Figure 1H). Negative effects of Cd observed by Paunov et al. (2018) in durum wheat also involved five-fold suppression of the efficiency of energy transformation in PSII, and a disruption in oxygen-evolving complex. This effect on ET_o/CS and F_v/F_0 was manifested mainly in cv. Tamaroi.

A decline in P_n occurred in SMH87 line at 5 mg Cd/kg DW and in BC_5Nax_2 line exposed to 3 mg Cd/kg DW (Figure 2A). In cv. Tamaroi no Cd effect on P_n was observed, although all ChlF parameters were clearly reduced in this cultivar. Transpiration rate decreased in all plants only at higher cadmium concentration (Figure 2B). Stomatal conductance dropped gradually along with increasing Cd dose in cv. Tamaroi and BC_5Nax_2, while in SMH87 the stomata were more closed in the plants exposed to 5 mg Cd/kg DW (Figure 2C). This caused a dramatic reduction of internal CO_2 concentration (C_i) (Figure 2D). Hart et al. (1998) stated that stomata closure is one of the mechanisms preventing Cd influx into the transpiration stream. Significant correlation was found between C_i and g_s and E and it amounted to $r = 0.824$ ($P < 0.05$) and $r = 0.794$ ($P < 0.05$), respectively, but C_i did not correlate with P_n. Harley and Sharkey (1991) reported that P_n may decline at high CO_2 concentration.

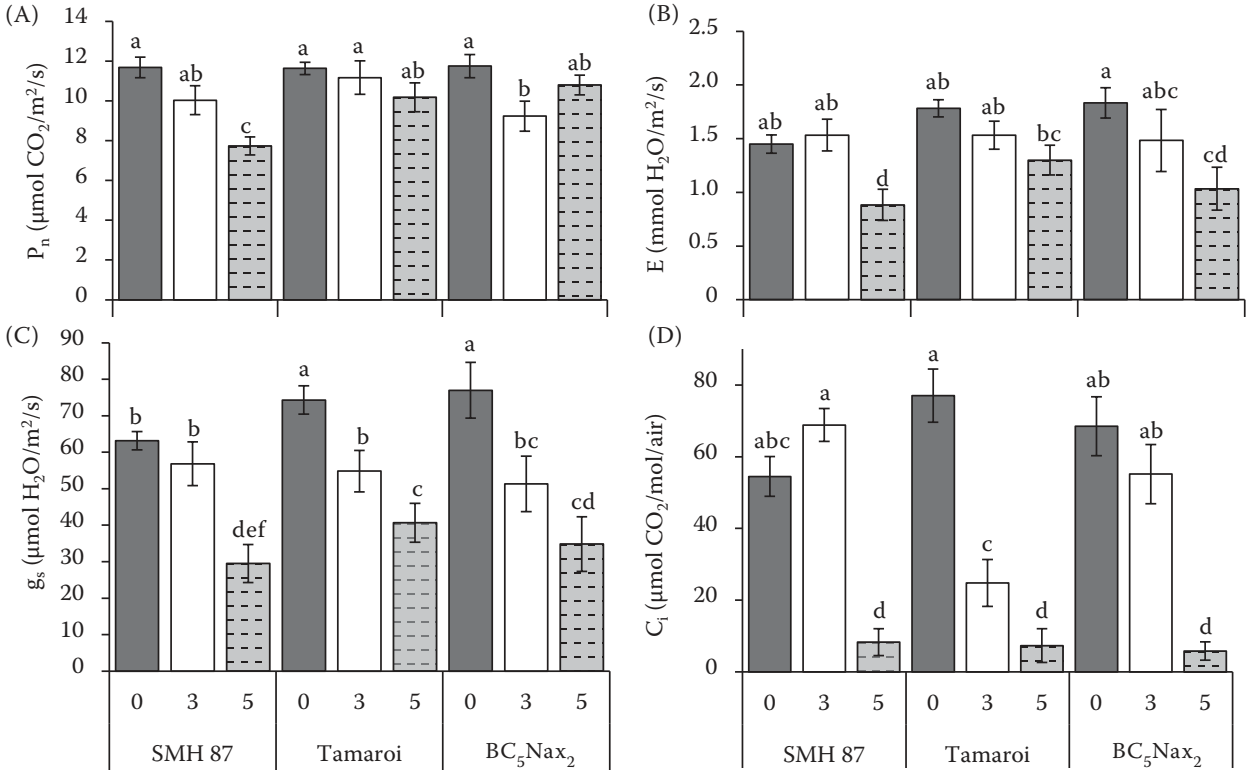

Figure 2. (A) Net photosynthesis (P_n); (B) transpiration rate (E); (C) stomatal conductance (g_s) and (D) internal CO_2 concentration (C_i) in the leaves of durum wheat genotypes grown in the soil with cadmium (Cd) at 0 mg (control), 3 mg/kg DW (dry weight) and 5 mg/kg DW. Values represent means ($n = 10$) ± standard error. Different superscript letters indicate significant differences between means (Duncan's multiple range test, $P < 0.05$)

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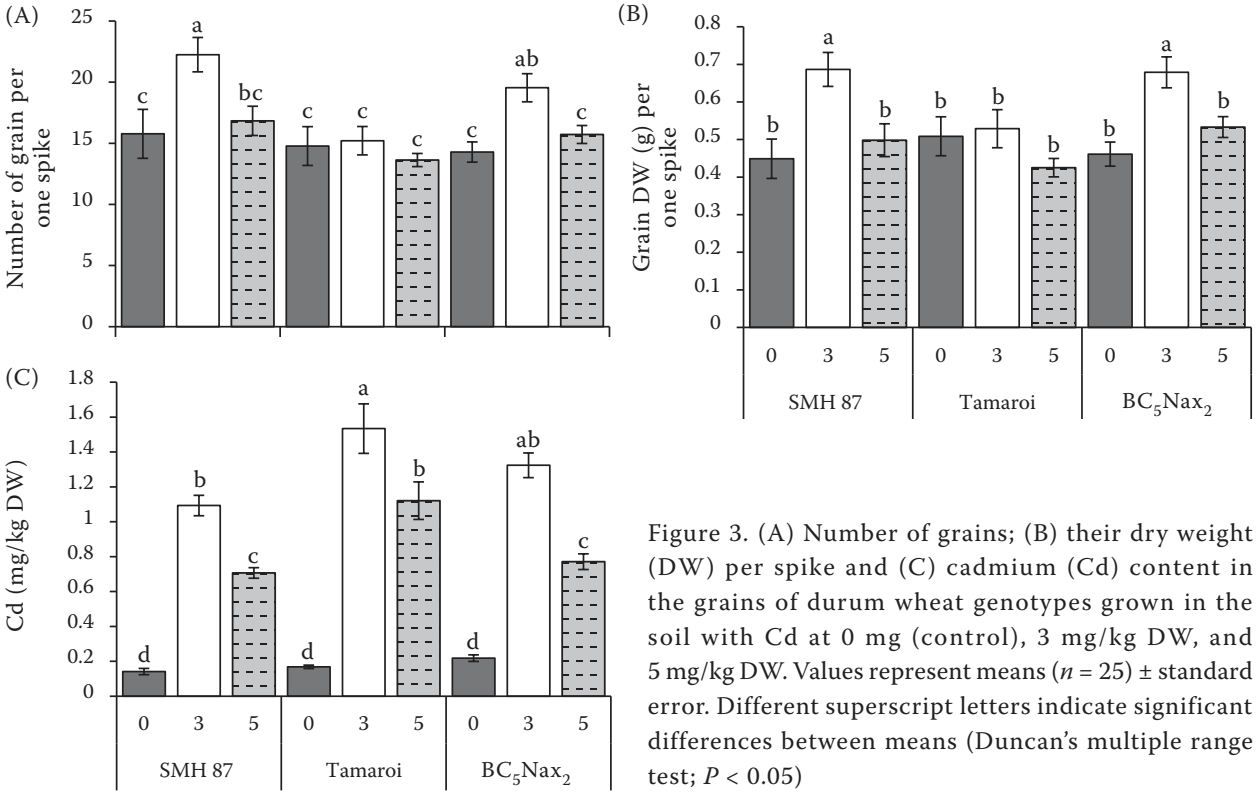

Figure 3. (A) Number of grains; (B) their dry weight (DW) per spike and (C) cadmium (Cd) content in the grains of durum wheat genotypes grown in the soil with Cd at 0 mg (control), 3 mg/kg DW, and 5 mg/kg DW. Values represent means (n = 25) ± standard error. Different superscript letters indicate significant differences between means (Duncan's multiple range test; $P < 0.05$)

Cadmium did not affect the yield of cv. Tamaroi (Figures 3A,B). The number of grains and their dry weight were the highest in SMH87 and BC_5Nax_2 lines grown at 3 mg Cd/kg DW, as compared with the control and the plants exposed to 5 mg Cd/kg DW. Moral et al. (1994) reported that Cd may provoke phytotoxic effects even at relatively low concentrations. In the studied plants, Cd content in the grains increased at both applied Cd doses *vs*. control (Figure 3C). The grains of the plants grown at 5 mg Cd/kg DW accumulated less Cd than those of the plants grown at 3 mg Cd/kg DW. Cv. Tamaroi grains accumulated greater Cd amounts at 3 mg Cd/kg DW as compared with SMH87, and greater Cd amounts at 5 mg Cd/kg DW than SMH87 and BC_5Nax_2 lines. Cadmium accumulation in the seeds correlated negatively with most of the studied ChlF parameters (Table 1). Moral et al. (1994) demonstrated lowered tomato yield in Cd presence but no changes in fresh weight of the fruit. Dai et al. (2017) revealed that the introduction of nutrients, namely nitrogen and sulphur, could be an important factor of wheat response to Cd. Coordination between N and S assimilation can strengthen plant defence mechanisms and effectively alleviate Cd negative effects (Khan et al. 2016). Gill et al. (2012) reported that high Cd concentrations in the soil disturb photosynthesis and coordination between carbon, nitrogen, and sulphur metabolism. On the other hand, *Fagopyrum tararicum* plants exogenously treated with sulphur increased Cd uptake in the root vacuoles (Lu et al. 2019). Decreased cadmium translocation to the leaves can result from its chelation and vacuolar sequestration by non-

Table 1. Correlation coefficients for cadmium (Cd) amount in the grains (mg/kg dry weight) and chlorophyll fluorescence (ChlF) parameters of all studied durum wheat plants grown in the presence of Cd

ChlF	Cd amount in grains
PI	0.588***
ABS/CS	0.446**
TR_o/CS	0.530***
ET_o/CS	0.448**
DI_o/CS	0.465***
RC/CS	0.577***

**$P < 0.01$; ***$P < 0.001$; PI – performance index; ABS/CS – energy absorption; TR_o/CS – reduction of plastoquinone; ET_o/CS – quantum yield of photosynthetic electron transport chain; DI_o/CS – energy dissipation; RC/CS – reaction centres

https://doi.org/10.17221/61/2020-PSE

protein thiols, and its inhibited transport from roots to shoots. We can assume also that higher Cd amount was toxic enough to block the transport of assimilates and ions to the seeds. Hart et al. (1998) showed that Cd uptake rates in xylem translocation to the shoots of durum wheat were not responsible for increased Cd accumulation in mature grains. Lachman et al. (2015) observed the effect of cadmium on essential elements transport into the plant. Especially, cadmium had an antagonistic influence of calcium and manganese. The adverse effects of heavy metal ions, including cadmium, are similar to the influence of the salinity caused by sodium and chlorine ions, on damage to cytoplasmic membranes and induction of osmotic stress. According to Munns and Tester (2008) osmotic stress evokes stronger effects than ionic stress. Both salinity and heavy metal ions cause cell dehydration, an increase in plasma membrane permeability, a decrease in photosynthesis rate and generation of oxidative stress, which evoke a decrease in yield (Munns and Tester 2008, Li et al. 2010, Kalaji et al. 2011). We showed a negative effect of salinity on the growth and the photosynthetic efficiency of *Miscanthus* × *giganteus* in previous work (Płażek et al. 2014). Moreover, our experience gained in research on the effects of high and low temperature indicates that the parameters of chlorophyll fluorescence strongly correlate with the yield obtained from plants grown under these stress conditions (Płażek et al. 2018, Hornyák et al. 2020). The photosynthetic apparatus is very sensitive to various environmental stresses, and therefore the plant's ability to maintain normal photosystems function correlates with the plant tolerance to unfavourable environmental conditions. Summing up, Cd accumulation was the highest in the grains of the salt sensitive cultivar. The decrease in the efficiency of the photosynthetic apparatus could play an important role in Cd deposition in the seeds. It may be supposed that the tolerance to Cd and NaCl stresses has a common physiological background.

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Research Article

Jakub Pastuszak*, Przemysław Kopeć, Agnieszka Płażek, Krzysztof Gondek, Anna Szczerba, Marta Hornyák, Franciszek Dubert

Antioxidant activity as a response to cadmium pollution in three durum wheat genotypes differing in salt-tolerance

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Abstract: Durum wheat is commonly used in various food industry industries and cultivated worldwide. A serious problem with the species cultivation is its capability to accumulate cadmium (Cd) in the grains. The aim of this study is to investigate whether antioxidant activity may be used as a marker of Cd tolerance in durum wheat. The experiment involved three durum wheat genotypes/lines differing in salt tolerance. The plant response to Cd was appraised based on the activity of ascorbate–glutathione (AsA–GSH) cycle enzymes, ascorbate-to-dehydroascorbate ratio, reduced-to-oxidized glutathione ratio (GSH:GSSG), as well as Cd content in the seeds. The highest activity of dehydroascorbate reductase, monodehydroascorbate reductase, and glutathione reductase was noted in control plants of salt-sensitive cultivar "Tamaroi." In the presence of Cd, activity of these enzymes was considerably reduced. "Tamaroi" plants demonstrated also the highest Cd content in the grain. In conclusion, we identified the cultivar "Tamaroi" as most susceptible to cadmium, and the level of durum wheat sensitivity to the element can be evaluated based on a significant decrease in the activity of AsA–GSH cycle enzymes and GSH:GSSG ratio.

Keywords: antioxidant enzymes, ascorbate–glutathione cycle, heavy metal stress, cadmium, durum wheat

*** Corresponding author: Jakub Pastuszak,** Department of Plant Breeding, Physiology and Seed Science, University of Agriculture, ul. Podłużna 3, 30-239 Kraków, Poland,
e-mail: jakub.pastuszak@urk.edu.pl
Przemysław Kopeć, Franciszek Dubert: Department of Cell Biology, The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, ul. Niezapominajek 21, 30-239 Kraków, Poland
Agnieszka Płażek, Anna Szczerba, Marta Hornyák: Department of Plant Breeding, Physiology and Seed Science, University of Agriculture, ul. Podłużna 3, 30-239 Kraków, Poland
Krzysztof Gondek: Department of Agriculture and Environmental Chemistry, University of Agriculture, Al. A. Mickiewicza 21, 31-120 Kraków, Poland

1 Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is one of the cereals most commonly used in the food industry and cultivated around the world [1]. The species is highly valued for its grain protein content, especially gliadin and gluten, high fiber content, low glycemic index, and high levels of vitamins and valuable micronutrients [2,3]. Tolerance to salinity is a crucial factor in durum wheat cultivation [4]. Salinity tolerance mechanisms in plants causes excretion of salt ions, control of ion uptake by roots and their transport to the leaves, and activation of the antioxidant system [5]. Australian researchers identified two loci, *Nax1* and *Nax2*, in *Triticum monococcum*, with genes responsible for the excretion of sodium ions from xylem, and consequently limited the accumulation of Na^+ in the leaves. A cross with a salt-sensitive cultivar "Tamaroi" produced a new line containing these genes and tolerant to high salt concentrations [4]. Durum wheat accumulates large amounts of cadmium (Cd) ions in the grains [6]. The European Union has proposed a limit of Cd concentration in food that should not exceed 0.2 mg kg^{-1} fresh weight of a product [7]. In humans, even small amounts of Cd can be toxic and cause permanent organ damage [8,9]. Crop plants growing at a higher content of Cd show many physiological disorders, such as inhibition of seed germination and plant growth [10], leaf rolling, chlorosis and necrosis [11], disturbed distribution of nutrients [12], reduction of photosynthesis efficiency and chlorophyll content, and imbalance of water uptake and stomatal closure [13,14]. Zook et al. [15]

and Jalil et al. [16] notified that durum wheat plants are more effective accumulators of Cd in the grain than *Triticum aestivum* L. They reported significant disparities between species and cultivars in the amount of absorbed Cd content and their tolerance to its poisonous effects. Some cultivars of durum wheat have a genetically determined potential for accumulation of significant amounts of Cd [17]. For example, one of the major genes in *Cdu1* locus is responsible for Cd tolerance of durum wheat [18–21]. Hart et al. [22] reported that the Cd level in the grain was regulated not only by genetic propensity but also by physiological factors including Cd absorption from the soil solution, ion transport from root to shoot, Cd sequestration in subcellular compartments, and phloem movement into the grain during fruit development. The analysis of Cd accumulation mechanisms should account for the strong influence of soil properties on the content of bioavailable forms of this element [23]. To become tolerant to Cd toxicity, plants have developed a number of protective mechanisms, including higher activity of enzymatic and nonenzymatic antioxidants [24], adjustment of the influx and efflux of heavy metals [25], and regulation of the levels of heavy metal chelators, phytochelatins, and metallothioneins [26]. Cd does not participate in redox reactions but triggers overproduction of reactive oxygen species (ROS), including H_2O_2 [27]. Cd increases lipid peroxidation, protein oxidation, and nucleic acid oxidation. Also, Cd inactivates several enzymes by binding with their sulfhydryl groups (–SH) and increases free Fe concentration by its replacement with various proteins [28,29]. Some studies reported that exposure to Cd induces specific alterations in the mitochondrial structure and function in animals [30]. Tolerance to toxic Cd content depends on plant capacity to scavenge or detoxify activated oxygen species. The key molecules in these processes are glutathione (GSH) and ascorbate (AsA), i.e., nonenzymatic antioxidants. They are components of the cellular antioxidant defense system, i.e., the ascorbate–glutathione cycle (AsA–GSH cycle) [31,32]. They act as cofactors for numerous enzymes and signaling molecules regulating pivotal cellular processes. Ascorbate reacts with singlet oxygen, hydrogen peroxide, superoxide, and hydroxyl radicals. Glutathione is a tripeptide with a sulfhydryl group (L-γ-glutamyl-L-cysteinyl-glycine) and is recognized as a key molecule in the detoxification system. The fundamental function of GSH consists of thiol-disulphide interactions, in which reduced glutathione (GSH) is continuously oxidized to a disulphide form (GSSG) [31]. The AsA–GSH pathway comprises four enzymes: ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [33]. According to Hasanuzzaman et al. [34], all enzymes of the ascorbate–glutathione cycle work closely together, which may additionally improve Cd tolerance. Figure 1 shows a simplified scheme of the ascorbate–glutathione cycle.

Our hypothesis was that the degree of durum wheat tolerance to salinity caused by NaCl coincides with the tolerance to Cd and that the antioxidant activity of AsA-GSH cycle maybe a marker of this tolerance. We compared the response to soil contamination with Cd applied at 3 and 5 mg kg^{-1} DM (dry matter) in three genotypes of durum wheat: Polish line SMH87 with moderate tolerance to salinity stress, Australian NaCl-sensitive cultivar "Tamaroi," and NaCl-resistant line BC_5Nax_2. The defense response of the studied durum wheat genotypes included evaluation of AsA–GSH cycle enzyme activity, ascorbate-to-dehydroascorbate ratio, reduced-to-oxidized glutathione ratio, and Cd content in the seeds.

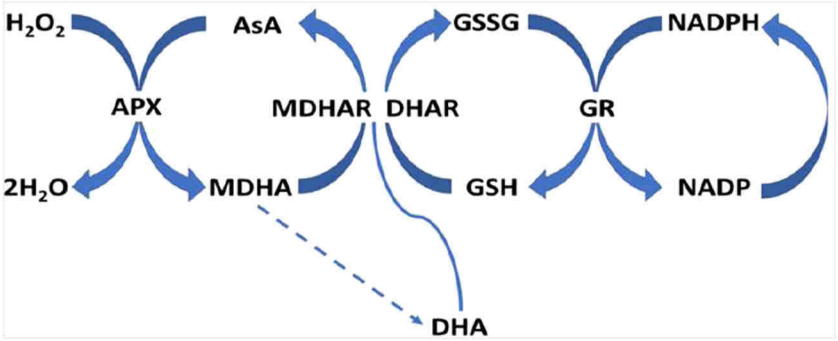

Figure 1: Schematic representation of antioxidant enzymes in the ascorbate–glutathione cycle investigated in this study (according to Szymańska and Strzałka [35]).

2 Materials and methods

2.1 Plant material

This study involved three genotypes of spring durum wheat. SMH87 line was obtained from Dr Jarosław Bojarczuk from Plant Breeding Center in Smolice, Plant Breeding and Acclimatization Institute Group (Poland). In our preliminary study, we identified SMH87 as moderately tolerant to salinity. BC_5Nax_2 line and cultivar "Tamaroi" were obtained from Dr Richard A. James from CSIRO Plant Industry (Australia). The Australian genotypes of durum wheat differed in their salt tolerance: cultivar "Tamaroi" was sensitive, while BC_5Nax_2, containing *Nax2* locus with salt tolerance genes, was tolerant to salt stress.

2.2 Experimental treatments

Seeds were sterilized with 70% ethanol for 1 min and placed in Petri dishes (Ø = 9 cm) on filter paper wetted with distilled water. The seeds germinated in the dark at 4°C for 21 days. Next, they were sown into pots (4 dm^3) filled with soil classified as degraded chernozem, formed from loess, the first soil quality class of very good wheat complex. Preliminary analysis of the soil used in the experiment revealed the presence of Cd ions at a concentration of 0.43 mg Cd kg^{-1} DM of soil. The Cd content in the soil was determined according to Baran et al. [36]. Based on data published by Tóth et al. [37], a dose of 3 mg Cd kg^{-1} DM was used in the experiment as average soil pollution with Cd ions, while a dose of 5 mg Cd kg^{-1} DM of soil corresponded to the maximum Cd content determined in Europe. The germinating seeds were sown into the soil contaminated with 3 $CdSO_4$ 8 H_2O at three concentrations: 0 (control), 3, and 5 mg of pure Cd per 1 kg DM of soil. Cd salt was evenly distributed in the entire volume of the pot. After adding the salt at both concentrations, soil salinity did not exceed 0.2 mS cm^{-1}. Its conductivity was measured according to Płażek et al. [38]. Each pot harbored five seedlings. Each treatment (genotype/Cd dose) contained 10 pots. The plants were cultivated to full seed ripening phase in air-conditioned glasshouse at 22 ± 3°C/18 ± 1°C day/night, in daylight (March–May) supplemented with light intensity (AGRO Philips sodium lamps) of 400 µmol m^{-2} s^{-2} PPFD (photosynthetic photon flux density), up to a 16 h photoperiod. Relative humidity was 65 ± 2%/75 ± 1% day/night. The plants were fertilized with Hoagland medium [39] once a week to ensure proper nutrition. The experiment was performed twice in 2018 and 2019, and the data presented are the means of the results obtained.

2.3 Measurements

2.3.1 Hydrogen peroxide assay

Hydrogen peroxide in flag leaf material was determined using the Amplex Red Hydrogen Peroxide/Peroxidase Assay kit of Invitrogen (Oregon, USA). Leaf material (0.1 g) was homogenized in 0.5 cm^3 of 50 mM potassium phosphate buffer (pH 7.5). The homogenates were centrifuged (19,000 *g*, 10 min, 4°C), and H_2O_2 concentration was measured colorimetrically in the supernatant according to the manufacturer's protocol. Hydrogen peroxide content was calculated from the standard curve prepared with H_2O_2 solutions. Results are expressed in µM H_2O_2 g^{-1} fresh weight. The measurements were taken in five replicates for each genotype/treatment with using Synergy 2 Microplate Reader (BioTek, USA).

2.3.2 Antioxidant assays

The concentration of reduced AsA, DHA, reduced (GSH) and oxidized (GSSG) glutathione, and the activity of APX, DHAR, and GR in flag leaves were measured spectrophotometrically as described by Harrach et al. [40]. The activity of MDHAR was determined according to Hossain et al. [41]. All assays were performed at 25°C using the Ultrospec 2100 pro UV/visible spectrophotometer (Amersham, Umeå, Sweden). The measurements were taken in five replicates for each genotype/treatment.

2.3.2.1 Low-molecular-weight antioxidant assays

For determination of low-molecular-weight antioxidant content, flag leaf material (0.1 mg) was homogenized in 0.5 cm^3 of 5% (w/v) metaphosphoric acid at 4°C and centrifuged (19,000 *g*, 30 min, 4°C). The supernatant was used for the assays. The content of AsA was detected as described by Foyer et al. [42] using ascorbate oxidase. Metaphosphoric acid extracts (125 µL) were neutralized with 25 µL of 1.5 M triethanolamine. The reaction mixture contained 133.3 µL of 150 mM sodium phosphate buffer (pH 7.4), 66.7 µL of H_2O, 2 cm^3 of 100 mM sodium

phosphate buffer (pH 5.6), and 1 unit of ascorbate oxidase. The extinction was measured immediately at 265 nm after the preparation of solution, and then, there was a decrease in the absorbance. The level of DHA was calculated as a difference between total ascorbate and AsA according to Harrach et al. [40]. Total ascorbate was determined after a reduction of DHA with dithiothreitol. Neutralized leaf extracts (45 µL) with 54 µL of 150 mM sodium phosphate buffer (pH 7.4) and 27 µL of 10 mM dithiothreitol were incubated for 15 min at room temperature. Total ascorbate levels were measured as mentioned earlier. The standard curve was created by known concentrations of AsA and DHA prepared in 5% metaphosphoric acid. GSH and GSSG were determined by the recycling method, using GR, according to Law et al. [43]. Metaphosphoric acid extracts (100 µL) were neutralized with 36 µL of 1 M triethanolamine. Initially, total glutathione was estimated, and then, to determine GSSG, GSH was derivatized with 2-vinylopyridine to the neutralized samples. The amount of GSH was estimated as a difference between these two assays. To determine total glutathione content, we prepared 1 cm^3 of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.5), 2.5 mM EDTA-Na_2, 1 mM 5,5′-dithio-bis (2-nitrobenzoic acid), 1 unit of GR, 0.2 mM NADPH, and 20 µL of the neutralized sample. Absorbance increases at 412 nm after the addition of GR and NADPH. Before measuring GSSG concentration, the neutralized samples were mixed with 8 µL of 2-vinylopyridine and incubated at 25°C for 1 h. Oxidized glutathione was determined as described earlier, using a 50 µL sample and the reaction mixture of a total volume of 1 cm^3. Total glutathione and GSSG content were estimated based on a standard curve generated with stock solutions of GSH and GSSG prepared in 5% metaphosphoric acid.

2.3.2.2 Antioxidant enzyme activity assays

For the detection of AsA–GSH cycle enzyme activity, flag leaf material (0.1 mg) was homogenized at 4°C in 0.5 cm^3 50 mM Tris–HCl buffer (pH 7.8) containing 1 mM EDTA-Na_2 and 7.5% (w/v) soluble polyvinylpyrrolidone. The suspension was centrifuged (12,000 *g*, 20 min, 4°C), and the supernatant was used to measure the total soluble enzyme activity. The APX activity was determined by following the oxidation of ascorbic acid at 290 nm (extinction coefficient of ascorbic acid was 2.8 $mM^{-1}\,cm^{-1}$) according to Nakano and Asada [44]. The reaction mixture (2.25 cm^3) consisted of 2 cm^3 of 50 mM Tris–HCl buffer (pH 7.8), 100 µL of 5.7 mM ascorbic acid, 100 µL of 11.25 mM H_2O_2, and 50 µL of the leaf extract. The control reaction was performed using the buffer instead of H_2O_2 solution. Results of the APX activity are expressed in nMAsA mg^{-1} protein min^{-1}. The DHAR activity was estimated by following the reduction of DHA at 265 nm (extinction coefficient of ascorbic acid was 14 $mM^{-1}\,cm^{-1}$), as described by Klapheck et al. [45]. The assay mixture contained 2 cm^3 of 50 mM sodium phosphate buffer (pH 7.5) with 2.5 mM EDTA-Na_2, 100 µL of 22.8 mM GSH, 100 µL of 11.5 mM DHA, and 100 µL of the leaf extract. The control reaction mixtures contained the buffer instead of the supernatant. Results of the DHAR activity are expressed in nM AsA mg^{-1} protein min^{-1}. The measurement of the MDHAR activity was based on monitoring the consumption of NADH at 340 nm (extinction coefficient of NADH was 6.2 $mM^{-1}\,cm^{-1}$). The reaction mix consisted of 2 cm^3 of 50 mM Tris–HCl buffer (pH 7.8), 100 µL of 22.7 mM ascorbic acid, 100 µL of 2.6 mM NADH, 6.6 units of ascorbate oxidase, and 100 µL of the leaf extract. The control reaction mix contained the buffer instead of ascorbate oxidase. Results of the MDHAR activity are expressed in nM NADH mg^{-1} protein min^{-1}. The GR activity was assayed by the decrease in absorbance at 340 nm due to the oxidation of NADPH (extinction coefficient of NADPH was 6.2 $mM^{-1}\,cm^{-1}$) according to Klapheck et al. [45]. The reaction mixture contained 2 cm^3 of 50 mM Tris–HCl buffer (pH 7.8), 100 µL of 2.4 mM NADPH, 300 µL of 4.6 mM GSSG, and 100 µL of the leaf extract. The control reaction was performed with the buffer instead of GSSG solution. Results of the GR activity are expressed in nM NADPH mg^{-1} protein min^{-1}.

2.3.3 Determination of Cd content in the grains

The content of Cd was determined as described by Ostrowska et al. [46]. The grains were collected from plants in each treatment and dried separately in an air flow dryer at 65°C for 48 h, weighed, and powdered in a ball mill MM400 (Retsch, Haan, Germany). For determination of hygroscopic water, the ground samples were dried at 105°C and later mineralized in a chamber furnace at 450°C for 12 h. The residue was dissolved in diluted nitric acid (acid:water ratio of 1:2; v/v). The content of the element was determined using the ICP-OES method in PerkinElmer Optima 7300DV apparatus (Norwalk, CT, USA). Reference material NCS DC73348 (China National Analysis Center for Iron & Steel) was applied to each analytical series as described by Fuentes et al. [47]. Analyses of chemical element content were done in five replicates for each Cd treatment/wheat line.

2.3.4 Statistical analysis

Two-way analysis of variance and Duncan's multiple range test (at $P < 0.05$) were performed using the statistical software of STATISTICA 13.0 (Stat-Soft, Inc., Tulsa, OK, USA). Data were represented as means ± SE (standard error), and linear correlation coefficients (Pearson's) were putative as statistically significant at $P < 0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Visual symptoms of Cd treatment

All cultivated plants showed no symptoms of Cd toxicity such as necrosis or leaf rolling; however, Cd accelerated seed maturation and early plant drying (Figure 2). In some cases, the plants grown in soil contaminated with 3 mg Cd kg^{-1} DM, and even with the higher dose showed greater vigor than control plants. This effect was most evident before flowering. Most often, however, the observed differences between plants resulted from their genotypic diversity.

Our observations showed that plants growing in soil contaminated with 5 mg kg^{-1} DM Cd generally did not differ in appearance from those grown at 3 mg kg^{-1} DM Cd, and even some of them looked more viable. It is difficult to unambiguously explain this phenomenon. It might be due to so-called hormesis effect. Hormesis involves stimulation of various parameters in living organisms by stress factors of low intensity [36,48]. The effect was reported in plants treated with low concentrations of heavy metals [49]. Moral et al. [12] proved lower tomato yield during Cd presence but no differences in mean fresh weight of the fruit. However, these authors stated that Cd negatively affected the plant growth and root and stem length, and also fresh weight decreased with increasing concentrations of Cd. An analysis of Cd accumulation mechanisms should account for a strong influence of soil properties on the content of bioavailable forms of this element [23]. The study by Dai et al. [23] revealed that the introduction of nutrients, namely, nitrogen and sulfur, could be a significant factor determining wheat response to Cd contamination in the soil. It should be noted that by increasing the dose of Cd used as sulfate, the dose of sulfur also increased. Khan et al. [50] stated that coordination between the main N and S assimilation pathways can strengthen plant defense mechanisms and effectively alleviate Cd negative effects. Gill et al. [51] notified that high Cd content in soil affects photosynthesis process and alignment between carbon, nitrogen, and sulfur metabolism.

3.2 Hydrogen peroxide content

Hydrogen peroxide production under Cd stress was specific for each studied genotype (Figure 3). The lowest level of this compound was recorded in control plants of SMH87, and its content in the leaves gradually increases with an increase in Cd dose. "Tamaroi" control plants produced the highest amount of H_2O_2, and contrary to

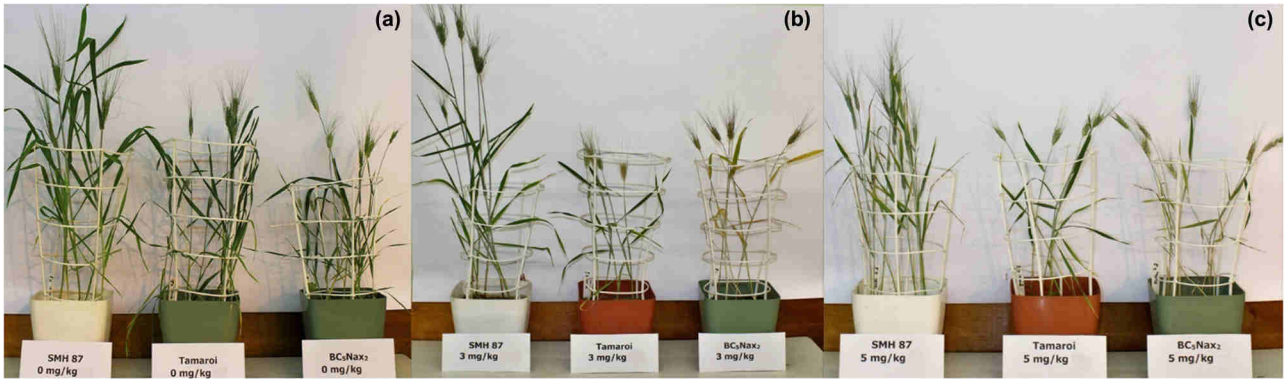

Figure 2: Plants of the studied genotypes, in the heading phase, growing in the soil without Cd (control) (a) and in the soil contaminated with 3 mg Cd kg^{-1} DM (b) and 5 mg Cd kg^{-1} DM (c).

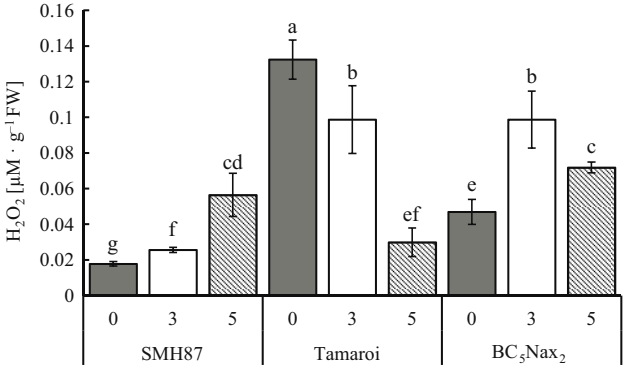

Figure 3: Hydrogen peroxide level in the flag leaves of three durum wheat genotypes grown in the soil contaminated with Cd at 0 mg (control), 3 and 5 mg kg^{-1} DM. Data are represented as mean ± SE in five replicates, which are significantly different at $P < 0.05$ using Duncan multiple range test.

SMH87 and BC_5Nax_2, growing Cd pressure reduced its content. This response of "Tamaroi" to Cd depended on the decrease in APX, GR, and MDHAR activities noted in plants of this cultivar (Figure 4a–c). In BC_5Nax_2 line, 3 mg Cd kg^{-1} DM enhanced the hydrogen peroxide amount, while higher metal dose reduced its level, which was still higher than that of the control.

Cd is not a redox metal, and it cannot catalyze Fenton reaction that affects the ROS production in plant cells. Higher H_2O_2 level noticed in plants of SMH87 and BC_5Nax_2 lines cultivated in contaminated soil presumably results from the decreased H_2O_2 scavenging rate or the increased H_2O_2 synthesis in enzymatic or nonenzymatic reactions. Hydrogen peroxide production might be associated with cellular integration processes and/or adaptation to environmental conditions [52]. Sarker and Oba [53] detected very low amounts of hydrogen peroxide in drought-sensitive genotype of *Amaranthus* sp. versus more tolerant genotypes. As described further in the article, salt-sensitive "Tamaroi" was recognized as considerably more sensitive to Cd ions than SMH87 and BC_5Nax_2, so the reduction in hydrogen peroxide under Cd stress may be a marker of plant sensitivity to various environmental stresses. Similar

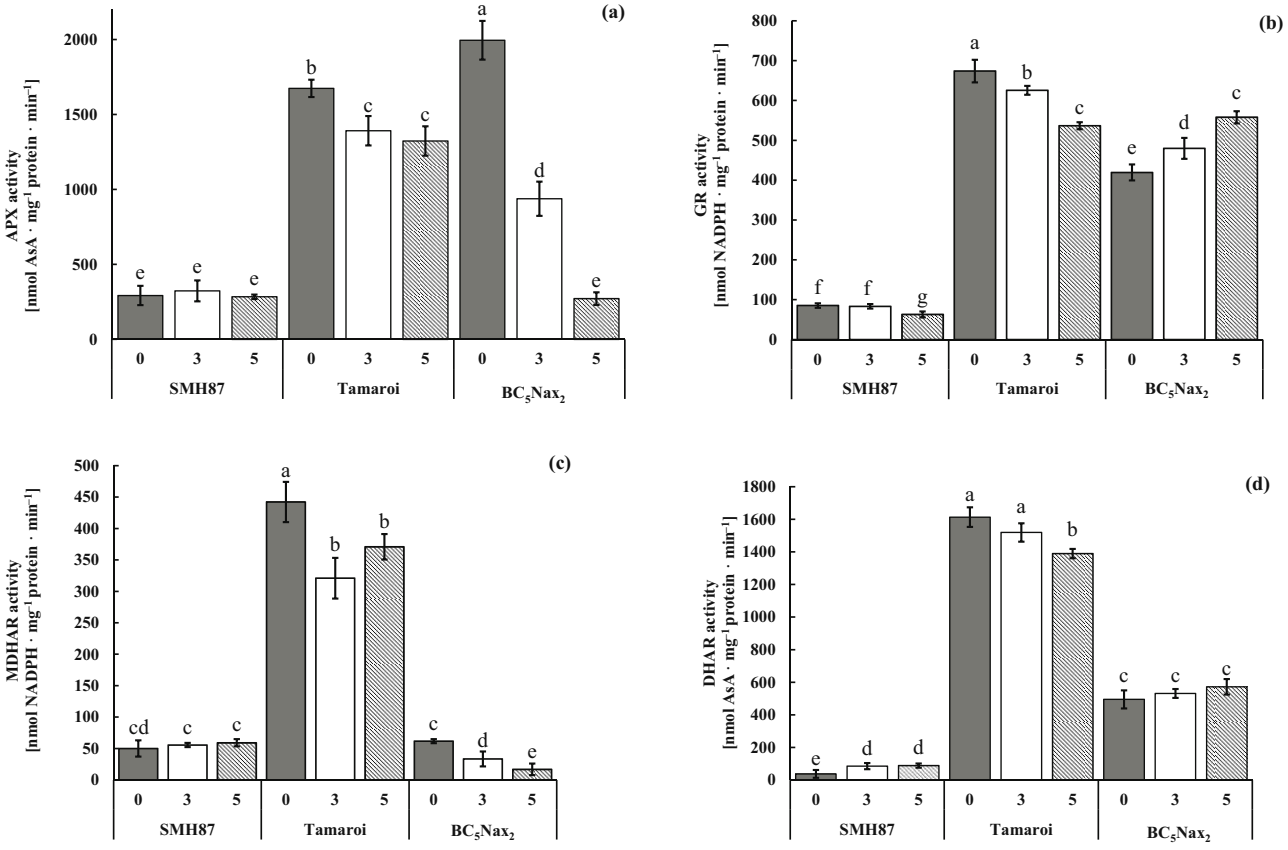

Figure 4: Activity of ascorbate–glutathione cycle enzymes in the flag leaves of three durum wheat genotypes grown in the soil contaminated with Cd at 0 mg (control), 3 and 5 mg kg^{-1} DM. Data are represented as mean ± SE in five replicates. Means with different letters (a, b, c,…) are significantly different at $P < 0.05$ using the Duncan multiple range test.

results were reported by Płażek and Żur [54]. These authors concluded that crop resistance to pathogens depended on low activity of catalase and high amount of hydrogen peroxide.

3.3 Enzyme activity

The lowest APX activity was recorded in SMH87 plants, and it was unaffected by increasing Cd concentration (Figure 4a). "Tamaroi" plants showed a decrease (by 21%) in the APX activity under both Cd concentrations. The highest APX activity was detected in the leaves of BC_5Nax_2 line, and it declined rapidly (by about 86%) with the increasing Cd amount in the soil.

The pattern of the GR activity was specific for each studied genotype (Figure 4b). It was the lowest in SMH87 and did not change in the presence of 3 mg Cd kg^{-1} DM but dropped at 5 mg Cd kg^{-1} DM. Very high GR activity in control "Tamaroi" plants gradually decreased in the plants grown in the contaminated soil. Only in BC_5Nax_2 plants, the increasing Cd amount enhanced the GR activity. The MDHAR activity was the highest in "Tamaroi" plants, while in SMH87 and BC_5Nax_2 plants, it was considerably lower (Figure 4c). Cd inhibited the MDHAR activity in "Tamaroi" and BC_5Nax_2 plants, while in SMH87 line, it remained unaffected. Cd contamination did not influence the DHAR activity in SMH87 and BC_5Nax_2 lines, while in "Tamaroi," we saw a decline in the activity of DHAR only at 5 mg Cd kg^{-1} DM (Figure 4d). Similarly, as for the highest MDHAR activity, the highest activity of DHAR was observed in "Tamaroi" plants. Figure 5a presents ascorbate-to-dehydroascorbate ratio (AsA:DHA). Predominance of ascorbic acid over its oxidized form was visible mainly in control plants of SMH87 and BC_5Nax_2 lines, while in "Tamaroi" control, DHA level was significantly higher than that of AsA. The AsA:DHA ratio decreased in all plants exposed to Cd, and a particularly strong response was noticed in both Australian genotypes. The ratio depended strongly on APX and DHAR activities (Figure 4a and d).

Similarly as for GR, the ratio of reduced to oxidized glutathione (GSH:GSSG) was specific for each studied genotype (Figure 5b). In SMH87 plants, the ratio increased at both applied Cd levels compared with that of the control. Control plants of cv. "Tamaroi" exhibited the highest GSH:GSSG ratio that rapidly (by 75%) declined in plants grown in the soil containing 3 mg Cd kg^{-1} DM. Higher Cd dose boosted GSH amount and in consequence GSH:GSSG ratio. In the case of BC_5Nax_2, only 5 mg Cd kg^{-1} DM increased the ratio, which indicated an increase in GSSG accumulation. The quantity of hydrogen peroxide correlated positively with the activity of all studied enzymes and with GSH:GSSG ratio, while the latter correlated only with the quantity of H_2O_2 amount (Table 1). GR activity correlated positively with APX, DHAR, and MDHAR activities. Sarker and Oba [53] observed a minute increase in ascorbate–glutathione content, ascorbate–glutathione redox, and ascorbate–glutathione cycle enzyme activities, which correlated with dramatic increment in hydrogen peroxide in drought-sensitive genotype of *Amaranthus tricolor*.

Linear correlation coefficients (Pearson's) were assumed statistically significant at $P < 0.05$ (ns – not statistically significant).

According to Cuypers et al. [30], Cd can induce oxidative stress by inhibiting antioxidants, but it also

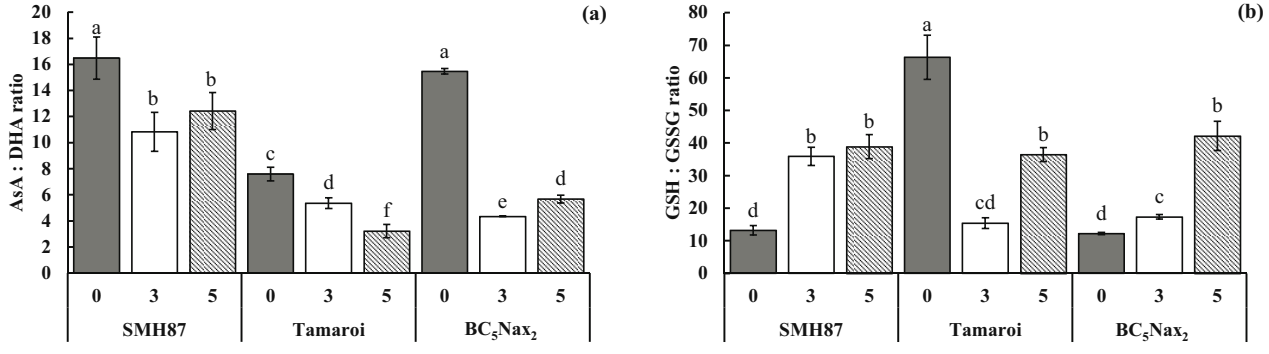

Figure 5: Ascorbate (AsA)-to-dehydroascorbate (DHA) ratio (a) and reduced-to-oxidized glutathione ratio (GSH:GSSG) (b) in the flag leaves of three durum wheat genotypes grown in the soil contaminated with Cd at 0 mg (control), 3 mg kg^{-1} DM and 5 mg kg^{-1} DM. Data are represented as mean ± SE in five replicates. Means with different letters (a, b, c,…) are significantly different at $P < 0.05$ using the Duncan multiple range test.

Table 1: Correlation between the activity of enzymes involved in the ascorbate–glutathione cycle, GR, reduced-to-oxidized glutathione ratio (GSH:GSSG), and hydrogen peroxide (H_2O_2) determined in all studied durum wheat plants grown under Cd pollution

Variable	GR	GSH:GSSG	H_2O_2
APX	0.711	ns	0.463
DHAR	0.877	ns	0.593
MDHAR	0.607	ns	0.381
GR	—	ns	0.685
GSH:GSSG	ns	—	0.385
H_2O_2	0.686	0.385	—

activates several antioxidant compounds. In the present experiment, the investigated genotypes showed considerable differences in Cd-induced response. The activity of individual enzymes of ascorbate–glutathione cycle and AsA:DHA and GSH:GSSG ratios were specific for each genotype. In the case of control "Tamaroi" plants, the activity of most studied enzymes was many times higher than in the remaining genotypes. Also, these plants responded to the increasing Cd content by a reduction of APX, MDHAR, DHAR, and GR activities. This effect was not visible or was considerably weaker in SMH87 and BC_5Nax_2 lines. Particular attention should be paid to the activity of ascorbate peroxidase that occurs in chloroplasts and cytoplasm. Hydrogen peroxide in chloroplasts is mainly removed by APX. In chloroplast stroma, APX concentration is about 37 μM, which is a high value for an enzyme [55]. In our experiments, the highest APX activity was noted in control plants of BC_5Nax_2; however, increasing doses of Cd in this line severely decreased the enzyme activity by up to 87%, while in "Tamaroi," it dropped by only 21%. Large differences in the APX activity in the plants treated with Cd were additionally emphasized by the response of SMH87 plants in which no changes in the activity of this enzyme were observed. In "Tamaroi," both Cd doses drastically decreased glutathione pool, while a reverse pattern developed in SMH87 and BC_5Nax_2 lines. The response to Cd pollution in salt-resistant BC_5Nax_2 seemed more similar to moderately salt-tolerant SMH87 and differed from that of salt-sensitive "Tamaroi." Considering the results described earlier, it could be concluded that significant inhibition of ascorbate–glutathione cycle activity may be a marker of durum wheat sensitivity to Cd. Our findings also suggest that the most salt-sensitive cultivar is also the most sensitive to Cd contamination. This result was confirmed by the analyses of Cd accumulation in the grains. The Cd content was the highest in the grains of the salt-sensitive cultivar and the lowest in the lines moderately tolerant and durable to salinity. The results of our study did not confirm previous reports [31,32] that tolerance to the toxic effects of Cd ions depends on the increased activity of the AsA–GSH cycle enzymes and nonenzymatic low-molecular antioxidants like ascorbic acid. Conversely, the more Cd tolerant cultivars showed a quantitative advantage of glutathione over oxidized glutathione.

3.4 Cd content in the grains

In all studied plants, Cd content in the seeds increased significantly at both applied Cd doses compared with the control (Figure 6). The grain of all plants grown at 5 mg Cd kg^{-1} DM are concentration less Cd than those produced by plants grown at 3 mg Cd kg^{-1} DM. Cd accumulation in the grains correlated only with the GSH:GSSG ratio and only for salt-sensitive "Tamaroi" ($r = -0.929$; $P < 0.05$) (Figure 7), while in the case of SMH87 and BC_5Nax_2, this correlation was insignificant.

Cd amount increased significantly in the seeds of all the studied genotypes exposed to the metal; however, the highest Cd ion content in the grain was demonstrated by plants growing at lower Cd concentration in the soil (3 mg kg^{-1} DM). This observation is difficult to explain. We can assume that higher Cd concentration (5 mg kg^{-1} DM) was toxic enough to block the processes involved in the transport of assimilates and ions to the

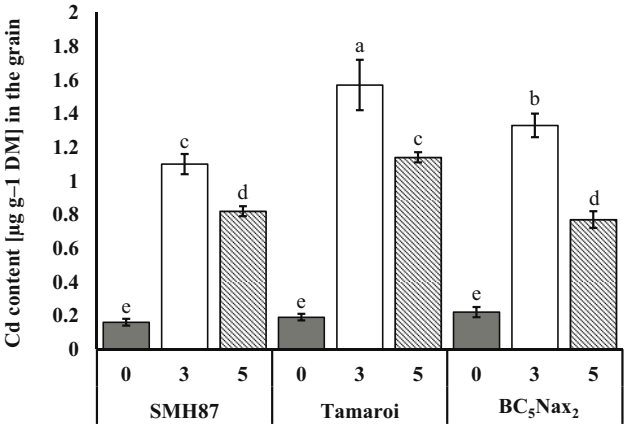

Figure 6: Cd content (μg g^{-1} DM) in the grains of three durum wheat genotypes grown in the soil contaminated with Cd at 0 mg (control), 3 and 5 mg kg^{-1} DM. Data are represented as mean ± SE in five replicates. Means with different letters (a, b, c,…) are significantly different at $P < 0.05$ using the Duncan multiple range test.

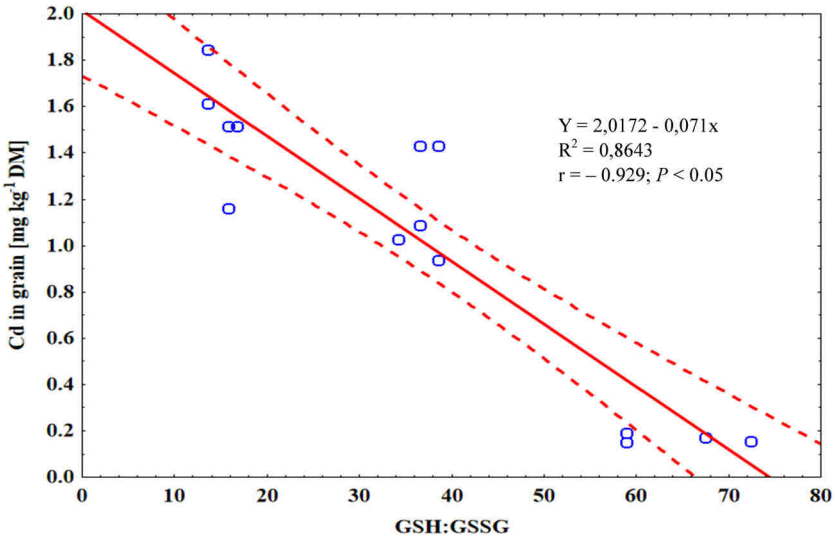

Figure 7: Correlation between Cd accumulation in the grains (mg kg^{-1} DM) and GSH:GSSG ratio in cv. "Tamaroi." Linear correlation coefficients (Pearson's) were assumed statistically significant at $P < 0.05$.

seeds. Lu et al. [56] observed a similar phenomenon in *Fagopyrum tataricum*. They stated that in plants exogenously treated with sulfur, increased Cd uptake in root vacuoles and its decreased translocation to the leaves can result from enhanced Cd binding by cell walls, chelation and vacuolar sequestration with nonprotein thiols, and inhibited transport of Cd from roots to shoots. Hart et al. [22] demonstrated that Cd uptake rates in the roots and xylem translocation to the shoots of durum wheat were not accountable for higher Cd accumulation in mature grains. Cd content in the grains correlated negatively with the GSH:GSSG ratio, which indicates that Cd accumulation is accompanied by intense oxidative stress, as evidenced by greater amount of oxidized form of glutathione. Cultivar "Tamaroi" is highly sensitive to both NaCl salinity and Cd pollution. This might indicate that tolerance to these stresses has a common physiological background. However, SMH87 line, which is similar to "Tamaroi," does not possess *Nax2* locus, demonstrated Cd tolerance at the level of BC_5Nax_2 line. Thus, it can be assumed that the NaCl-resistant genes in this locus do not contribute to Cd tolerance.

4 Conclusion

Durum wheat genotypes differed considerably in the activity of ASA–GSH cycle enzymes in the presence of Cd. Salt-sensitive cv. "Tamaroi" demonstrated the strongest Cd-induced decline in their activity and in the GSH:GSSG ratio. This cultivar accumulated also the highest amounts of Cd in the seeds compared with other genotypes under the study. We concluded that salt-sensitive "Tamaroi" was more susceptible, while moderately salt-tolerant SMH87 and salt-resistant BC_5Nax_2 were more tolerant to Cd contamination. The study showed that the inhibited activity of AsA–GSH cycle enzymes and a significant decrease in the GSH:GSSG ratio induced by Cd can be recognized as markers of durum wheat sensitivity to this metal. In the future, we plan to explore other mechanisms that block the accumulation of Cd in the seeds. First, we will study durum plants for the expression of genes located at *Cdu1* locus in the presence of Cd.

Abbreviations

APX	ascorbate peroxidase
AsA	ascorbate
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
DM	dry matter
GSH	glutathione
GSSG	oxidized glutathione
GR	glutathione reductase
MDHAR	monodehydroascorbate reductase
NADH	nicotinamide adenine dinucleotide, reduced form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form

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Physiological and Biochemical Parameters of Salinity Resistance of Three Durum Wheat Genotypes

Jakub Pastuszak [1,*], Michał Dziurka [2], Marta Hornyák [3], Anna Szczerba [1], Przemysław Kopeć [2] and Agnieszka Płażek [1,*]

[1] Department of Plant Breeding, Physiology and Seed Science, University of Agriculture, Podłużna 3, 30-239 Kraków, Poland; anna.szczerba21@gmail.com
[2] The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Kraków, Poland; m.dziurka@ifr-pan.edu.pl (M.D.); p.kopec@ifr-pan.edu.pl (P.K.)
[3] Władysław Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland; marta.hornyak@botany.pl
* Correspondence: jakub.pastuszak@urk.edu.pl (J.P.); rrplazek@cyf-kr.edu.pl (A.P.)

Abstract: The area of farming lands affected by increasing soil salinity is growing significantly worldwide. For this reason, breeding works are conducted to improve the salinity tolerance of important crop species. The goal of the present study was to indicate physiological or biochemical parameters characterizing three durum wheat accessions with various tolerance to salinity. The study was carried out on germinating seeds and mature plants of a Polish SMH87 line, an Australian cultivar 'Tamaroi' (salt-sensitive), and the BC_5Nax_2 line (salt-tolerant) exposed to 0–150 mM NaCl. Germination parameters, electrolyte leakage (EL), and salt susceptibility index were determined in the germinating caryopses, whereas photosynthetic parameters, carbohydrate and phenolic content, antioxidant activity as well as yield were measured in fully developed plants. The parameters that most differentiated the examined accessions in the germination phase were the percentage of germinating seeds (PGS) and germination vigor (*Vi*). In the fully developed plants, parameters included whether the plants had the maximum efficiency of the water-splitting reaction on the donor side of photosystem II (PSII)–F_v/F_0, energy dissipation from PSII–DI_o/CS_m, and the content of photosynthetic pigments and hydrogen peroxide, which differentiated studied genotypes in terms of salinity tolerance degree. Salinity has a negative impact on grain yield by reducing the number of seeds per spike and the mass of one thousand seeds (MTS), which can be used as the most suitable parameter for determining tolerance to salinity stress. The most salt-tolerant BC_5Nax_2 line was characterized by the highest PGS, and *Vi* for NaCl concentration of 100–150 mM, content of chlorophyll *a*, *b*, carotenoids, and also MTS at all applied salt concentrations as compared with the other accessions. The most salt-sensitive cv. 'Tamaroi' demonstrated higher H_2O_2 concentration which proves considerable oxidative damage caused by salinity stress. Mentioned parameters can be helpful for breeders in the selection of genotypes the most resistant to this stress.

Keywords: antioxidant enzyme activity; chlorophyll fluorescence; durum wheat; gas exchange; germination vigor; salinity

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1. Introduction

The problem of soil salinity is important due to the increased demand for food for the global population, growing every year. The main anthropogenic factors causing soil salinity are the intensification of agriculture, and inappropriate agronomic practices, which include the use of high doses of artificial fertilizers, inadequate irrigation, and deforestation [1–3]. Around 20% of agricultural lands are at risk of increased soil salinity. Rapid climate changes cause long-term droughts that affect more and more areas in Europe. This situation forces farmers to more frequently irrigate their crops with only partly desalinated water, which at growing air temperatures increases the salinity of the upper soil layers [4]. Sodium ions

(Na^+) in the soil decrease water osmotic potential, which in consequence reduces water uptake capacity by imbibed seeds and roots of plants cultivated in saline areas [5]. The Na^+ and chlorine (Cl^-) ions exert a toxic effect on plants and induce osmotic and ionic stress. The ionic stress limits the uptake of potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and nitrate (NO_3^-) ions important for plant functioning [6,7]. A high concentration of Na^+ in the soil complex can lead to many permanent disorders at every stage of plant development. Salinity stress worsens numerous yield parameters [8–10]. The increased amount of toxic Na^+ in the soil solution damages cell membranes in germinating seeds [11]. It also limits the biosynthesis of all chlorophyll fractions, lowers photosynthesis efficiency and stomatal closure, and has a negative impact on gas exchange [12,13]. An increased accumulation of reactive oxygen species (ROS) is also observed during salinity stress. High amounts of ROS evoke lipid peroxidation leading, i.e., to increased membrane fluidity and permeability, denaturation of DNA, changes in hormonal profile, and enzyme inactivation [14–16].

Osmotic stress can be evoked by not only salinity but also by drought and heavy metal ions. These stress factors change morphological traits causing the reduction in leaf size and vegetative growth, a decline in photosynthesis rate, stomatal conductance, and alter stem anatomical features [17–21]. To counteract the negative effects of direct exposure to salinity, plants have developed internal defense mechanisms. There are two types of mechanisms. The first is named the 'avoidance strategy', and it consists of avoiding salt stress by creating barriers that prevent the penetration of toxic ions into the plant. The second mechanism involves 'salinity tolerance'. It is based on the development of intracellular mechanisms that minimize damage and make it possible to repair the negative consequences of stress [22]. Cellular mechanisms of resistance to salinity regulate ion transport. This is possible due to the Na^+/H^+ antiports, proton pumps, and ion channels. The presence and participation of membrane transporters are significant in order to maintain intracellular homeostasis [23–25]. To maintain a low concentration of toxic Na^+ inside the cytoplasm, active transport of the ions outside the cell through the plasmalemma and their compartmentalization in the vacuole is necessary [4,24,26].

The defense mechanism against ROS is based on the production of enzymatic (catalase, peroxidases, superoxide dismutase) and non-enzymatic (ascorbic acid, glutathione, carotenoids, anthocyanins) antioxidants [27,28]. The first discovered and described antioxidant enzyme was catalase (CAT). Its essential role is to remove the excess H_2O_2 and degrade it in the cellular organelles during, e.g., fatty acid oxidation or photorespiratory oxidation [29,30]. Peroxidase (POX) also contributes to H_2O_2 removal from different cellular organelles, especially from the cell wall. POX utilizes apoplastic H_2O_2 in the lignification process [31,32]. Superoxide dismutase (SOD) is considered to be the first line of defense against oxidative stress in plants. The pivotal role of SOD is to catalyze the dismutation of $O_2^{\bullet -}$ (superoxide) and $HO_2^{\bullet}$ (hydroperoxide radical) to H_2O_2 and H_2O and to maintain redox balance in the defense response of plants exposed to stress [33]. Hydrogen peroxide plays a signaling role that may facilitate plant response to various stimuli in plant cells and is involved in cellular signaling transduction pathways and gene expression modulations [34].

The cereal most commonly used in the food industry is durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.). It is considered to be the tenth most important and cultivated cereal around the world. It is estimated that the global durum wheat production reaches up to 40 million tons. The world's largest producers are Canada, America, and Turkey. In Europe, it is cultivated in the countries of the Mediterranean region [35]. Recent years have brought increased interest in durum wheat cultivation in Poland. The widespread use of durum wheat grains includes pasta production, and to a lesser extent bread and groats. The grain is characterized by a high content of grain protein and fiber, a low glycemic index, and a high level of vitamins and other valuable micronutrients [36,37]. Durum wheat is more sensitive to salinity stress than common wheat. In comparison with durum wheat, common wheat has well-developed mechanisms of salinity tolerance consisting of the excretion of salt ions from the cytosol [4]. Genetic analysis of

many common wheat genotypes has identified one 'Line 149' which has two major Na^+ exclusion loci named *Nax*$_1$ and *Nax*$_2$. According to Huang et al. [38], some members of the HKT family (high-affinity K^+ transporter) as sodium transporters play an important role in the regulation of Na^+ content in the roots and shoots. HKT transporters appear important in the control of Na^+ transport in bread wheat and may also transport sodium contributing to salt tolerance in durum wheat. The function of the *Nax*$_1$ gene is to remove Na^+ from the xylem in the roots and lower parts of the leaves and leaf sheaths, whereas *Nax*$_2$ removes sodium ions from the xylem only in the roots [39,40]. The locations of the *Nax*$_1$ (chromosome 2A) and *Nax*$_2$ (chromosome 5A) genes were confirmed by quantitative trait locus (QTL) analysis and identified by fine-mapping the Na^+ transporter from the *HKT* gene families—*HKT7* for *Nax*$_1$ and *HKT8* for *Nax*$_2$ [38,41]. Ibrahimowa et al. [21] studied the response of two *T. aestivum* genotypes differing in terms of salinity tolerance. These authors observed an increased expression level of the *TaHKT1;5* genes in the roots of the salt-sensitive genotype, and its decrease in the salt-tolerant one.

The research hypothesis of our work was that it will be possible to choose physiological or biochemical markers of salinity tolerance useful for selection among the parameters studied in our paper. The research material consisted of two well-characterized Australian spring durum wheat accessions: the cv. 'Tamaroi' was found to be very sensitive to a high concentration of NaCl, whereas the BC_5Nax_2 line was created by crossing the cv. 'Tamaroi' with the 149 line, carrying the *TmHKT1;5* gene responsible for preventing the transport of sodium ions from the roots to shoots [42]. Additionally, the Polish SMH87 line was used, which was not examined in terms of salinity tolerance, so far. Until now, in Poland, the research on the resistance of durum wheat to salinity was conducted by our team, but on other genotypes [43]. The aim of the present work was to indicate the most important physiological or biochemical parameters shaping the durum wheat tolerance to salinity. The study was carried out on three accessions: Polish line SMH87 and two Australian genotypes: cv. 'Tamaroi' and the BC_5Nax_2 line. We performed two experiments: a laboratory test on germinating seeds exposed to 0, 50, 100, 125, and 150 mM NaCl (Experiment 1), and analyses performed on plants grown in soil watered with a saline solution containing 0, 100, 125, and 150 mM NaCl (Experiment 2). In Experiment 1, the responses of the studied durum wheat accessions to salinity were evaluated by determination of the percentage of germinating seeds, germinating vigor, coleoptile length, salt susceptibility index, and electrolyte leakage from germinating seeds. Experiment 2 involved the analyses of the kinetics of chlorophyll *a* fluorescence, gas exchange parameters, content of chlorophyll *a* and *b*, carotenoids, total soluble carbohydrates, total phenolic compounds, and cell-wall bound phenolics, the activity of superoxide dismutase, catalase, peroxidase, hydrogen peroxide content, and finally the evaluation of yield parameters.

2. Results

2.1. Experiment 1

2.1.1. Percentage of Germinated Seeds (PGS), Germination Vigor Index (*Vi*), Coleoptile Length (CL) and Salt Susceptibility Index (SSI)

The seeds were germinated in the presence of 50, 100, 120, and 150 mM NaCl. The percentage of germinated seeds (PGS) was calculated two days after sowing. The highest percentage of germinating seeds at all NaCl doses was seen in the BC_5Nax_2 line (Figure 1A). NaCl treatment significantly reduced the germination in the SMH87 line and cv. 'Tamaroi' at all applied doses. The lowest dose of NaCl (50 mM) did not reduce PGS only in the BC_5Nax_2 line. In the SMH87 plants, the lowest PGS was obtained at 125 mM NaCl, and it was by 13.9% lower than that of the control. A significantly lower PGS was observed in cv. 'Tamaroi' treated with 125 and 150 mM NaCl, and it was lower by respectively 21 and 26% than in the control. In the case of the BC_5Nax_2 line, 125 and 150 mM significantly reduced PGS by 11 and 10%, respectively. Moreover, treatments with 125 and 150 mM NaCl increased seed ability to germinate in the case of BC_5Nax_2, as compared with the other studied accessions.

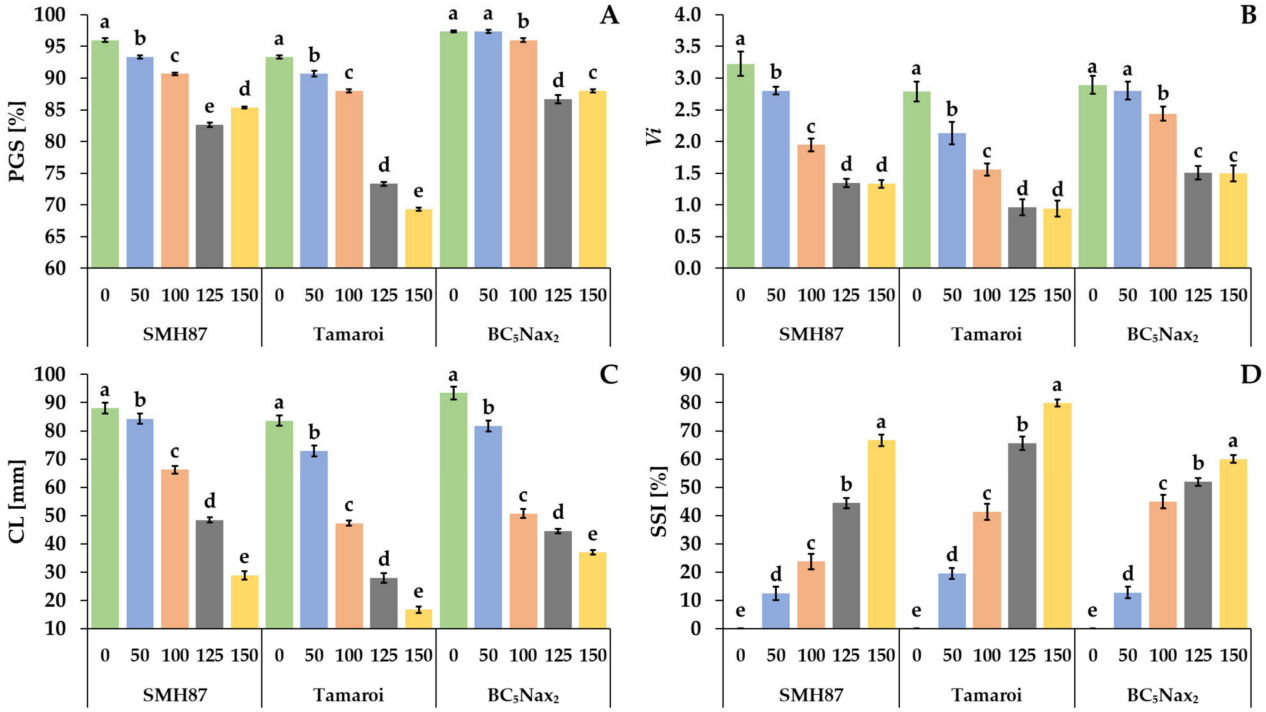

Figure 1. Effects of NaCl treatments (0, 50, 100, 125, 150 mM) on the PGS—percentage of germinated seeds (**A**), *Vi*—germination vigor index (**B**), CL—coleoptile length (**C**) and SSI—salt susceptibility index (**D**). The values represent means (n = 5) ± SE (standard error) for PGS and *Vi*; (n = 20) ± SE for CL and SSI within each accession. Mean values marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

The vigor of germinating seeds (*Vi*) was compatible with the results obtained for the PGS (Figure 1B). Moreover, the lowest NaCl dose (50 mM) reduced *Vi* of the SMH87 line and cv. 'Tamaroi', whereas *Vi* did not change in plants of the BC_5Nax_2 line. The greatest reduction in *Vi* was observed in cv. 'Tamaroi', where NaCl at 125 and 150 mM reduced the parameter by 65 and 66%, respectively, as compared with the control. In SMH87 and BC_5Nax_2 seeds, 125 and 150 mM NaCl reduced *Vi* by over 58 and 48%, respectively, as compared with the control.

With increasing salinity, the coleoptile length (CL) decreased in all studied accessions (Figure 1C). At 50 mM NaCl, a significant reduction by more than 12% versus the control coleoptiles was observed in cv. 'Tamaroi' and the BC_5Nax_2 line. The highest NaCl dose had a significant effect on the CL reduction in all studied accessions, whereas the greatest CL reduction (80%) was noted in cv. 'Tamaroi'.

Salt susceptibility index (SSI) increased with growing NaCl doses in all accessions (Figure 1D). The treatment with 50 mM NaCl had a similar effect on SSI in the SMH87 and BC_5Nax_2 lines. Their SSI was elevated by over 12%, whereas in cv. 'Tamaroi' it rose by 20%. The highest SSI was observed in cv. 'Tamaroi' at 125 and 150 mM NaCl. At 125 mM NaCl, the SMH87 line showed lower sensitivity to salinity than the other genotypes, whereas the BC_5Nax_2 line turned out the least sensitive to salinity (59.9%) at 150 mM NaCl.

2.1.2. Electrolyte Leakage (EL)

All NaCl treatments enhanced electrolyte leakage (EL) from germinating seeds (Figure 2). Even the lowest NaCl dose increased EL by more than 50% for each accession. The highest EL was observed in BC_5Nax_2 seeds at 150 mM NaCl. It was 6.2-fold higher than in the control. In the SMH87 line and cv. 'Tamaroi', the EL increased by 5.1 and 4.6 times, respectively.

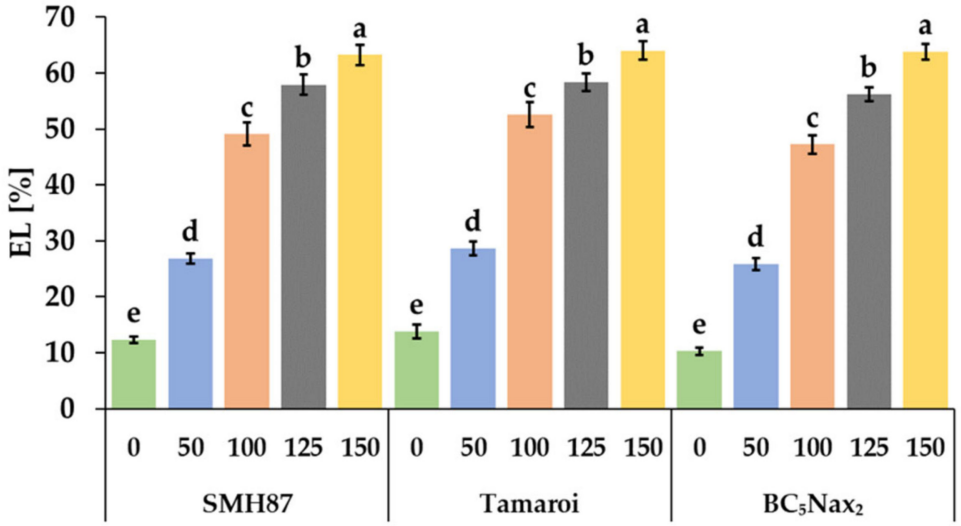

Figure 2. Effects of NaCl treatments (0, 50, 100, 125, 150 mM) on the electrolyte leakage (EL). The values represent means (n = 10) ± SE within each accession, and those marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

2.1.3. Correlation Analysis

The CL correlated negatively with the SSI and EL (Table 1). A positive correlation between CL and PGS and *Vi* was found. SSI positively correlated with EL, whereas a negative correlation was obtained between PGS and *Vi*. A negative correlation between EL and PGS, and between EL and *Vi* was observed. A positive correlation was also found between PGS and *Vi*.

Table 1. Pearson coefficients of linear correlation ($p < 0.05$) between CL—coleoptile length, SSI—salt susceptibility index, EL—electrolyte leakage, PGS—percentage of germinated seeds, *Vi*—vigor of germinating seeds.

Parameter	CL	SSI	EL	PGS
SSI	−0.954			
EL	−0.863	0.848		
PGS	0.63	−0.666	−0.572	
Vi	0.795	−0.822	−0.864	0.778

2.2. Experiment 2

2.2.1. Measurements of Chlorophyll *a* Fluorescence (ChlF)

The energy absorbed by the antennas (ABS/CS_m) was different for the studied accessions exposed to salinity (Figure 3A). In the SMH87 line, the ABS/CS_m decreased with increasing NaCl concentration. In cv. 'Tamaroi' plants this parameter increased at 100 and 125 mM NaCl, respectively, and at 150 mM NaCl, it decreased to the control value. The BC_5Nax_2 line was characterized by the highest ABS/CS_m at 100 mM, and the lowest at 150 mM. The excitation energy trapped in PSII (TR_o/CS_m) was different for each studied genotype (Figure 3B). The highest applied salinity dose (150 mM) significantly decreased TRo/CS_m in the SMH87 and BC_5Nax_2 lines. In cv. 'Tamaroi' plants, such changes were not observed. The highest TR_o/CS_m value was recorded for the BC_5Nax_2 line at 100 mM NaCl. The highest value of energy used for electron transport (ET_o/CS_m) was observed in all genotypes at 100 and 125 mM NaCl (Figure 3C). The lowest value of this parameter was detected at 150 mM NaCl in the SMH87 and BC_5Nax_2 lines. In cv. 'Tamaroi', this salinity dose did not change the ET_o/CS_m values as compared with the control. The energy dissipation from PSII (DI_o/CS_m) did not change under any of the NaCl treatments in cv. 'Tamaroi' and BC_5Nax_2 plants (Figure 3D). In the SMH87 line, 100 and 125 mM NaCl decreased the

value of this parameter, whereas 150 mM NaCl evoked its drastic increase. The number of active reaction centers (RC/CS_m) increased under 100 mM NaCl in all studied genotypes (Figure 3E). The higher doses of NaCl lowered the RC/CS_m value in all studied accessions but in the 'Tamaroi' and BC_5Nax_2 plants at 150 mM NaCl the number of reaction centers was lower than that of the control. The same type of changes under salinity was observed for the performance index (PI) (Figure 3F). The highest value of PI was seen at 125 mM NaCl in SMH87 plants, whereas the lowest was in the BC_5Nax_2 line at 150 mM NaCl. The most pronounced effect of salinity on the maximum efficiency of water-splitting reaction of the donor side of PSII (F_v/F_0) was noted in SMH87 plants at 150 mM NaCl (Figure 3G). In the case of other accessions, this concentration also reduced the value of this parameter.

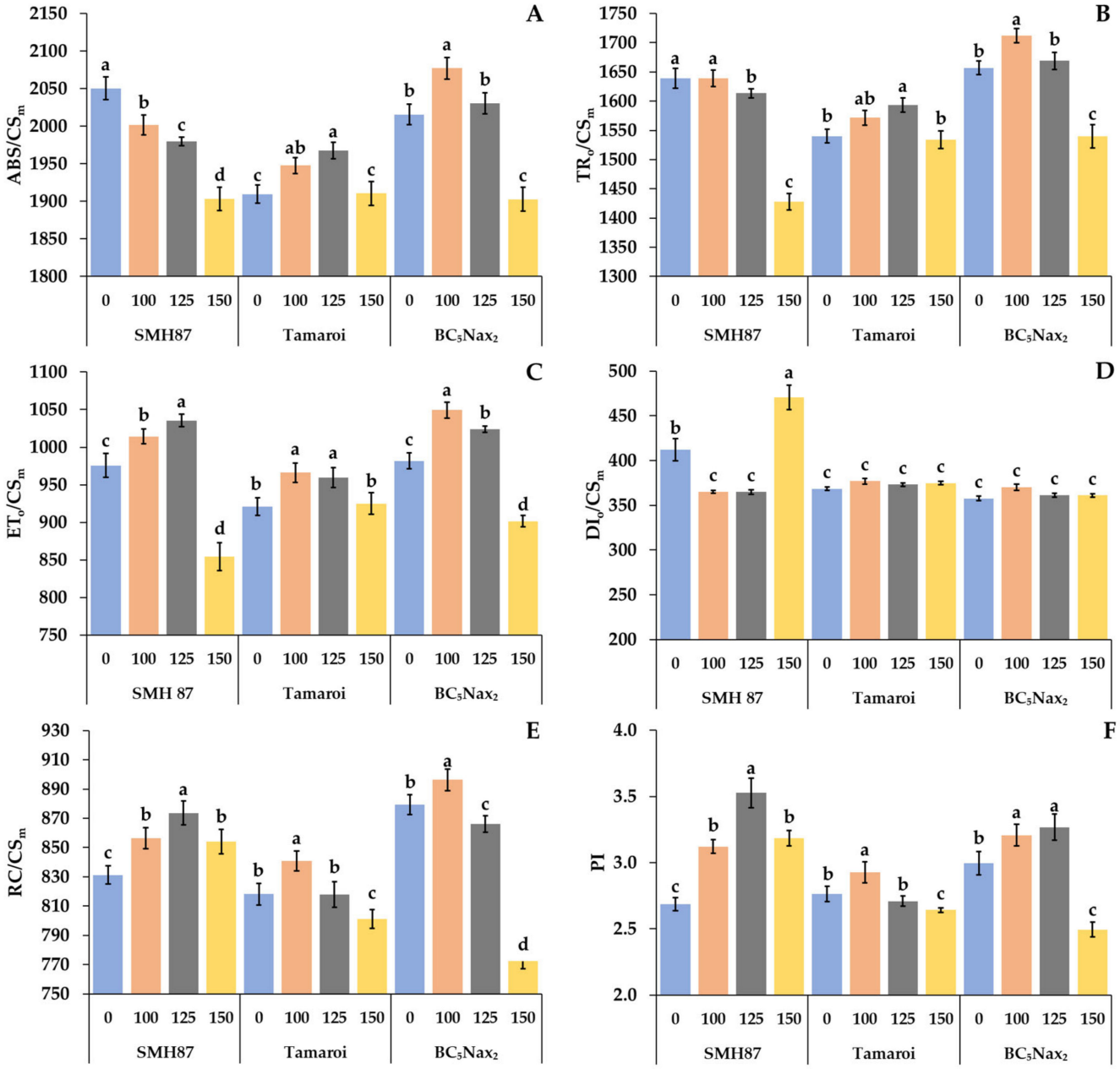

Figure 3. *Cont.*

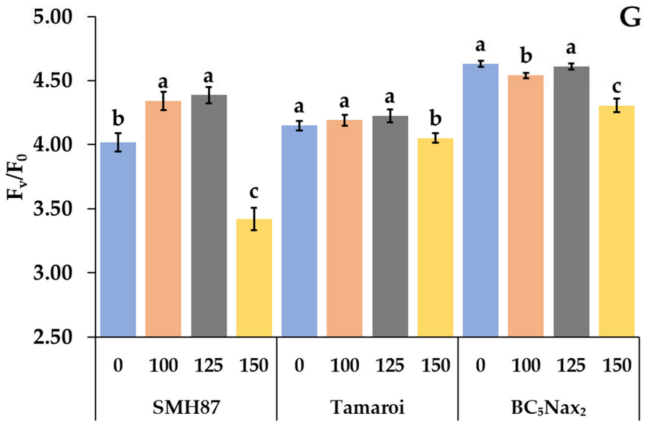

Figure 3. Effects of NaCl treatments (0, 100, 125, 150 mM) on chlorophyll *a* parameters: ABS/CS_m—energy absorption by antennas (**A**); TR_o/CS_m—excitation energy trapped in PSII (**B**); ET_o/CS_m—energy used for electron transport (**C**); DI_o/CS_m—energy dissipation from PSII (**D**); RC/CS_m—number of active reaction center (**E**); PI—performance index (**F**); F_v/F_0—maximum efficiency of water-splitting reaction of the donor side of PSII (**G**). The values represent means (n = 10) ± SE within each accession, and those marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

2.2.2. Measurements of Gas Exchange Parameters

Salinity had a significant impact on all gas exchange parameters (Figure 4). The net photosynthesis rate (*A*) was changed in all studied accessions under increasing salinity (Figure 4A). The increase in net photosynthesis rate in the SMH87 line was observed under all salt treatments, and it was over two-fold higher than in the control. In cv. 'Tamaroi' and the BC_5Nax_2 line, 100 mM NaCl diminished the net photosynthesis rate over two times. The increasing salinity boosted A significantly in cv. 'Tamaroi' plants. The highest photosynthetic efficiency of this cultivar was observed at 150 mM NaCl. In BC_5Nax_2 plants at 125 and 150 mM NaCl, this parameter was lower than in the control ones.

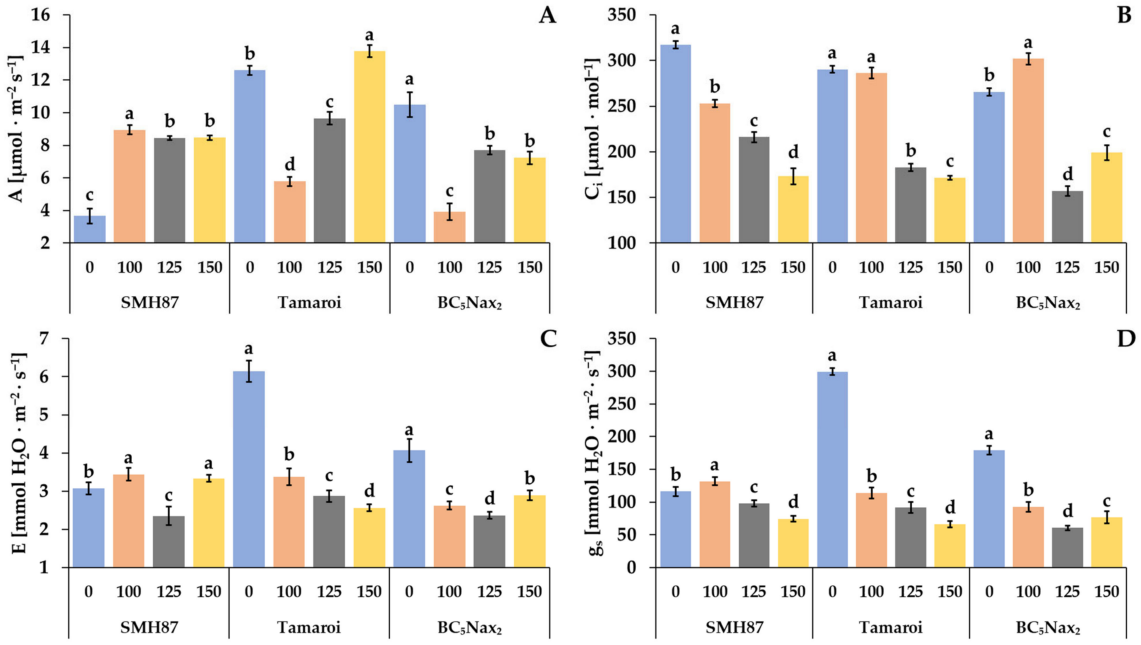

Figure 4. Effects of NaCl treatments (0, 100, 125, 150 mM) on the: *A*—net photosynthetic rate (**A**); C_i—intercellular CO_2 concentration (**B**); *E*—transpiration rate (**C**), g_s—stomatal conductance (**D**). The values represent means (n = 8) ± SE within each accession, and those marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

Along with increased NaCl treatment, the intracellular CO_2 concentration (C_i) decreased in the SMH87 line (Figure 4B). Treatment with 125 and 150 mM NaCl significantly reduced the C_i in cv. 'Tamaroi' and the BC_5Nax_2 line as compared with the control. In BC_5Nax_2 plants, C_i was the highest at 100 mM NaCl.

In the SMH87 line, the highest transpiration rate (E) was observed in plants grown in the presence of 100 and 150 mM NaCl, and it was respectively 12% and 9% higher than in the control. NaCl at 125 mM reduced E by 23%. In cv. 'Tamaroi' and the BC_5Nax_2 line, the highest transpiration rate was observed in the control plants. In these genotypes, the parameter decreased with increasing soil salinity. In cv. 'Tamaroi', the concentration of 100 mM NaCl reduced E by 45%, whereas 125 and 150 mM NaCl reduced the parameter by 53% and 58%, respectively, as compared with the control. In the BC_5Nax_2 line, the transpiration rate decreased significantly by 35% and 41% at respectively 100 and 125 mM NaCl, and at 150 mM NaCl, it was 28% lower than in the control. In the SMH87 plants, the stomatal conductance (g_s) increased at 100 mM NaCl but it was limited at 125 and 150 mM NaCl. In cv. 'Tamaroi' and the BC_5Nax_2 line, the changes in the stomatal conductance followed a similar pattern as for the transpiration rate, and g_s was the highest in the control plants.

2.2.3. Chlorophyll (*a*, *b*) and Carotenoid (Car) Content

The content of chlorophyll *a* (Chl *a*) differed under salt treatments in each accession (Figure 5A). In the SMH87 line, an increase in Chl *a* content was observed at 100 and 125 mM NaCl as compared with the control. In cv. 'Tamaroi', growing salinity reduced Chl *a* content. A decrease in Chl *a* was observed in the BC_5Nax_2 line at 100 and 125 mM NaCl versus the control, whereas at 150 mM NaCl a slight rise by 8% was detected. The SMH87 control plants and those grown at 125 mM NaCl showed comparable content of Chl *b* (Figure 5B). The doses of 100 and 150 mM NaCl reduced its content by 14% and 12%, respectively. In cv. 'Tamaroi', the increased soil salinity curbed Chl *b* content. In the BC_5Nax_2 line, treatments with 100 and 150 mM NaCl elevated Chl *b* content by 10% and 21%, respectively. A reduced carotenoid content (Car) was observed in SMH87 plants at 100 and 150 mM NaCl, as compared with the control (Figure 5C). In cv. 'Tamaroi', increasing salinity drastically reduced Car content, whereas an opposite effect was observed in BC_5Nax_2 plants, where 150 mM NaCl increased this pigment level by 11% as compared with the control.

2.2.4. Total Soluble Carbohydrate (TSC) Content

In all accessions, increased salinity significantly enhanced TSC content (Figure 5D). In SMH87 plants, the highest TSC content was observed at 150 mM NaCl, and it was 46% greater than in the control plants. In cv. 'Tamaroi', increasing salinity resulted in a TSC spike, and its highest content was noted at 100 mM NaCl (it was 92% higher than in the control). In the BC_5Nax_2 line, the highest content of TSC was observed at 125 mM NaCl, and it was 70% greater than in the control.

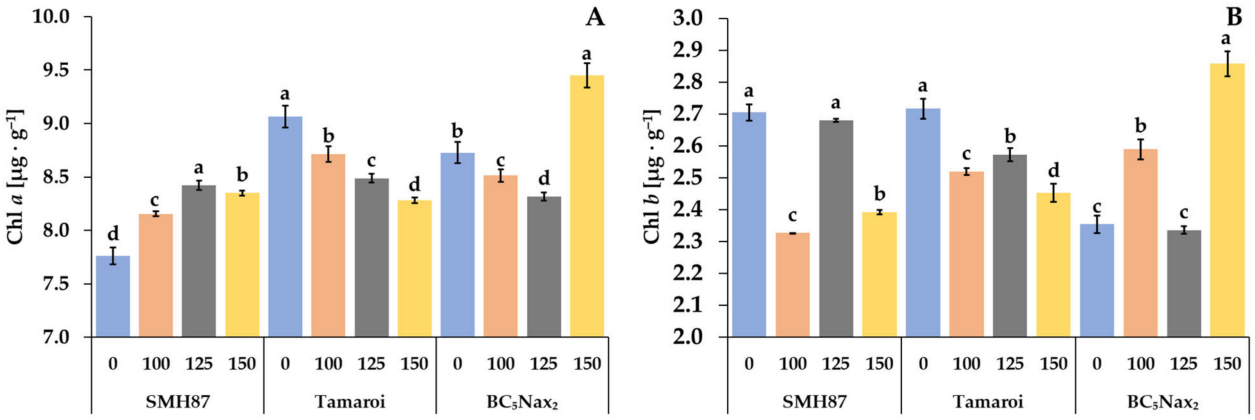

Figure 5. *Cont.*

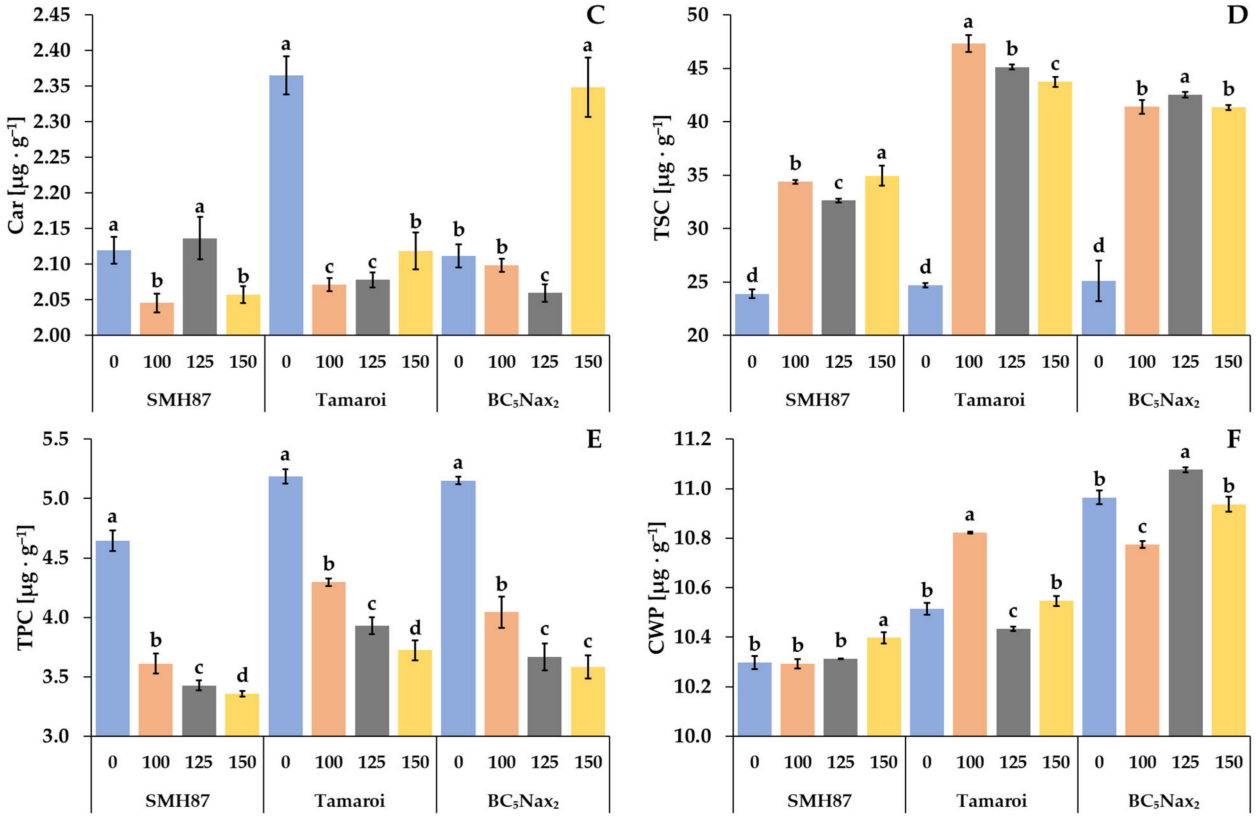

Figure 5. Effects of NaCl treatments (0, 100, 125, 150 mM) on Chl *a*—chlorophyll *a* (**A**), Chl *b*—chlorophyll *b* (**B**), Car—carotenoids (**C**), TSC—total soluble carbohydrates (**D**), TPC—total phenolic compounds (**E**), CWP—cell wall-bound phenolics (**F**). The values represent means (n = 3) ± SE within each accession, and those marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

2.2.5. Total Phenolic Compound (TPC) Content

The lowest amount of TPC was noted in the leaves of control SMH87 plants (Figure 5E). Increasing soil salinity resulted in a gradual decline in TPC in all studied genotypes. At 100 mM NaCl, TPC decreased in the SMH87 line by 22%, in cv. 'Tamaroi' by 17%, and in the BC_5Nax_2 line by 22% in relation to the control. In all accessions, the lowest TPC in the leaves was found at the highest salt concentration.

2.2.6. Cell Wall-Bound Phenolic (CWP) Content

The increasing salinity affected CWP content in the leaves of each accession under study (Figure 5F). Line SMH87 showed no changes in CWP content at 100 and 125 mM NaCl, whereas at 150 mM NaCl a slight increase, albeit significant, was found. In the leaves of other plants, CWP amount varied non-specifically under different salt concentrations. The highest content of CWP in cv. 'Tamaroi' was recorded at 100 mM NaCl, whereas in the BC_5Nax_2 line at 125 mM NaCl.

2.2.7. Superoxide Dismutase (SOD) Activity

The highest SOD activity was observed in SMH87 plants treated with 125 mM NaCl, and it was 22% higher than the control value (Figure 6C). In the case of cv. 'Tamaroi' and the BC_5Nax_2 line, the increased salinity declined the activity of this enzyme and it was the lowest at 150 mM NaCl.

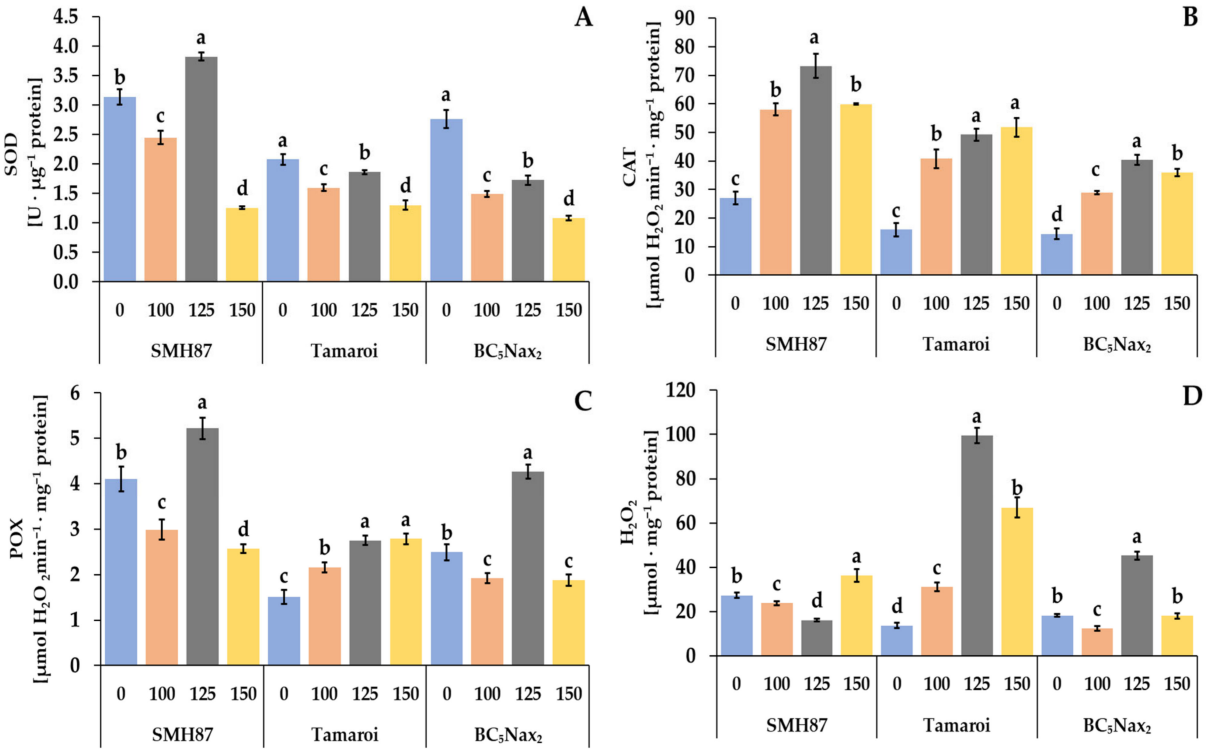

Figure 6. Effects of NaCl treatments (0, 100, 125, 150 mM) on SOD—superoxide dismutase (**A**), CAT—catalase (**B**), POX—peroxidase (**C**) activity, and H_2O_2—hydrogen peroxide (**D**) content. The values represent means (n = 3) ± SE within each accession, and those marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

2.2.8. Catalase (CAT) Activity

In all studied genotypes, increasing salinity enhanced the activity of CAT as compared with the control (Figure 6A). In SMH87 plants, treatment with 125 mM NaCl resulted in the highest CAT activity which was 2.7 times higher than in the control. In cv. 'Tamaroi', the highest activity of this enzyme was observed at 125 and 150 mM NaCl, and it was over 3-fold higher than in the control plants. In the BC_5Nax_2 line, the highest CAT activity was detected in plants treated with 125 mM NaCl, and it was 2.8-fold higher than in the control plants.

2.2.9. Peroxidase (POX) Activity

Along with increasing salinity, POX activity changed non-specifically in all plants under the study. The 125 mM NaCl treatment significantly enhanced POX activity in SMH87 and BC_5Nax_2 plants, whereas at the other salt concentrations it was significantly lower than in the control (Figure 6B). In cv. 'Tamaroi', increased POX activity was observed under all NaCl treatments.

2.2.10. Hydrogen Peroxide (H_2O_2) Content

The hydrogen peroxide content of SMH87 plants was significantly higher than in the control ones only under 150 mM NaCl treatment. In cv. 'Tamaroi', the growing doses of NaCl increased the production of H_2O_2, and at 125 mM NaCl its content was 7.32-fold higher than in the control plants. The same relationship was noted in the BC_5Nax_2 line but then the H_2O_2 content was 2.5-fold higher than in the control.

2.2.11. Yield Parameters

The salt treatments reduced the number of seeds per spike in all studied accessions (Figure 7A). The most drastic reduction was visible in the cv. 'Tamaroi', where a decrease of 23%, 40%, and 59% was observed at 100, 125, and 150 mM NaCl, respectively. The

lowest reduction in the number of seeds per spike was shown by plants of the BC_5Nax_2 line at 150 mM NaCl; 32.3% fewer grains per spike were observed as compared with the control seed number. Salinity also had a negative impact on the dry weight (DW) of grain calculated per spike (Figure 7B). A decrease in seed DW along with the increasing NaCl concentration was observed. Drastic reduction in seed DW per spike was determined in cv. 'Tamaroi' at 100 mM NaCl, and amounted to 59.3% of the control. The higher NaCl concentrations declined grain DW by 67–75% as compared with the control. The mass of one thousand seeds (MTS) also decreased under increasing salinity. In the SMH87 line, the greatest decline in MTS was noted at 125 and 150 mM NaCl, and it was 35% and 45%, respectively, of the control. In cv. 'Tamaroi', a drastic 41% drop in MTS was already noted at 100 mM NaCl. At 125 and 150 mM NaCl, MTS significantly decreased by respectively 57% and 67% in relation to the control. BC_5Nax_2 plants showed the smallest loss in MTS in salt-treated soil. Moreover, at 125 and 150 mM NaCl, no significant differences between MTS were observed in this line.

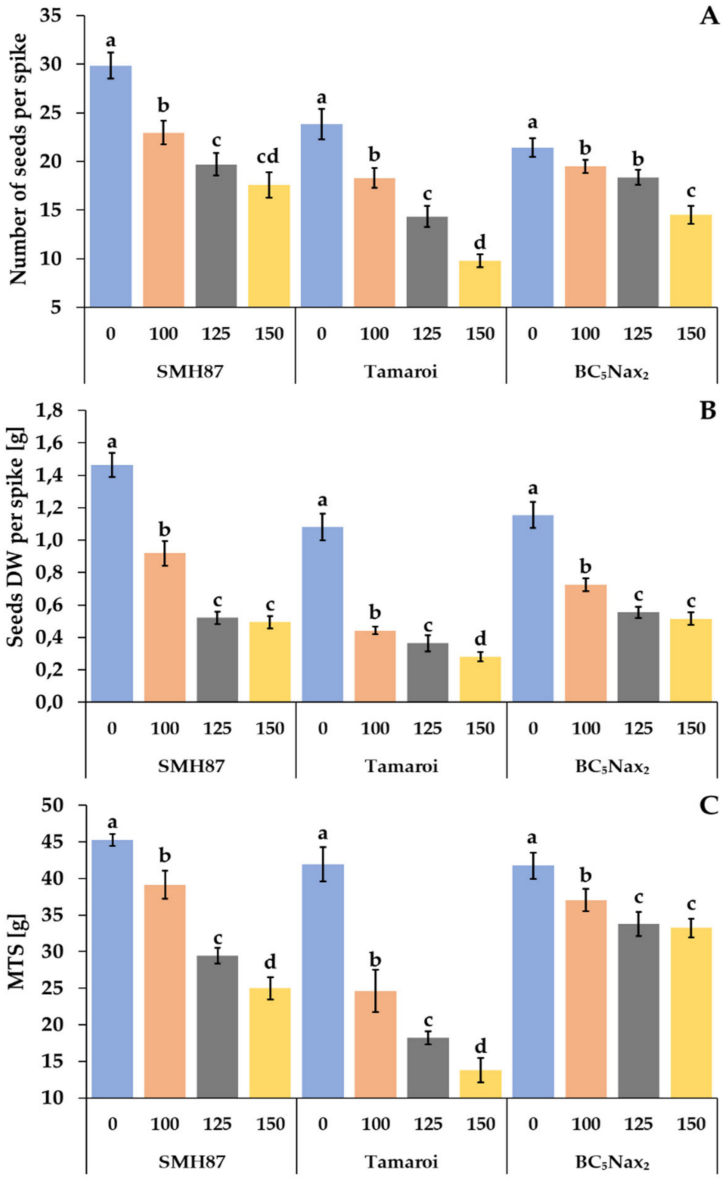

Figure 7. Effects of NaCl treatments (0, 100, 125, 150 mM) on yield components: number of seeds per spike (**A**), seed DW per spike (**B**), and MTS—mass of one thousand seeds (**C**). The values represent means (n = 3) ± SE within each accession, and those marked with the same letters do not differ statistically according to multiple range Duncan's test ($p < 0.05$).

2.3. Correlation Analysis

A negative correlation between Ci and A, and a positive correlation between E and g_s and other gas exchange parameters were found (Table 2).

Table 2. Pearson coefficients of linear correlation ($p < 0.05$) for gas exchange parameters.

Parameter	*A*	*Ci*	*E*
Ci	−0.197		
E	0.664	0.408	
g_s	0.656	0.509	0.909

A—net photosynthetic rate; *Ci*—intercellular CO_2 concentration; *E*—transpiration rate, g_s—stomatal conductance.

H_2O_2 content positively correlated with CAT and POX only in cv. 'Tamaroi' and BC_5Nax_2 line plants (Table 3).

Table 3. Pearson coefficients of linear correlation ($p < 0.05$) between H_2O_2 and antioxidant enzyme activity in the leaves of three durum wheat accessions.

Accession	Parameter	CAT	POX	SOD
SMH87		ns	ns	ns
Tamaroi	H_2O_2	0.451	0.551	ns
BC_5Nax_2		0.640	0.712	ns

CAT—catalase; POX—peroxidase; SOD—superoxide dismutase; H_2O_2—hydrogen peroxide; ns—not significant.

A negative correlation between TPC and CAT activity in all examined accessions was detected (Table 4). In all accessions, TPC positively correlated with SOD activity. The content of H_2O_2 correlated negatively with TPC only in cv. 'Tamaroi'. No correlation between H_2O_2 and POX in all accessions was found.

Table 4. Pearson coefficients of linear correlation ($p < 0.05$) between TPC and antioxidant enzyme activity and H_2O_2 content in the leaves of three durum wheat accessions.

Accession	Parameter	CAT	POX	SOD	H_2O_2
SMH87		−0.884	ns	0.441	ns
Tamaroi	TPC	−0.533	ns	0.424	−0.410
BC_5Nax_2		−0.763	ns	0.553	ns

TPC—total phenolic compounds; CAT—catalase; POX—peroxidase; SOD—superoxide dismutase; H_2O_2—hydrogen peroxide; ns—not significant

3. Discussion

3.1. Experiment 1

3.1.1. Percentage of Germinating Seeds, Germination Vigor, and Cell Membrane Permeability

Seedlings are the plant developmental stage the most sensitive to stresses occurring at the air–soil level. These stresses include, i.e., salinity and drought. An important aspect of salinity tolerance is the possibility of seeds germinating in saline soil and their ability to continue development. In their research, Płażek et al. [43] investigated the salinity tolerance of four wheat accessions. They found a difference in seed germination ability in common wheat cultivars in the saline soil. Contrary to that, two durum wheat accessions did not differ in the percentage of germinating seeds and they germinated even at 250 mM NaCl. However, the coleoptile length of both wheat species definitely decreased already at 70 mM NaCl. The authors concluded that salinity tolerance did not depend on wheat species but on their genotype.

In the present experiment, we also observed significant differences in seed germination ability under salinity for all studied accessions. The lowest percentage of germinated seeds (PGS) was recorded in the most sensitive to salinity cv. 'Tamaroi', whereas the highest germination capacity was observed in the salt-resistant BC_5Nax_2 line. In the SMH87 line, the PGS decreased with increasing soil salinity. A similar relationship was

demonstrated for CL and *Vi*. Brini et al. [3] also reported reduced PGS of durum wheat at 200 mM NaCl. Borlu et al. [44] found a large diversity in durum wheat cultivars germinated during exposure to 0 to 200 mM NaCl. Datir et al. [45] published similar findings for common wheat.

The electrolyte leakage is a common method used to evaluate cell membrane permeability under various stresses, i.e., under salinity [46]. In this study, the EL from the seeds of all accessions increased significantly with increasing salinity. Similar results were obtained by Płażek et al. [43] for two *Triticum durum* accessions. This parameter most strongly differentiates the response of durum wheat genotypes to salinity treatment. A positive correlation between CL, *Vi*, and PGS, and a negative correlation between CL, SSI, and EL were found. The study of Płażek et al. [43] yielded similar results. In our research, the parameters that most strongly differentiated the degree of salt sensitivity in durum wheat accessions from 100 mM NaCl upwards were the percentage of germinated seeds and germination vigor. Borlu et al. [44] reported considerable differences already at 75 mM NaCl. One of the reasons for the reduction in coleoptile growth under salinity could be combined osmotic-oxidative-toxic stress, which causes a disturbance of cell division, and modification of the structure of cell organelles [47]. In addition, salt causes the effect of plasmolysis, which is manifested by reduced turgor pressure, which compresses the cell cytoplasm resulting in a disturbance of the growth and shape of the emerging organs. Optimum cell hydration is essential for cell growth and its division [48].

Durum wheat is generally considered more sensitive to salinity than common wheat [49]. However, the results obtained by Płażek et al. [43] indicated that durum wheat was more tolerant to salinity at the germination stage, whereas common wheat showed better salt tolerance at the seedling stage. The salt susceptibility index (SSI) is a general parameter used for plant phenotyping as well as for determining the other components of physiological traits that provide information on the plant sensitivity or tolerance to stresses [41,42]. From an agricultural perspective, SSI is an important parameter taking into account the agronomic performance of the genotype under stress in relation to its yield under non-stress conditions [43]. In the present research, the highest values of SSI were recorded for salt-sensitive cv. 'Tamaroi' at both 125 and 150 mM NaCl. Płażek et al. [36] also observed an increase in SSI in durum wheat under increasing NaCl concentration, whereas a significant increase in this parameter was already observed at 70 mM NaCl. Thus, it can be concluded that the increased SSI reflects greater sensitivity to salinity.

3.2. Experiment 2

3.2.1. Photosynthetic Efficiency

Kalaji and Pietkiewicz [44] reported that the analysis of chlorophyll fluorescence provides a quick insight into the photochemical efficiency of plants grown under different field conditions. Most of the examined parameters of ChlF significantly decreased due to increasing soil salinity. The parameters that most strongly differentiated the studied accessions under salinity were TR_o/CS_m, ET_o/CS_m, and DI_o/CS_m. The SMH87 line showed the largest decrease in the excitation energy trapped in PSII (TR_o/CS_m) and energy used for electron transport (ET_o/CS_m) at 150 mM. The most interesting result was a significant increase in the energy dissipation of PSII (DI_o/CS_m) only in the case of the SMH87 line at 150 mM. This result suggests that higher DI_o/CS_m, in this case, was an effect of a significant decrease in ET_o/CS_m and TR_o/CS_m. The reduction in ET_o/CS_m may be related, among other things, to the inactivation of the enzymatic complex responsible for water photodissociation. In addition, the F_v/F_0, maximum efficiency of water-splitting reaction at the donor side of PSII in SMH87 plants was almost two-fold lower at 150 mM than in the control and was significantly lower than in the other studied accessions. Therefore, it could be assumed that a low value of this parameter, as well as very high DI_o/CS_m, indicated a relatively low plant tolerance to salinity at the heading phase. Moradi and Ismail [50] showed that tolerance to salinity can be determined by higher dissipation of excess energy. According to Kalaji et al. [51], an increase in the dissipation of excessive energy could also

indicate damage to the photosynthetic apparatus. These two statements indicate a difficult interpretation of changes in this parameter under stress conditions and suggest that it should be analyzed together with other parameters concerning energy absorption and electron transport within PSII. In SMH87 and BC_5Nax_2 plants, the performance index (PI) was the highest at 125 mM NaCl, whereas at 150 mM in the Polish SMH87 line it was higher than in cv. 'Tamaroi' and BC_5Nax_2 plants. These results do not clearly confirm if PI can be used as a salinity tolerance marker. A similar outcome was obtained by Płażek et al. [43]. However, Kalaji et al. [51] used PI to predict the seed yield of barley seven days after salt stress application. Płażek et al. [52] reported that PI turned out to be an excellent indicator of higher yield for narrow-leaf lupine germinating at low temperatures. It is worth noting that this parameter was measured at the seedling phase, much earlier than the seeds were harvested.

The photosynthesis rate depends on stomatal conductance and the availability of carbon dioxide [20]. In C4 plants or under stress conditions, mainly under osmotic stress, photosynthesis proceeds despite the closing of the stomata, caused by the plant's defense response to the loss of turgor pressure. Carbonic anhydrase plays a key role in the photosynthesis rate through its effects on CO_2 diffusion and other processes in photosynthetic organisms. It is mainly known to catalyze the CO_2 to HCO_3^- equilibrium. This reversible conversion has a clear role in sustaining the CO_2 concentration at the site of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [53]. In C3 plants, stomatal closure is recognized as a major protective mechanism against osmotic stress that decreases CO_2 availability and photosynthetic activity. The lack of clear reduction in photosynthetic efficiency observed in durum wheat plants with closed stomata may suggest a significant use of C4-type primarily carboxylation via phosphoenolpyruvate carboxylase (PEPC) [54].

In terms of gas exchange intensity, the studied accessions responded specifically to rising salinity. The SMH87 line showed greater CO_2 assimilation at all NaCl treatments than the control plants, whereas in cv. 'Tamaroi' and the BC_5Nax_2 line, the lowest A value was determined at 100 mM NaCl, and at higher salinity, the photosynthetic efficiency increased. In cv. 'Tamaroi' plants the highest net photosynthetic rate was achieved by the plants at 150 mM NaCl. Although the net photosynthetic rate correlated positively with stomatal conductance ($r = 0.656$; $p < 0.05$), this correlation was not observed at 150 mM NaCl. It is very interesting that a higher assimilation rate was provided when stomata were more closed. Zeeshan et al. [55] observed A and E reduction in both salt-sensitive and tolerant common wheat. This fact can be interpreted as a plant response to the osmotic stress in order to limit transpiration and reduce the amount of intercellular CO_2 (Ci) in the stomata. According to Kalaji et al. [46], the first stage of the salinity effect on the photosynthesis of barley plants is the closing of the stomata (a decrease in g_s parameter) rather than a reduction in PSII activity. In our experiment, a strong correlation was found between the transpiration rate and stomatal conductance ($r = 0.909$; $p < 0.05$). A decrease in transpiration rate may be a positive mechanism that can help to conserve water and reduce salt loading with the transpiration flux in the plant [56]. In the SMH87 line, the transpiration rate did not decrease at 150 mM NaCl, similarly to the net photosynthetic rate. In C3 plants, stomatal closure is recognized as a major protective mechanism against osmotic stress that decreases CO_2 availability and photosynthetic activity. Non-reduction in photosynthetic efficiency with closed stomata in durum wheat plants may suggest a similarity to C4 plants, which can cope much better with stomatal closure, thanks to phosphoenolpyruvate carboxylase (PEPC) [50]. A weak but significant negative correlation was detected between CO_2 concentration in the stomata (Ci) and the net photosynthesis rate (A) ($r = -0.195$; $p < 0.05$). This result suggests that in the studied accessions of durum wheat, CO_2 from photorespiration could be used in the photosynthesis process. According to Garcia et al. [57] (2019), alternative carbon sources are important under stressful situations that reduce the uptake of atmospheric CO_2 due to partial stomatal closure respiration and photorespiration stimulates internal sources of CO_2.

Zeeshan et al. [55] compared two common wheat genotypes (salt-tolerant and salt-sensitive). Chlorophyll content decreased in the salt-sensitive genotype due to increasing salinity, whereas the net photosynthesis rate and intercellular CO_2 concentration decreased significantly in both genotypes. Stomatal conductivity was also drastically reduced. Saqib et al. [58] observed a decrease in all gas exchange parameters at 150 mM NaCl in both resistant and sensitive common wheat genotypes. In our study, the gas exchange parameters did not differentiate the studied genotypes.

According to Bose et al. [59], a high concentration of carotenoids may protect plants from ROS damage during various environmental stresses. The SMH87 line showed an increase in Chl *a* content with increasing salinity, whereas the other accessions showed the opposite tendency; however, BC_5Nax_2 plants had the highest Chl *a* and *b* content at 150 mM NaCl. A similar effect in this accession was observed for the carotenoid content. It is interesting that in these salt-resistant plants the content of the photosynthetic pigments was the highest at the highest NaCl concentration. Zheng et al. [60] showed that under increasing salinity, the content of chlorophyll and carotenoids decreased more in salt-sensitive genotypes of common wheat. In our study, the content of photosynthetic pigments was also a good parameter differentiating the degree of salinity sensitivity of the studied accessions.

Total carbohydrate content in the flag leaf of all studied accessions, regardless of salt sensitivity degree, was higher than in the control. A different result was published by Azizpour et al. [61] in an experiment on durum wheat genotypes treated with salt in the range of 0–200 mM NaCl. Geissler et al. [55] did not find an increase in carbohydrate content in the leaves of a halophyte Aster pripolium in any salt treatment. Based on these results, it can be supposed that carbohydrate content in the leaves is not a valid parameter differentiating plants in terms of their resistance to salinity.

3.2.2. Antioxidant System Phenolic Content, Antioxidant Enzymes, and H_2O_2

Abiotic stresses as high light, drought, salinity, anoxia, heavy metal ions induce plant immune responses through increased ROS production. The antioxidant compounds include enzymes as well as low-molecular phenolic compounds, e.g., flavonoids. [20,55,59,62–64]. The role of these compounds is ROS detoxification and protecting the organic compounds such as nucleic acids, proteins, and lipids. The synthesis of phenolics is generally induced in plants as a response to various stresses including salinity [56,57]. A reduced phenolic content was observed in Cynara cardunculus leaves under saline conditions [58]. According to Bose at al. [59], polyphenols are the key antioxidants limiting ROS damage in halophytes. Sharma et al. [65] stated that polyphenol oxidase activity is the strongest in salt-sensitive wheat and barley cultivars, intermediate in salt-tolerant genotypes, and the weakest in halophytes. These authors suggest that salt stress induces tissue damage in glycophytes but not in halophytes. In our study, the content of phenolics was also significantly affected by salt stress.

Low molecular weight antioxidants include mainly phenolic compounds [66]. In our work, we studied the content of total phenolic compounds (TPC) and cell wall-bound phenolic compounds (CWP). The latter plays a role in the plant's response to various stresses by strengthening the cell wall or preventing excessive water leakage. Unfortunately, the content of both types of phenolic compounds did not significantly differentiate the studied durum wheat accessions in terms of salinity tolerance. In the leaves of all accessions, TPC was reduced due to salinity, whereas in terms of CWP each accession responded specifically. Ashraf et al. [67] observed that in the salt-sensitive genotype of common wheat, phenolic content decreased at 150 mM NaCl, as compared with that of the control.

Overproduction of ROS in the organisms due to salinity stress contributes to the oxidation of proteins that play a signaling role in many metabolic processes. Studies on various crops, such as peas, rice, wheat, barley, and tomatoes confirm that high soil salinity contributes to oxidative stress [8,62,63]. ROS cause lipid peroxidation in cellular membranes, DNA damage, protein denaturation, carbohydrate oxidation, decrease pigment content,

and inhibit enzymatic activity [64,65]. Sairam et al. [68] found that wheat accessions differing in their salinity tolerance show different activity of antioxidant enzymes. Drought and salinity stress may induce light-dependent inactivation of the primary photochemistry associated with PSII. One of the most important antioxidant enzymes is CAT, which scavenges H_2O_2 in peroxisomes. In our study, all accessions showed an increase in CAT activity under increased soil salinity. POX activity varied specifically for each accession, although in all studied genotypes the highest POX activity was observed at 125 mM. Other authors obtained similar results. A reduction in POX activity due to salinity was observed in *Raphanus sativus* by Muthukumarasamy et al. [69], and in *Catharanthus roseus* (L.) by Jaleel et al. [70]. Decreasing POX activity under increasing salinity may indicate that this enzyme does not play a key role in defense mechanisms against salinity. It should be noted that POX is a more sensitive enzyme, activated at much lower concentrations of H_2O_2 than CAT. Peroxidases are activated at micromolar and CAT at millimolar concentrations of H_2O_2 [71]. Datir et al. [45] also observed an increase in CAT activity in wheat plants, especially in the salt-tolerant cultivar. Dioniso-Sese and Tobit [72] and Jaleel et al. [70] reported that higher NaCl concentrations reduced the activity of SOD in rice and *Catharanthus roseus*. In our study, a decrease in SOD activity under NaCl was also observed. Latef [73] claimed that in the salt-sensitive wheat genotype, the activity of SOD decreased under increasing salinity but it intensified in the intermediate and more salt-tolerant genotypes.

The concentration of H_2O_2 strongly differentiated the studied accessions. This result is opposite to that obtained by Sairam et al. [68], who reported an increase in H_2O_2 at 100 and 200 mM NaCl in wheat cultivars, regardless of their sensitivity to salinity. In the most salt-sensitive, cv. 'Tamaroi', at 125 mM NaCl, H_2O_2 concentration increased more than 7-fold in relation to the control, and was 2-fold higher than that recorded at the same salt concentration in the BC_5Nax_2 line, and 7-fold higher than in the SMH87 line. The rapid increase in the H_2O_2 level in cv. 'Tamaroi' leaves at 125 mM NaCl did not affect CAT activity, although for all NaCl doses a positive correlation between these parameters was confirmed. It could be speculated that the source of additional H_2O_2 in the flag leaf was photorespiration. Increased photorespiration may be a defensive response to the toxic impact of Na^{2+} and Cl^- on the photosystems. Voss et al. [74] suggested that photorespiration plays a major role in the regulation of redox homeostasis under drought and salinity. Hydrogen peroxide is recognized as a signal molecule taking part in, e.g., lignin synthesis and activation of transcription factors, so its high level is in fact required in defense response to stress [75]. However, its too high concentration can be dangerous for the cell. A very high concentration of H_2O_2 in cv. 'Tamaroi' and CAT activity at 125 mM NaCl probably indicated that the enzyme activity was too low to alleviate oxidative stress. Only in this cultivar, a negative correlation between H_2O_2 and TPC ($r = -0.410$; $p < 0.05$) was found, which may suggest that phenolic compounds were engaged in H_2O_2 scavenging. Despite the presence of two types of antioxidants, such high levels of hydrogen peroxide could cause oxidative damage. In conclusion, an extremely high concentration of H_2O_2 under salt stress, much higher than in the other accessions, may be an indicator of higher susceptibility to this stress.

3.3. Yield Parameters

In the studied accessions, all yield parameters decreased under increasing salinity. The greatest reduction in the yield parameters was observed in cv. 'Tamaroi', where a drastic drop was noted at 125 and 150 mM NaCl. The lowest loss in the grain yield was observed in the most salt-resistant BC_5Nax_2 line at all salt doses. Among all studied yield parameters, MTS was the most differentiating for the studied accessions in terms of salt tolerance. Yield limitation is a common effect of salt stress and it is observed in many crop plants [76]. James et al. [39] reported a smaller decrease in the grain yield of durum wheat in plants with *Nax*$_2$ locus than in the other genotypes, along with increasing NaCl concentration in the soil. Similar results for durum wheat were obtained by Husain et al. [77], whereas Poustini and Siosemardeh [78] described the same relationship in common wheat. Francois et al. [79]

also found a significant grain yield reduction in durum wheat with an increasing salt concentration in the soil. Moreover, the decrease was much greater than in common wheat genotypes.

4. Materials and Methods

4.1. Plant Material

The study was performed on three spring durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) accessions differing in their salt tolerance. They were two Australian accessions: the salt-sensitive cv. 'Tamaroi' and salt-resistant BC_5Nax_2 line obtained from Dr. Richard A. James from CSIRO Plant Industry, and a Polish SMH87 line obtained from Dr. Jarosław Bojarczuk from the Plant Breeding Centre in Smolice, Plant Breeding and Acclimatization Institute Group.

4.2. Experimental Design

Plant response to salinity was examined in two separate experiments (Figure 8). The first experiment was carried out in laboratory conditions in Petri dishes, where the plant response was determined in the germination phase. The analyses included the percentage of germinated seeds (PGS), germination vigor index (*Vi*), electrolyte leakage from seeds (EL), coleoptile length (CL), and salt susceptibility index (*SSI*). The second experiment was performed under controlled greenhouse conditions on plants in the phase of a fully developed flag leaf (BBCH–39) [80–82]. The flag leaves were used for the following analyses: chlorophyll *a* fluorescence, chlorophyll *a*, *b*, and carotenoid content (Chl *a*, *b* and Car), gas exchange, total soluble carbohydrate content (TSC), total phenolic content (TPC), cell wall-bound phenolic content (CWP), the activity of antioxidant enzymes, such as catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), and hydrogen peroxide content (H_2O_2). Moreover, yield parameters such as seed number per spike, seed dry weight per spike, and mass of one thousand seeds (MTS) were determined.

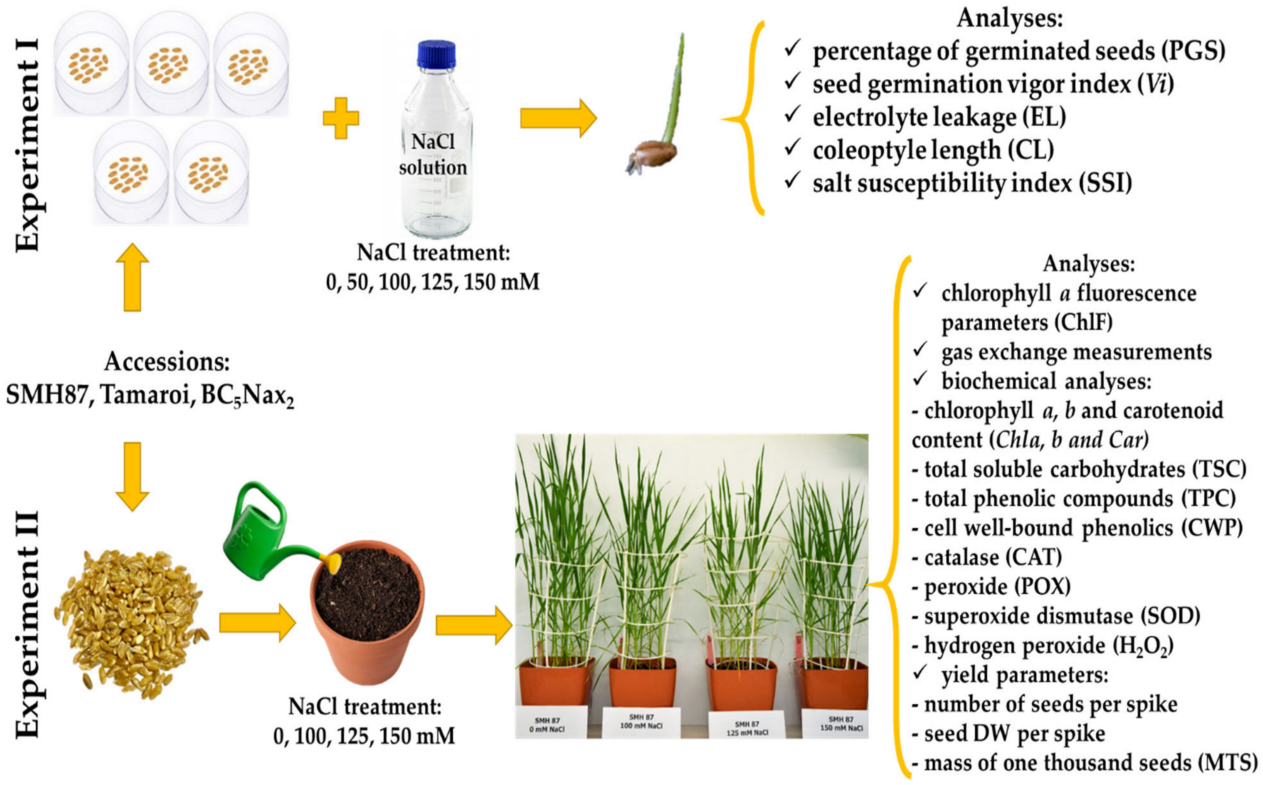

Figure 8. Design of the experiments.

4.3. Experiment 1—Laboratory Conditions

4.3.1. Percentage of Germinated Seeds (PGS), Germination Vigor Index (*Vi*), Coleoptile Length (CL), and Salt Susceptibility Index (SSI)

Seeds of each accession were surface sterilized with 70% ethanol for 1 min, rinsed three times for 2 min with sterile water, and placed into Petri dishes (∅ = 9 cm) with filter paper wetted with four sodium chloride (NaCl) solutions: 0 (control), 50, 100, 125, and 150 mM; (10 plates × 15 seeds × 4 NaCl solutions for each accession). The seeds were germinated in a growth chamber (ST 5 C Smart, Pol-Aura Aparatura, Wodzisław Śląski, Poland), at 25 °C without access to light. The percentage of germinated seeds (PGS) and germination vigor index (*Vi*) were evaluated two days after sowing in five replicates for each durum wheat accession and NaCl solution. *Vi* was evaluated on the basis of a visual scale of coleoptile length (CL) according to Płażek et al. [43].

A visual scale was used, where 0—no germination; 1—coleoptile length of 1 mm; 2—coleoptile length of 2–3 mm; 3—coleoptile length of 4–7 mm; and 4—coleoptile length greater than 7 mm. The *Vi* index was calculated according to the formula:

$$Vi = (n_0 \times 0 + \cdots + n_4 \times 4)/N$$

where: n_x—number of seeds assigned to a given coleoptile length; N—total number of seeds in the dish. *Vi* was assessed in five replicates for each accession and NaCl treatment.

Based on coleoptile length, SSI was estimated six days after sowing, according to the method described by Płażek et al. [43]. Coleoptile length (CL) was measured in 20 replicates for each NaCl treatment and accession. The influence of NaCl treatment on the percentage of germinated seeds and coleoptile length was presented as SSI using the following formula:

$$SSI = (1 - G_1/G_2) \times 100$$

where: G_1—seeds germinated in NaCl solution, G_2—seeds germinated in water (control). SSI for coleoptile length was calculated in a similar way. The results shown are the means of two independent experiments performed at the same time.

4.3.2. Electrolyte Leakage (EL)

To determine the plasma membrane permeability of the germinated seeds, three two-day-old germinated seeds with visible 2–3 mm coleoptile were collected from each accession and NaCl treatment. Next, the seeds were washed in distilled water and put into plastic vials containing 10 cm^3 of ultra-pure water and shaken for 24 h at 150 rpm at room temperature. After that, electrical conductivity (EL_1) was measured using a conductometer (CI 317, Elmetron, Zabrze, Poland), and then the samples were frozen at −80 °C for 24 h to achieve complete tissue degradation and to release all electrolytes. After 24 h, the samples were thawed and shaken again prior to the second measurement (EL_2). Electrolyte leakage from the seeds was expressed as a percentage of total EL according to the formula:

$$EL = (EL_1 \times 100)/EL_2$$

All the measurements were performed in 10 replicates for each accession and NaCl treatment.

4.4. Experiment 2—Greenhouse Conditions

4.4.1. Plant Cultivation

The seeds from each accession were sown into plastic pots (20 × 20 × 25 cm; five seeds per pot), in ten replicates (pots) for each accession and NaCl treatment (0, 100, 125, 150 mM). The plants were cultivated in commercial soil substrate, pH 5.8 (Ekoziem, Jurkow, Poland), mixed with sand (1:1; *v*/*v*). They were watered every day with the same volume of NaCl solution (300 cm^3), and once a week with Hoagland's medium [83]. Before sowing, the seeds were sterilized according to the procedure described in Experiment 1. The plants

were grown until seed maturity in a greenhouse (50° 04′ 10.195′′ N, 19° 50′ 44.763′′ E) at 22/20 ± 1 °C (day/night), in daylight (May–August). To determine the actual NaCl concentration in the soil, the soil conductivity was estimated for each NaCl treatment according to the method described by Płażek et al. [43]. The results of the soil conductivity test for each NaCl treatment are presented in Table 5.

Table 5. Conductivity of commercial soil watered with NaCl solution of 0, 100, 125, 150 mM.

NaCl (mM)	dS $m^{-1} \cdot s^{-1}$
0	1.695
100	11.150
125	13.640
150	17.560

4.4.2. Measurements of Chlorophyll *a* Fluorescence (ChlF)

Chlorophyll a fluorescence measurements were done with a plant efficiency analyzer (PEA; Hansatech Ltd., King's Lynn, UK). The measurements involved the flag leaves after 25 min of adaptation to darkness (clips with a hole 4 mm in diameter). Before the measurements, the LED light source of a fluorimeter was calibrated using an SQS light meter (Hansatech Ltd., King's Lynn, UK). Excitation irradiance had an intensity of 3 µmol m^{-2} s^{-1} (peak at 650 nm). Changes in fluorescence were recorded during irradiation between 10 µs and 1 s. During the initial 2 ms, the data were collected every 10 µs with 12-bit resolution. After this period, the frequency of measurements was reduced automatically. The data were used to calculate the following parameters based on the theory of energy flow in PSII and the JIP test [84,85]: ABS/CS_m—energy absorption by antennas, TR_o/CS_m—excitation energy trapped in PS II, ET_o/CS_m—energy used for electron transport, DI_o/CS_m—energy dissipation from PS II, RC/CS_m—number of active reaction centers, PI—performance index of PS II, F_v/F_0—maximum efficiency of water-splitting reaction of the donor side of PSII. All measurements were taken in ten replicates for each accession and NaCl treatment.

4.4.3. Measurements of Gas Exchanges Parameters

Gas exchange parameters measured in the flag leaves included photosynthesis rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). The analyses were performed with a CIRAS-3 infrared gas analyzer (PP Systems, Amesbury, MA, USA), with a Parkinson leaf chamber (PLC6). The irradiation system consisting of halogen lamps was applied. The airflow rate with a constant CO_2 concentration of 400 µmol mol^{-1} through the assimilation chamber was 300 cm^3 min^{-1}. The temperature of the leaves was 22 °C, the air humidity was 40%, and the irradiance was 800 µmol photon m^{-2} s^{-1}. To ensure the same conditions, all measurements were carried out between 9 am and 12 pm. All the measurements were taken in eight replicates for each accession and NaCl treatment.

4.4.4. Chlorophyll (*a*, *b*) and Carotenoid (Car) Content

The measurements of chlorophyll a, b, and carotenoid content were performed in the flag leaves using the method described by Czyczyło-Mysza et al. [86] with a 96-well plate spectrophotometer (Synergy II, Biotek, Winooski, VT, USA). Absorbance was read at 470, 648, and 664 nm, and the total concentration of the measured pigments was calculated according to the formulas described by Lichtenthaler and Buschman [87]:

$$\text{Chl}a\ (\mu g/cm^3) = 13.36\ A_{664} - 5.19\ A_{648}$$

$$\text{Chl}b\ (\mu g/cm^3) = 27.43\ A_{648} - 8.12\ A_{664}$$

$$\text{Car}\ (\mu g/cm^3) = (1000\ A_{470} - 2.13\ \text{Chla} - 97.64\ \text{Chlb})/209$$

where: Chl*a*—chlorophyll *a*, Chl*b*—chlorophyll *b*, A_{470}—absorbance at 470 nm, A_{664}—absorbance at 664 nm, A_{648}—absorbance at 648 nm. All the measurements were taken in three biological replicates for each accession and NaCl treatment.

4.4.5. Total Soluble Carbohydrate Content (TSC)

Total water-soluble carbohydrate content was analyzed in the flag leaves by the phenol-sulfuric method according to Dubois et al. [88], with the modification described by Bach et al. [89]. Carbohydrate estimation was done in the samples collected from the flag leaves and prepared as described in Section 4.4.3. To this end, 10 µL of the extract was diluted with water to 200 µL, 200 cm^3 of 5% phenol solution (*w*/*w*) was added, and 1 cm^3 of concentrated H_2SO_4 was dispensed. Then the samples were vortexed and incubated for 20 min at room temperature and transferred to 96-well plates. Absorbance was read spectrophotometrically at 490 nm (Synergy II, Biotek, Winooski, VT, USA). The sugar content was finally calculated using a calibration curve where a glucose solution was used as a calibration standard. All the measurements were taken in three replicates for each accession and NaCl treatment.

4.4.6. Total Phenolic Compound Content (TPC)

Estimation of total phenolic compounds was performed in the flag leaves using a method described by Singleton et al. [90], with a modification from Bach et al. [89]. To the extracts prepared as described in Section 4.4.3. A Folin–Ciocalteu phenol reagent diluted with water (5:2, *v*/*v*) was added and left for 10 min. Then, saturated sodium carbonate (c.a. 25% *w*/*w*) was added. The ratio of these compounds in the samples was 100/400/400 µL (*v*/*v*/*v*). Next, the samples were incubated for 2 h at room temperature in the dark. After that, they were centrifuged (21,000× *g*, for 15 min at 15 °C) and transferred to 96-well plates. The absorbance was read spectrophotometrically at 760 nm (Synergy II, Biotek, Winooski, VT, USA). All measurements were taken in three replicates for each accession and NaCl treatment.

4.4.7. Cell Wall-Bound Phenolic Content (CWP)

Content of CWP was assessed in the flag leaves according to Hura et al. [91]. The pellets obtained after pigment extraction (Section 4.4.3) were rinsed with 99.8% ethanol. Then, the samples were hydrolyzed with 3 M NaOH at room temperature overnight. Subsequently, concentrated HCl was added for the sample neutralization, and the samples were diluted with 1 cm^3 of ethanol. The resulting solutions were analyzed for released phenolics similarly to soluble forms, as described in Section 4.4.6. All the measurements were taken in three replicates for each accession and NaCl treatment.

4.4.8. Activity of Antioxidant Enzymes

Fresh plant material was collected from the flag leaves and homogenized in a hand mortar with 50 mM phosphate-potassium buffer (pH = 7.0) containing 0.1 mM EDTA (100 mg of FW plant material per 1 cm^3 of the buffer). After centrifugation (10,000× *g*, 15 min at 4 °C, 32R, Hettich, Germany), the supernatant was subsampled and transferred to a 96-well plate format (Synergy II, Biotek, Winooski, VT, USA). The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by the cytochrome reduction method according to McCord and Fridovic [92]. Catalase activity (CAT, E.C. 1.11.1.6) was measured at 240 nm according to the Aebi [93] method with H_2O_2 as a substrate. Peroxidase activity (POX, EC 1.11.1.7) was determined using the Lück [94] method with *p*–phenylenediamine as a substrate and absorbance was read at 485 nm. The analyses were conducted as described by Grudys et al. and references are cited therein [95–97]. The enzyme activities were calculated into protein content. The protein was analyzed using the Bradford [98] method. All the measurements were taken in three replicates for each accession and NaCl treatment.

4.4.9. Hydrogen Peroxide Content (H_2O_2)

The method of plant material homogenization was the same as for the enzyme activity analyses (Section 4.4.6). The homogenization was performed at 4 °C in 50 mM phosphate-potassium buffer (pH = 7.0) containing 0.1 mM EDTA. The content of hydrogen peroxide was assessed using a commercial Amplex Red (10-acetyl-3,7-dihydro-xyphenoxazine) reagent kit [99] from Invitrogen (Waltham, MA, USA), according to the method provided in the manufacturer's manual [100]. Pre-prepared samples of plant material were diluted with the reaction buffer, and the working solution containing a fluorescence probe precursor (Amplex Red). Next, 0.2 $U \cdot cm^{-3}$ of horseradish peroxidase was added and the samples were incubated for 30 min. Then they were transferred to a 96-well plate format (Synergy II, Biotek, Winooski, VT, USA) and fluorescence was read at Ex/Em 530/590 nm. The results were quantitated based on a calibration curve made for H_2O_2. All the measurements were taken in three replicates for each accession and NaCl treatment.

4.4.10. Yield Parameters

After the harvest, ripe seeds were collected and the yield parameters were evaluated. The number and dry weight (DW) of seeds per spike were calculated in 30 replicates, and then the mass of one thousand seeds (MTS) for each accession and NaCl treatment was evaluated.

4.5. Statistical Analyses

The experiments were arranged and performed with the application of a completely randomized design. The normal distribution of data was analyzed using the Shapiro–Wilk test. The two-way analysis of variance (ANOVA) and Duncan's multiple range test (at $p < 0.05$) were performed using the statistical package Statistica 13.3 (Stat–Soft, Inc., Tulsa, OK, USA). The data were presented as means ± SE (standard error). Pearson's correlation coefficients were assumed as statistically significant at $p < 0.05$. MS Excel 2016 was used for drawing figures.

5. Conclusions

1. The percentage of germinated seed (PGS) and the germination vigor (*Vi*) are the parameters most differentiating the durum wheat accessions in terms of salt tolerance in the germination phase.
2. Chlorophyll fluorescence parameters, such as maximum efficiency of water-splitting reaction of the donor side of photosystem II (PSII)—F_v/F_0 and energy dissipation from PSII—DI_o/CS_m can be used as non-invasive parameters differentiating durum wheat accessions in terms of salinity tolerance.
3. Salinity has a negative impact on grain yield by reducing the number of seeds per spike and the mass of one thousand seeds (MTS). The latter can be used as the most suitable parameter for determining tolerance to salinity stress.
4. The salt-resistant BC_5Nax_2 line is characterized by the highest percentage of germinated seeds (PGS) and germination vigor (*Vi*) at the germination stage, and the highest content of chlorophyll *a*, *b*, and carotenoids, and MTS at the heading stage.
5. The salt-sensitive cv. 'Tamaroi' shows significantly higher hydrogen peroxide levels at 125 and 150 mM NaCl, which proves considerable oxidative damage caused by salinity stress.
6. From among the examined parameters, we chose those that most effectively differentiate durum wheat genotypes in terms of their salinity tolerance. These results can be helpful for breeders in the selection of genotypes the most resistant to this stress.
7. Future research will include the analysis of proline content, hormonal profile in leaves, and the content of elements, especially the Na^+/K^+ ratio in the durum wheat genotypes examined in this study under salt stress.

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Abbreviations

A	*Net* photosynthetic rate
ABS/CS_m	Energy absorbed by the antennas
Car	Carotenoid
CAT	Catalase
ChlF	Chlorophyll *a* fluorescence
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
C_i	Intercellular CO_2 concentration
CL	Coleoptile length
CS_m	Excited cross section of a leaf
CWP	Cell wall-bound phenolic content
DI_o/CS_m	Energy dissipation from PSII
DW	Dry weight
E	Transpiration rate
EL	Electrolyte leakage
EL_1	Initial electrolyte leakage
EL_2	Final conductivity
ET_o/CS_m	Energy used for electron transport
F_v/F_0	Maximum efficiency of water-splitting reaction of the donor side of PSII
g_s	Stomatal conductance
H_2O_2	Hydrogen peroxide
MTS	Mass of one thousand seeds
TR_o/CS_m	Excitation energy trapped in PSII
RC/CS_m	Number of active reaction centers
PI	Performance index of PSII photochemistry
PGS	Percentage of germinated seeds
POX	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SSI	Salt susceptibility index
TPC	Total phenolic content
TSC	Total soluble carbohydrate content
Vi	Germination vigor index

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Physiological and Biochemical Response to *Fusarium culmorum* Infection in Three Durum Wheat Genotypes at Seedling and Full Anthesis Stage

Jakub Pastuszak [1,*], Anna Szczerba [1], Michał Dziurka [2], Marta Hornyák [1,3], Przemysław Kopeć [2], Marek Szklarczyk [4] and Agnieszka Płażek [1]

[1] Department of Plant Breeding, Physiology and Seed Science, University of Agriculture, Podłużna 3, 30-239 Kraków, Poland; anna.szczerba21@gmail.com (A.S.); marta.hornyak@botany.pl (M.H.); rrplazek@cyf-kr.edu.pl (A.P.)
[2] Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Kraków, Poland; m.dziurka@ifr-pan.edu.pl (M.D.); p.kopec@ifr-pan.edu.pl (P.K.)
[3] Polish Academy of Sciences, W. Szafer Institute of Botany, Lubicz 46, 31-512 Kraków, Poland
[4] Faculty of Biotechnology and Horticulture, University of Agriculture, 29 Listopada 54, 31-425 Kraków, Poland; marek.szklarczyk@urk.edu.pl
* Correspondence: jakub.pastuszak@urk.edu.pl

Abstract: *Fusarium culmorum* is a worldwide, soil-borne plant pathogen. It causes diseases of cereals, reduces their yield, and fills the grain with toxins. The main direction of modern breeding is to select wheat genotypes the most resistant to *Fusarium* diseases. This study uses seedlings and plants at the anthesis stage to analyze total soluble carbohydrates, total and cell-wall bound phenolics, chlorophyll content, antioxidant activity, hydrogen peroxide content, mycotoxin accumulation, visual symptoms of the disease, and Fusarium head blight index (FHBi). These results determine the resistance of three durum wheat accessions. We identify physiological or biochemical markers of durum wheat resistance to *F. culmorum*. Our results confirm correlations between FHBi and mycotoxin accumulation in the grain, which results in grain yield decrease. The degree of spike infection (FHBi) may indicate accumulation mainly of deoxynivalenol and nivalenol in the grain. High catalase activity in the infected leaves could be considered a biochemical marker of durum sensitivity to this fungus. These findings allowed us to formulate a strategy for rapid evaluation of the disease severity and the selection of plants with higher level, or resistance to *F. culmorum* infection.

Keywords: antioxidant enzymes; deoxynivalenol; *Fusarium culmorum*; mycotoxins; nivalenol; *Triticum durum*

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1. Introduction

Fungi of *Fusarium* species are responsible for numerous diseases in wheat and other small grain cereals cultivated worldwide. *Fusarium culmorum (Wm.G. Sm.) Sacc.* is a threat to plants at every stage of their development. The infection evoked by this pathogen is a serious problem in cereal agriculture. The most common symptoms of Fusarium wilt in wheat include Fusarium seedling blight (FSB), root rot, and Fusarium head blight (FHB). These symptoms have especially disadvantageous effects on plant growth, development, grain yield, and its quality [1–3]. The yield reduction is an outcome of damaged kernels which appear discolored and shriveled. *Fusarium culmorum* belongs to the fungi producing numerous dangerous toxins, such as deoxynivalenol (DON) (Figure 1A), nivalenol (NIV) (Figure 1B), T-2 toxin (Figure 1C), and zearalenone (ZEN) (Figure 1D). These mycotoxins represent the trichothecenes family, i.e., epoxy-sesquiterpenoid metabolites responsible for pathogenic virulence and protein synthesis [4,5]. Food products and fodder contaminated with secondary metabolites of *F. culmorum* may evoke severe and chronic harm to human and domestic animal health [6–8]. In the food industry, grain infected with *Fusarium*, in which the level of mycotoxins exceeds the permissible EU standards, must be discarded.

The maximum limit of toxins are: 750 μg·kg^{-1} DON and 75 μg·kg^{-1} ZEN in flour, and 500 μg·kg^{-1} DON and 50 μg·kg^{-1} ZEN in bread. The toxin levels are also established for feed production at 900 μg·kg^{-1} DON for pigs and 100 μg·kg^{-1} ZEN for piglets [9,10].

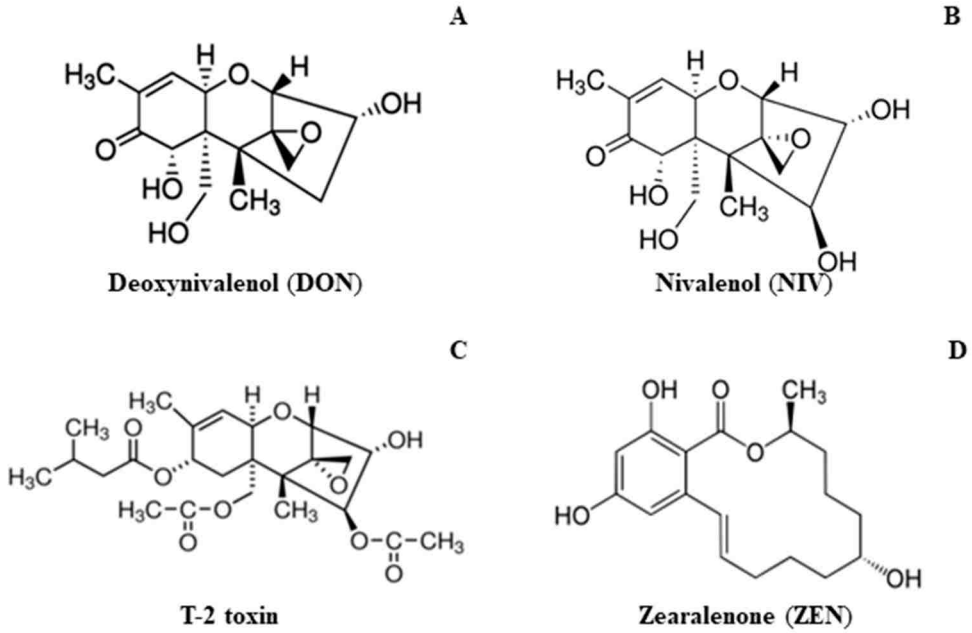

Figure 1. Secondary metabolites (mycotoxins) produced by *Fusarium culmorum*: (**A**) deoxynivalenol (DON), (**B**) nivalenol (NIV), (**C**) T-2 toxin, (**D**) zearalenone (ZEN).Source: Sigma-Aldrich.

Resistance to *Fusarium* head blight is a complex, quantitative trait. Several types (mechanisms) of resistance were identified, and they were described as: Type I—resistance to an initial infection; type II—resistance to the pathogen spread within the host; type III—kernel damage; type IV—tolerance to trichothecene toxins; type V—resistance to toxin accumulation [11,12]. In response to the presence of the pathogen, the host plant activates defense processes, e.g., alters the production of some biochemical components, such as soluble sugars, phenolic compounds, hormones, or reactive oxygen species (ROS) [13]. Sugars play a pivotal role in the immune processes, especially in pathogen attacks, by initiating a signal transduction pathway and regulating the osmotic potential [14–16]. Increased concentration of phenolic compounds is toxic to pathogens and prevents further infection. Phenolics are involved in the lignification of the cell wall, which increases the structural barrier that hinders the spread of the pathogen within the host plant tissue. The lignification may reduce the transfer of nutrients from the host plant cell to the pathogen [17]. Due to their toxic nature, phenolic compounds, such as phytoalexins, are considered activators of pathogen resistance genes and modulators of pathogen toxicity [18]. Another way to prevent pathogen infection is a mechanism that involves the production of enzymatic and non-enzymatic antioxidants, and scavenging of reactive oxygen species (ROS) [19]. The ROS includes non-radical molecules, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), as well as free radicals, such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) [20]. Reactive oxygen species can perform three functions: They can act as cell-damaging agents, signal transduction molecules, and can provide protection against pathogenic microbes [21]. Excessive production of ROS is often called an oxidative burst. Overproduction of ROS can lead to protein and chlorophyll oxidation, damage to nucleic acids, lipid peroxidation, or initiation of programmed cell death [22,23]. Reactive oxygen species accumulation is counteracted by the activation of enzymatic antioxidants, such as catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), and non-enzymatic antioxidants, such as low molecular weight (LMW) phenolics and carotenoids [21,24,25]. Catalase is responsible for the decomposition of H_2O_2 into H_2O and O_2, as well as for

the regulation of H_2O_2 concentration in plant tissues. This enzyme is involved in plant development, but also plays an important role in plant resistance to pathogens and aging processes [26]. Peroxidases have a similar function to CAT, as they are involved in scavenging ROS in response to pathogen-plant interactions. In addition, POXs are responsible for the oxidation of phenolics, making them more toxic towards pathogens, lignin biosynthesis, suberization, and growth of the plant cell walls [27]. Superoxide dismutase plays an equally pivotal role in maintaining redox balance and defense response in plants exposed to stress. Its task is to catalyze the dismutation of $O_2^{\bullet -}$ and $HO_2^{\bullet}$ (hydroperoxide radical) to H_2O_2 and H_2O. Superoxide dismutase is the first line of defense against a pathogen attack and protects plants from oxidative stress [28]. Hydrogen peroxide also plays a significant role in pathogen defense. Thanks to its antimicrobial properties, it can induce local and systemic resistance to pathogen infection in plants [29].

Pathogen presence can also affect the level of chlorophyll pigments and their activity, resulting in altered efficiency of photosystem II (PS II) [30]. Similar observations were reported by other authors examining the photosynthetic pigment content after *F. culmorum* infection in tomato [31] and barley [32]. The investigated pathogen predominates in cooler areas of northern, central, and western Europe, and it infects wheat, barley, and oats [33]. Grain of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is used primarily in the production of pasta and to a lesser extent in the production of bread and groats. Although, durum wheat originates from the Mediterranean region and the countries of the Middle East is also very sensitive to *F. culmorum* [34]. Recent years have brought increased interest in durum wheat cultivation in Poland. Major problems with this crop include its high sensitivity to drought, soil salinity, cadmium accumulation, and *Fusarium* infections [34–37]. Durum wheat, as compared with common wheat (*T. aestivum*), is characterized by higher sensitivity to *Fusarium* infection. This is attributed to its morphological traits, such as early flowering, longer awn, another retention inside the floret, spike compactness, and genetic differences, such as the presence of type I rather than type II resistance genes [38,39].

In the presented study, three durum wheat accessions were assessed in terms of resistance to *Fusarium* diseases at two stages of their ontogenesis: Two-week-old seedlings and full anthesis stage—65 BBCH scale [40]. The defense response of the studied durum genotypes included evaluation of the resistance degree in the seedlings by means of visual inspection of the leaves and roots, and fresh weight measurements. We also determined the content of total soluble carbohydrates, total soluble phenolics and cell wall-bound phenolics, chlorophyll pigments, hydrogen peroxide, and antioxidant enzymes activity. At the full anthesis stage, we visually evaluated the resistance to *Fusarium* head blight, and measured the content of mycotoxins (deoxynivalenol, nivalenol, T–2 toxin, and zearalenone), and yield parameters. The main objective of the study was to identify physiological or biochemical markers of resistance to *F. culmorum* at both developmental stages in three durum wheat accessions. The investigation was carried out on Polish line SMH87 and two Australian accessions: cv. 'Tamaroi' and BC_5Nax_2 line. The selected genotypes differed in the degree of resistance to salinity and were the subject of our earlier studies on cadmium accumulation in the grain of durum wheat [36,37].

2. Results

2.1. Experiment I

2.1.1. Disease Rating (DR) and Fresh Weight (FW) Loss

The visual disease rating (DR) included only the seedlings infected with *F. culmorum*, so control seedlings were not evaluated. In this experiment, the vigor of plant seedlings at the three-leaf stage was assessed. All analyses, described in Section 2.1., were done on material collected in this developmental stage. The leaves and roots of SMH87 line and cv. 'Tamaroi' showed higher sensitivity to the pathogen infection than those of the BC_5Nax_2 line (Figure 2). The highest percentage of leaf DR was observed in SMH87 plants, while the lowest in the BC_5Nax_2 line. Root infection results were similar in SMH87 and cv. 'Tamaroi'.

Roots of BC_5Nax_2 were infected to the same degree as in cv. 'Tamaroi', but lower than in SMH87.

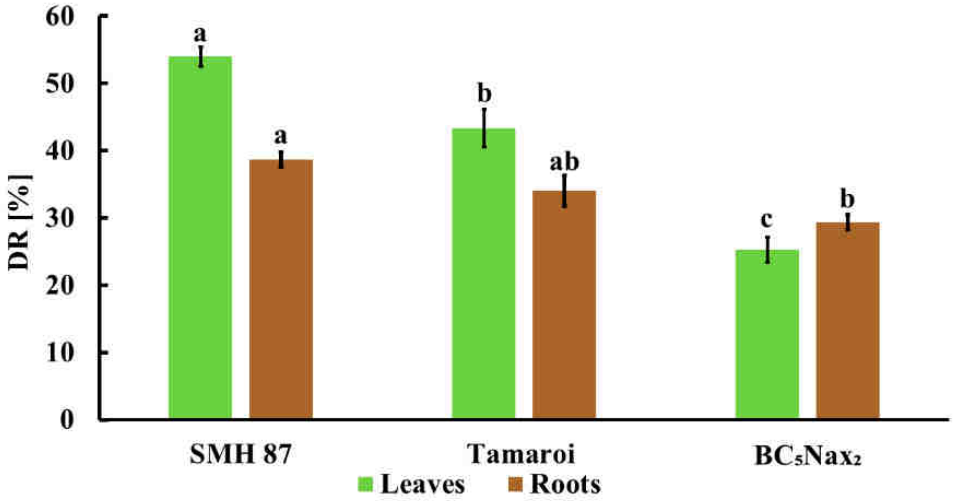

Figure 2. Disease rating (DR) in the leaves and roots of three durum wheat genotypes infected with *F. culmorum*. The values represent means (n = 30) ± SE (standard error). Different superscript letters (a–c) for each organ indicate significant differences between means (Duncan's multiple range test; $p < 0.05$).

Fusarium culmorum infection negatively affected leaf and root FW in all studied accessions (Figure 3). The effects were more pronounced in the roots than in the leaves. In SMH87 and cv. 'Tamaroi' FW reduction of the infected leaves was greater than in BC_5Nax_2. *Fusarium* infection caused a 37% decrease in leaf FW in both SMH87 and cv. 'Tamaroi' as compared with control. The infected roots of SMH87 and cv. 'Tamaroi' demonstrated a 66% decrease in FW in comparison with control. BC_5Nax_2 line did not show FW reduction in the infected leaves, but in the infected roots, FW loss amounted to 39% versus that of control.

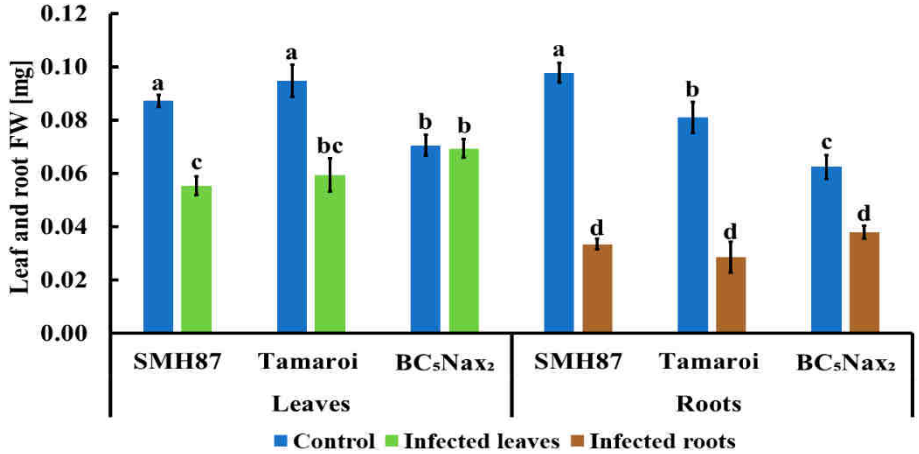

Figure 3. Effects of *F. culmorum* infection on fresh weight (FW) of the leaves and roots of three durum wheat accessions. The values represent means (n = 30) ± SE. Different superscript letters (a–d) for each organ indicate significant differences between means (Duncan's multiple range test; $p < 0.05$).

2.1.2. Chlorophyll *a*, *b*, and Carotenoid (Chl*a*, *b*, and car) Content

The infection reduced the contents of chlorophyll *a*, *b*, and carotenoids (Chl*a*, *b*, and car) in all studied durum wheat accessions (Figure 4A). The greatest decrease in all pigments was observed in SMH87 leaves. Chlorophyll *a* content dropped by 48%, Chl*b* by 40%,

and carotenoids by 44% as compared with control plants. The other studied accessions also showed pigment content reduction in the infected plants; however, these differences, although significant, were not as drastic as in the SMH87 line.

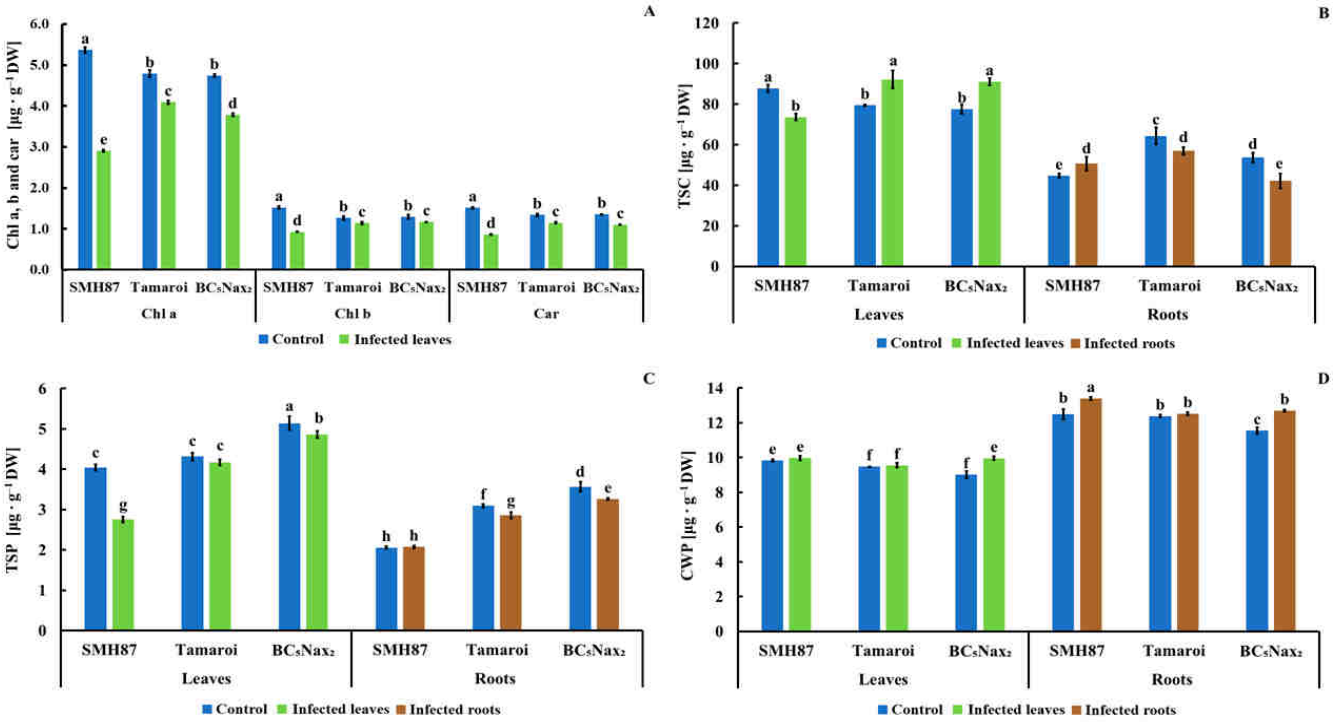

Figure 4. Effect of *F. culmorum* infection on the content of (**A**) chlorophyll *a*, *b* (Chl*a*, *b*) and carotenoids (Car), (B) total water-soluble carbohydrates (TSC), (**C**) total soluble phenolics (TSP), (**D**) cell wall-bound phenolics (CWP) in the leaves and roots of three durum wheat accessions. The values represent means (n = 30) ± SE. Different superscript letters (a–h) for each organ indicate significant differences between means (Duncan's multiple range test; $p < 0.05$).

2.1.3. Total Soluble Carbohydrates (TSC)

As a result of the infection, TSC content in SMH87 plants (Figure 4B) decreased in the leaves and increased in the roots, as compared with control. An opposite trend was observed in cv. 'Tamaroi' and BC_5Nax_2, where the infection triggered an increase in TSC in the leaves and a drop in the roots as compared with control.

2.1.4. Total Soluble Phenolics (TSP)

The studied genotypes differed in the content of total soluble phenolics (TSP) in the control and infected leaves and roots (Figure 4C). Line SMH87 showed a significant, almost 32% decrease of TSP content in the infected leaves, but no changes in the roots. In the infected leaves and roots of BC_5Nax_2 a reduction in TSP was observed, while in cv. 'Tamaroi' the infection decreased phenolic content in the roots. Total soluble phenolics levels in the leaves of this cultivar were unaffected by the infection.

2.1.5. Cell Wall-Bound Phenolics (CWP)

Fusarium infection boosted CWP only in the leaves of BC_5Nax_2 (Figure 4D). In the roots of SMH87 and BC_5Nax_2, an increase in CWP was observed upon inoculation. In other cases, the infection did not cause significant changes in the content of these compounds.

2.1.6. Catalase (CAT) Activity

The greatest increase in CAT activity is due to the infection was observed in SMH87 leaves. In cv. 'Tamaroi', it was diminished, and in the BC_5Nax_2 line, it was unaffected by the infection (Figure 5A). Catalase activity was considerably higher in control roots of cv.

'Tamaroi' and BC_5Nax_2 than in the infected ones. There was no difference in CAT activity in the infected and control SMH87 roots.

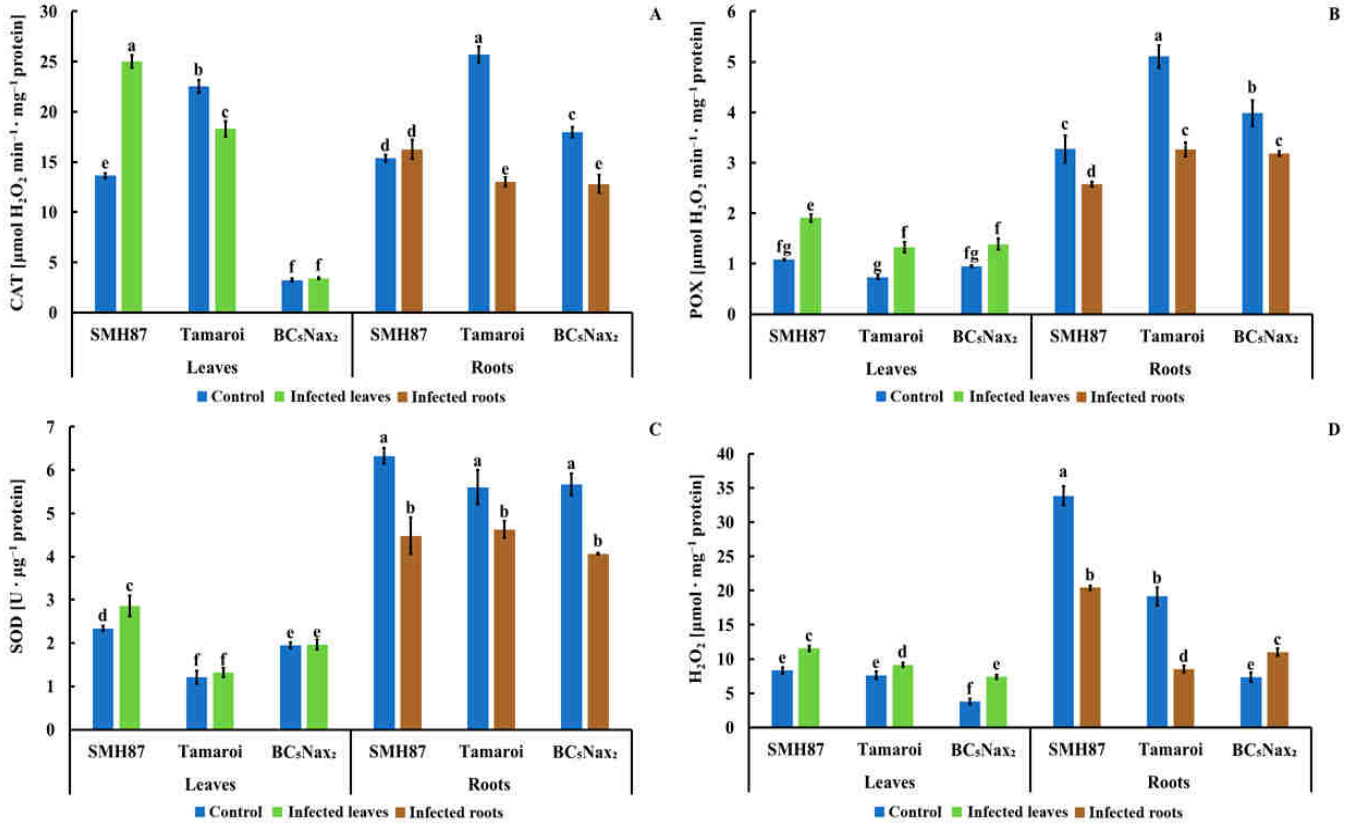

Figure 5. Effects of *F. culmorum* infection on catalase (**A**), peroxidases (**B**), and superoxide dismutase (**C**) activity, and H_2O_2 (**D**) content in the leaves and roots of three durum wheat accessions. The values represent means (n = 3) ± SE. Different superscript letters (**a–g**) for each organ indicate significant differences between means (Duncan's multiple range test; $p < 0.05$).

2.1.7. Peroxidases (POXs) Activity

An increase in POXs activity was observed in the leaves of all infected genotypes (Figure 5B). The leaves of SMH87 and cv. 'Tamaroi' showed a 44% increase in POXs activity as compared with control, while in BC_5Nax_2 leaves, this increase amounted to 31%. In the roots ofSMH87, 'Tamaroi', and BC_5Nax_2, the infection brought about a significant decrease in POXs activity by 23, 35, and 19%, respectively.

2.1.8. Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was significantly higher only in the infected leaves of the SMH87 line as an effect of *Fusarium* infection (Figure 5C). In the case of other accessions, the infection did not change SOD activity. In the infected roots of SMH87, cv. 'Tamaroi', and BC_5Nax_2 the infection decreased activity of this enzyme by 30%, 18%, 28%, respectively.

2.1.9. Hydrogen Peroxide (H_2O_2) Content

Hydrogen peroxide content increased in the leaves of all studied accessions as an effect of infection (Figure 5D). The highest increase in H_2O_2 level was noted in the infected leaves of BC_5Nax_2 plants. A rapid decrease in H_2O_2 content was noted in the infected roots of SMH87 (39%) and cv. 'Tamaroi' (55%), while in BC_5Nax_2 plants, the infection enhanced H_2O_2 level in the roots by 33% vs. control.

2.2. Correlation Analysis

The disease symptoms assessed with the visual disease rating (DR) negatively correlated with Chl*a*, *b*, and Car content, and with FW of the leaves and roots (Table 1).

Table 1. Pearson coefficients of linear correlation ($p < 0.05$) between the disease rating (DR) and chlorophyll *a, b* (Chl*a*, *b*), and carotenoid (Car) content and fresh weight (FW) of the leaves and roots.

Variable	Chl *a*	Chl *b*	Car	FW of Leaves	FW of Roots
DR in leaves	−0.859	−0.804	−0.870	−0.715	−0.755
DR in roots	−0.891	−0.811	−0.898	−0.672	−0.821

Significant correlations between H_2O_2 content and the investigated enzymes were found (Table 2).

Table 2. Pearson coefficients of linear correlation ($p < 0.05$) between H_2O_2 content in the leaves and roots and the activity of catalase (CAT), peroxidases (POX), superoxide dismutase (SOD), as well as total soluble phenolic (TSP) and cell wall-bound phenolic (CWP) content in the leaves and roots of three accessions of durum wheat.

Variable	H_2O_2 Content	
	Leaves	Roots
CAT in leaves	0.710	ns
CAT in roots	ns	ns
POX in leaves	0.688	ns
POX in roots	ns	ns
SOD in leaves	ns	ns
SOD in roots	ns	0.613
TPC in leaves	−0.863	ns
TPC in roots	−0.763	−0.658
CWP in leaves	0.679	ns
CWP in roots	0.861	ns

ns—values not significant.

In the leaves, CAT and POX positively correlated with H_2O_2 content. This relationship was not observed in the roots. Superoxide dismutase activity correlated positively with H_2O_2 only in the roots (r = 0.613, $p < 0.05$), proving that the higher SOD activity, the more H_2O_2 was produced. In the leaves, TPC content negatively correlated with H_2O_2 levels, while TPC content in the roots negatively correlated with H_2O_2 concentration in both studied organs (Table 2.). Significant correlation between TSP and TSC (r = 0.63118, $p < 0.05$) was found.

2.3. Experiment II

2.3.1. Fusarium Head Blight Index (FHBi) and Yield Parameters

Index FHBi showed a significant difference between the studied genotypes regarding *F. culmorum* resistance (Table 3). The time after infection on the spikes significantly impacted the disease development. The research revealed the fastest and the strongest spike infection (10%) in the SMH87 line seven days after the first inoculation. After 14 days, a 71% increase in FHBi of SMH87 spikes vs. the first evaluation was observed. The spike inoculation with *Fusarium* spores significantly reduced the amount of grain per spike in all studied genotypes (Table 3). A significantly higher grain reduction occurred in SMH87, where it was 83% greater than in control. A strong reduction in grain number (64%) was also observed in cv. 'Tamaroi'.

Table 3. *Fusarium* head blight index (FHBi) evaluated 7 and 14 days (DAI 7 and DAI 14) after spike inoculation with *F. culmorum* and yield components of three durum wheat genotypes.

Accession	Treatment	FHBi [%]		Amount of Grain Per Spike	Grain Mass Per Spike (g)	Mass of One piece of Grain (g)	MTS (g)
		DAI 7	DAI 14				
SMH87	Control	-	-	12.3 ± 1.4^{b}	0.328 ± 0.048^{b}	0.050 ± 0.003^{b}	36.437 ± 0.319^{b}
	Inoculum	10.1 ± 1.8^{a}	34.7 ± 4.1^{a}	2.0 ± 0.4^{d}	0.055 ± 0.016^{d}	0.012 ± 0.003^{d}	15.603 ± 0.348^{d}
Tamaroi	Control	-	-	16.8 ± 1.2^{a}	0.459 ± 0.047^{a}	0.049 ± 0.005^{b}	37.674 ± 1.077^{b}
	Inoculum	4.8 ± 0.9^{b}	8.3 ± 1.1^{b}	5.9 ± 1.0^{d}	0.099 ± 0.019^{d}	0.016 ± 0.004^{d}	16.200 ± 1.853^{d}
BC_5Nax_2	Control	-	-	13.6 ± 1.2^{b}	0.355 ± 0.042^{b}	0.061 ± 0.004^{a}	51.763 ± 0.602^{a}
	Inoculum	3.0 ± 0.7^{b}	10.7 ± 1.9^{b}	10.9 ± 1.1^{c}	0.209 ± 0.023^{c}	0.028 ± 0.004^{c}	28.209 ± 1.905^{c}

Values represent means ± SE. Fusarium head blight index (FHBi), amount of grain, grain mass per spike, and mass of one piece of grain were calculated in 45 replicates, while mass of one thousand seeds (MTS) were calculated in 3 replicates. Different superscript letters (a–d) FHBi and yield parameters indicate significant differences between means within columns (Duncan's multiple range test; $p < 0.05$).

BC_5Nax_2 line showed the lowest reduction of grain yield per spike in the inoculated plants. The analysis of yield parameters showed significant differences in grain mass per spike and in the mass of one piece of grain, as well as MTS of the control and inoculated plants (Table 3). A significantly higher (83%) reduction of grain mass per spike was observed in SMH87 plants. BC_5Nax_2 plants showed a significantly lower (by 40%) reduction in grain mass per spike as compared with the other studied accessions. Analyses of the mass of one piece of grain and of one thousand grains revealed significant differences between the studied accessions (Table 3). A significant loss of a single grain mass was observed in the SMH87 line, dropping by 75% vs. control plants. A 45% reduction in MTS was observed in BC_5Nax_2 plants. This genotype showed the lowest MTS reduction, while the highest MTS reduction was seen in SMH87.

2.3.2. Mycotoxin Content

Trace amounts of DON and ZEN were found in the grain of all control plants, while the concentration of T–2 was ten times higher (Table 4). The amount of NIV in control plants of 'Tamaroi' and BC_5Nax_2 was four times higher than that in SMH87. The infection considerably increased the levels of NIV, DON, and T–2 toxins. The highest amount of NIV was recorded in cv. 'Tamaroi' grain after inoculation and it was six times higher than that of the control. In the grain of the infected SMH87, the level of this toxin was 18 times higher than in control, while in the infected BC_5Nax_2 plants—four times higher. In BC_5Nax_2 grain, NIV level after inoculation increased four times as compared with control. As in the case of NIV, cv. 'Tamaroi' grain contained the highest level of DON. The grain content of ZEN was the lowest among the other studied toxins. Inoculation did not increase ZEN content in SMH87 seeds, while in cv. 'Tamaroi' and BC_5Nax_2, the level of this toxin was more than six times higher than in the control. In all cases, inoculation slightly increased the content of T–2 in the seeds as compared with that of the control. The highest increase in T–2 was found in cv. 'Tamaroi' (2.3×), while in SMH87 and BC_5Nax_2, the increase was 1.64 and 1.75×, respectively. To summarize, cv. 'Tamaroi' showed the highest toxin accumulation in grain.

Table 4. Content [μg kg^{-1}] of nivalenol (NIV), deoxynivalenol (DON[1]), T-2 toxin (T–2), and zearalenone (ZEN[2]) in the grain after *F. culmorum* spike inoculation.

Accession	Treatment	NIV	DON [1]	ZEN [2]	T-2
SMH87	Control	0.098 ± 0.008^{d}	0.093 ± 0.002^{c}	0.008 ± 0.001^{e}	0.101 ± 0.003^{e}
	Inoculum	1.785 ± 0.073^{b}	5.763 ± 0.137^{b}	0.002 ± 0.001^{e}	0.166 ± 0.013^{c}
Tamaroi	Control	0.414 ± 0.021^{c}	0.037 ± 0.003^{c}	0.004 ± 0.001^{d}	0.136 ± 0.006^{d}
	Inoculum	2.474 ± 0.109^{a}	8.052 ± 0.373^{a}	0.031 ± 0.001^{b}	0.317 ± 0.010^{a}
BC_5Nax_2	Control	0.406 ± 0.026^{c}	0.047 ± 0.002^{c}	0.022 ± 0.002^{c}	0.126 ± 0.004^{d}
	Inoculum	1.765 ± 0.018^{b}	6.208 ± 0.318^{b}	0.138 ± 0.002^{a}	0.221 ± 0.005^{b}

Values represent means ($n = 3$) ± SE. Different superscript letters (a–e) within columns indicate significant differences between means (Duncan's multiple range test; $p < 0.05$). DON [1] total amount of deoxynivalenol (DON), and 3–acetyldeoxynivalenol (3AcDON) and 15–acetyldeoxynivalenol (15AcDON); ZEN [2] total amount of zearalenone (ZEN); alpha–Zearalanol (α–ZAL), beta–Zearalanol (β–ZAL); alpha–Zearalanol (α–ZEL) and beta–Zearalenol (β–ZEL).

2.3.3. Correlation Analysis

Fusarium head blight index (FHBi) evaluated 7 and 14 days after inoculation negatively correlated with the number of seeds and their mass (Table 5). The content of NIV and DON significantly decreased all studied yield parameters, while ZEN reduced only the mass of a single grain. No correlation between the content of T–2 and the evaluated yield parameters was found. The latter two results can be explained by low content of both toxins.

Table 5. Pearson's coefficients of linear correlation ($p < 0.05$) between *Fusarium* head blight index (FHBi) evaluated 7 and 14 days after inoculation (DAI 7 and DAI 14), mycotoxin content in grain and yield parameters.

Variable	FHBi DAI 7	FHBi DAI 14	NIV	DON [1]	ZEN [2]	T–2
Number of grain per spike	−0.591	−0.575	−0.503	−0.549	ns	ns
Mass of grain per spike [g]	−0.589	−0.576	−0.566	−0.612	ns	ns
Mass of a single grain [g]	−0.754	−0.743	−0.863	−0.864	−0.714	ns
MTS [g]	−0.741	−0.848	−0.551	−0.589	ns	ns

DON [1] total amount of deoxynivalenol (DON), 3–acetyldeoxynivalenol (3AcDON) and 15–acetyldeoxynivalenol (15AcDON); ZEN [2] total amount of zearalenone (ZEN); alpha–Zearalanol (α–ZAL), beta–Zearalanol (β–ZAL); alpha–Zearalanol (α–ZEL) and beta–Zearalenol (β–ZEL); MTS-mass of thousand seeds; ns—values not significant.

Strong correlation was detected between the visual assessment of *Fusarium* head blight index (FHBi) evaluated in both terms (7 and 14 days after infection) and the grain content of all investigated mycotoxins, except for T–2 (Table 6). Index FHBi positively correlated with the content of DON and NIV, while in the case of ZEN, a significant correlation was found only seven days after the infection.

Table 6. Pearson's coefficients of linear correlation ($p < 0.05$) between *Fusarium* head blight index (FHBi) evaluated 7 and 14 days after the inoculation (DAI 7 and DAI 14) and mycotoxin content in the grain.

Variable	NIV	DON [1]	ZEN [2]	T-2 Toxin
FHBi DAI 7	0.731	0.733	0.484	ns
FHBi DAI 14	0.630	0.632	ns	ns

DON [1] total amount of deoxynivalenol (DON), 3–acetyldeoxynivalenol (3AcDON) and 15–acetyldeoxynivalenol (15AcDON); ZEN [2] total amount of zearalenone (ZEN); alpha–Zearalanol (α–ZAL), beta–Zearalanol (β–ZAL); alpha–Zearalanol (α–ZEL) and beta–Zearalenol (β–ZEL); MTS-mass of thousand seeds; ns—values not significant.

3. Discussion

Fusarium culmorum attacks plants at various developmental stages. The pathogenesis is responsible for the formation of seedling blight and root rot, which limit seedling emergence and plant development [32]. In our experiment, the infection caused a darkening of the roots and slower leaf growth. The studied genotypes differed more in the degree of leaf than root infestation. SMH87 line was the most, and $BC_5Nax_2$the least heavily infested. Medium infestation degree was observed in cv. 'Tamaroi'. This result was surprising, since the genotypes originating from a much warmer and drier climate were less severely infected than the original genotype from Poland. The infection degree was visible in leaf FW loss: BC_5Nax_2 genotype did not show changes in fresh leaf weight, while the decrease in root weight, although significant, was the smallest among the studied genotypes. Similar results were obtained by Grey and Mathre [41] in barley, by Wojciechowski et al. [42] in winter wheat, and by Warzecha et al. [43] in oats. These authors suggest that the most severe damage caused by *Fusarium* seedling blight appeared in the roots. It indicates that visual evaluation of root infestation may be more useful than leaf assessment. According to Malalaseker et al. [44] and Knudsen et al. [45], root rot may also develop, due to a prior infestation of hypocotyls and shoots. Root infection negatively affects proper plant

development and disturbs basic physiological processes, such as distribution of assimilates, water uptake and transport, and soil mineral absorption. These disturbances result in reduced seedling vigor and interrupted growth which negatively affects grain quality and yield.

Fungal mycelium penetrates the host-plant cells and limits access to nutrients and water. Released toxins disrupt metabolic and physiological processes. This leads to the reduction of photosynthetic pigment content and disturbances of photosynthesis [44]. Our study demonstrated that *F. culmorum* infection significantly decreased the content of chlorophyll *a*, *b*, and carotenoids in the leaves. Similar observations were published by other researchers examining the content of photosynthetic pigments after *F. culmorum* infection in tomato [31] or barley [32]. In our study, the results of visual assessment of DR in the leaves and roots negatively correlated with the content of Chl*a*, *b*, and Car.

Soluble sugars play an important role in plant development and metabolism, and therefore, their content fluctuates during plant infection. Soluble sugars in the host-plant cells are a source of carbon for the pathogen [46–48]. Sucrose was shown to induce defense mechanisms in the infected cells. The hexose, through signal transduction by hexokinase, increases the production of peroxidases and proteins directly related to pathogenesis [14,16]. Soluble sugars, as compounds with higher osmotic potential, limit the spread of the infection. Moreover, they isolate healthy cells from the infected ones and protect them against water loss [49]. Our analyzes of TSC showed that infection significantly increased TSC content in cv. 'Tamaroi' and BC_5Nax_2 leaves and decreased TSC levels in the roots. A contrary trend was observed in the leaves and roots of SMH87 line. Warzecha et al. [32] noted an increased sugar content in the leaves and their decrease in the roots of barley infected by *F. culmorum*. Morkunas et al. [16] reported that increased content of soluble sugars supported the resistance of *Lupinus luteus* L. to *F. oxysporum* infection, while Gaudet et al. [15] observed a similar correlation in wheat infested by snow mold fungi. Bani et al. [50] suggested that *Fusarium* species infection during seed germination disrupted sugar distribution between cotyledons and the tissues of embryo axis in the germinating seeds. Formela-Luboińska et al. [51] reported that soluble carbohydrates reduced sporulation of *F. oxysporum* f. sp. *lupini* and limited the production of moniliformin toxin synthesized by this *Fusarium* species. In our study, the more resistant Australian accessions (cv. 'Tamaroi', BC_5Nax_2) showed higher sugar content in the leaves of infected seedlings than Polish SMH87. Our research demonstrated that sugar content in the leaves was a stronger indicator of *F. culmorum* resistance than that in the roots.

Synthesis of phenolic compounds is a well-known defense response to pathogen attack. Their biosynthesis occurs both before and after the infection [52]. The defensive role of phenolics in fungal infections in plants was confirmed in our previous studies [53–55]. The phenolic compounds involved in the immune response to pathogen attack include salicylic and chlorogenic acids. Salicylic acid controls the content of the signal molecule hydrogen peroxide (H_2O_2) responsible for plant resistance to environmental stresses. Salicylic acid activates superoxide dismutase (SOD), which boosts H_2O_2 production and stimulates the synthesis of pathogenesis related proteins (PR)—chitinases and glucanases that decompose the cell wall of the fungal hyphae [56,57]. Salicylic acid participates in systemic acquired resistance (SAR). This reaction is triggered in the case of biotrophic fungi infection. The fungi from *Fusarium* species are classified as hemibiotrophic ones, which means that the pathogens initially behave like biotrophic fungi and then switch on to the optional parasitization mode [52]. Another group of compounds participating in the immune response to pathogens is phytoalexins, i.e., low molecular weight phenolics. They are derivatives of benzoic acid, stilbene, coumarin or quercetin [58,59]. The synthesis of phenolic compounds requires a large energy input, and therefore, it depends on the accumulation of the number of soluble sugars in the cells. We confirmed this correlation in our experiments. High correlation ($r = 0.631$; $p < 0.05$) between TSC and TPC may indicate the plant defense response to the infection consisting in the increase of TSC consumption for ATP synthesis and further use of this energy in the synthesis of phenolics. SMH87

plants, more sensitive to *F. culmorum*, showed a significant decrease in the phenolic content in the leaves as compared with the other accessions. Contrary to that, the most resistant BC_5Nax_2line was characterized by the highest content of phenolics in the leaves and roots of the control and infected seedlings. Hakulinen et al. [60] suggested that the lowered content of phenolics may be caused by the synthesis of lignin that is a polymer of oxidized phenolic alcohols. Lignin fortifies cell walls making them difficult for fungal hyphae to colonizing the host plant [61,62]. Datta and Lal [63] and Noman et al. [64] reported this phenomenon as a hypersensitivity reaction initiated as a plant defense mechanism to developing an infection. In our experiment, a decrease in leaf TPC was associated with higher content of cell-wall-bound phenolic compounds (CWP) only in BC_5Nax_2. The same line revealed a relationship between decreased root TPC and increased accumulation of CWP. The leaf CWP content positively correlated with the content of H_2O_2 ($r = 0.679$; $p < 0.05$). In the leaves, TPC content negatively correlated with H_2O_2 levels, while TPC content in the roots negatively correlated with H_2O_2 concentration in both studied organs. These negative correlations may suggest that during *Fusarium* infection, TPC acted as antioxidants and possibly reduced H_2O_2 amount.

Antioxidant enzymes, such as CAT, POXs, and SOD, form the first line of defense against ROS during the entire pathogenesis [65–67]. Superoxide dismutase (SOD) is responsible for the dismutation of the superoxide radicals to molecular oxygen and hydrogen peroxide. CAT and POX decompose H_2O_2. Some studies reported that *Fusarium* infections boosted the activity of the antioxidant enzymes [68–70]. In the investigated wheat seedlings, we detected greater POX and SOD activity in the roots than in the leaves. It can be explained by the fact that the in vitro infection started in the roots growing in the infected medium. In the roots we observed a correlation between SOD activity and H_2O_2 accumulation ($r = 0.613$; $p < 0.05$), while in the leaves there was a correlation between CAT and POX activity and H_2O_2 ($r = 0.710$ and $r = 0.688$; $p < 0.05$, respectively). We recorded high negative correlation between TPC content and CAT and POX activity in the leaves ($r = -0.788$ and $r = -0.515$; $p < 0.05$, respectively). These results may suggest a competition between antioxidant enzymes and phenolic compounds for H_2O_2, which can indicate the antioxidant properties of phenolics. We reported higher activity of the antioxidant enzymes and higher levels of H_2O_2 in the control leaves than in the control roots. The infection decreased the activity of the antioxidant system in the roots, but not in the leaves. The enzyme activity poorly differentiated the studied accessions regarding their resistance to *F. culmorum.* Only CAT activity was twofold higher in the infected SMH87 leaves, considered by us to be more sensitive to *Fusarium*, while in cv. 'Tamaroi' and BC_5Nax_2 line this activity was lower or remained unchanged. Płażek and Żur [71] indicated that low activity of CAT could be a marker of a plant resistance to a fungal infection, as CAT decomposes H_2O_2 that is necessary for the defense as a signal molecule.

Fusarium head blight causes huge yield losses in cereals, reaching over 40%. The disease reduces grain yield, its mass, nutritional value and leads to grain contamination with mycotoxins [6]. Our research confirmed that spike infection not only reduces the grain mass, but also lowers the final yield. The reduction of yield parameters is also associated with high concentrations of mycotoxins in the grain. Negative correlation between the examined yield parameters (amount of grain per spike, mass of grain per spike, mass of a single grain, and mass of thousand seeds) and the content of NIV and DON suggest the reduction of the yield is mainly, due to accumulated toxins. ZEN content only affected the mass of a single grain, and we found no relationships between T–2 toxin and the yield. The visual assessment of spike infestation degree (FHBi) was performed at two terms: Seven and fourteen days after infection. In both terms, FHBi highly negatively correlated with the yield parameters. It could be stated that FHBi, especially 7 days after the infection, is a reliable method to determine cereal resistance to the infection, as confirmed by other studies [72,73].The statistical analysis showed a strong correlation between FHBi 7 and 14 days after the infection, and DON accumulation in the grain ($r = 0.733$, $r = 0.632$, $p < 0.05$, respectively), and NIV content ($r = 0.731$, $r = 0.630$, $p < 0.05$,

respectively). A similar relationship between FHBi and DON accumulation was observed by Haidukowski et al. [74] in common wheat. Nowicki et al. [75] and Pascale et al. [76] claimed that FHBi can be used to predict the grain contamination degree with mycotoxins before performing detailed analyses. In our research, we used NIV-chemotype isolate of *F. culmorum*, which is considered a milder *Fusarium* chemotype than DON-chemotype or acetyl derivatives (3AcDON, 15AcDON) [77,78]. Desjardin and Plattner [79] reported that *F. culmorum* NIV-chemotypes can produce DON, but in amounts <1% of NIV, while DON-chemotypes are not capable of producing NIV [80]. In our experiments, we observed increased level of DON in relation to NIV, which contradicted the hypothesis presented by Dejsrdin and Plattner [79].

4. Materials and Methods

4.1. Plant Material and Experimental Design

Two experiments were performed in 2020. The first was an in vitro assay testing the plant resistance to *Fusarium*, and the other involved plants at the anthesis stage grown in an open foil tunnel. Three durum wheat accessions differing in salt–stress tolerance were used: Polish moderately sensitive SMH87 line (courtesy of Dr. Jarosław Bojarczuk, Plant Breeding Centre in Smolice, IHAR Group, Poland), sensitive Australian cultivar 'Tamaroi', and resistant BC_5Nax_2 line (courtesy of Dr. Richard A. James, CSIRO Plant Industry, Acton, Australia). These accessions were investigated in previous studies on durum wheat tolerance to salinity and cadmium.

4.1.1. Preparation of *Fusarium culmorum* Isolate

In both experiments, the plants or seeds were infected with IPO348–01 nivalenol chemotype mycelium of *F. culmorum* from the Plant Breeding Institute, Wageningen (Netherland). Mycelium box test was performed in in vitro conditions on seedlings grown on inoculated Potato Dextrose Agar (PDA) medium (Sigma–Aldrich, Poznań, Poland). The mycelium was grown in a microbiological thermostatic chamber (ST 5 Smart, Pol–Aura Aparatura, Wodzisław Śląski, Poland) at 21 °C, in darkness for seven days [32]. The mycelium production for the open tunnel experiment followed the method described by Wiśniewska et al. [81]. An Erlenmeyer glass flask (250 cm^3) was filled with 50 g of spring wheat seeds, and 15 cm^3 of water w added to obtain 40% humidity. After 24 h, the seeds were autoclaved at 101 325 Pa, at 121 °C for 30 min and then cooled. The infection was initiated by transferring three 1.5 cm discs of PDA medium. The glass flasks were placed in the microbiological thermostatic chamber (ST 5 Smart, Pol–Aura Aparatura, Wodzisław Śląski, Poland), and the mycelium was growing at 20 ± 1 °C for five to six weeks in darkness. The flasks were shaken thoroughly every day to prevent sticking the grain to the glass, and to also provide uniform inoculation of the grain.

4.1.2. Experiment I—Box Test Assay

This experiment was performed on the seedlings grown in Magenta GA–7 Boxes (Sigma–Aldrich, Poznań, Poland) under sterile conditions. The boxes were filled with 20 cm^3 of MS medium [82]. Discs (5 mm) of PDA medium overgrown with *Fusarium* mycelium were cut and transferred into magenta boxes on MS medium (five discs per box). The seeds were surface disinfected in 20% commercial bleach (active ingredient sodium hypochlorite) for 20 min, rinsed three times for 2 min with sterile water, and transferred into Petri dishes lined with wetted sterile filter paper for 24 h germination. The germinating seeds (five seeds per Magenta-Box) were placed on mycelium discs. The control seeds were placed on PDA medium discs free of the pathogen (Figure 6). The experiment was performed in six replicates for each accession/treatment (magenta with control and inoculated seeds) combination. Vegetation in both treatments was conducted on MS medium. Vegetation conditions were maintained for 14 days in a growth chamber at 22/20 °C (day/night), the light intensity of 150 µmol m^{-2}·s^{-1} PPFD (Photosynthetic Photon Flux Density) and 12 h/12 h (day/night) photoperiod with 100% air humidity.

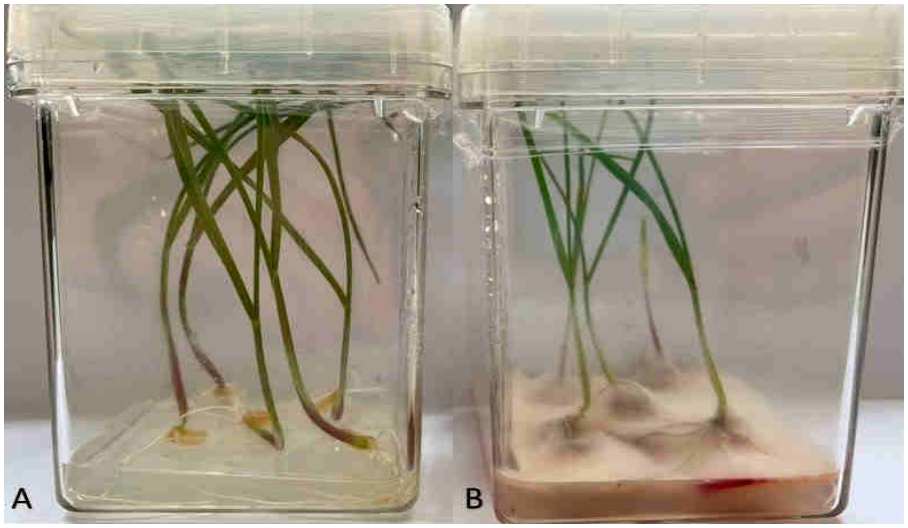

Figure 6. Two-week old durum wheat seedlings growing in pathogen-free medium (**A**) and the medium infected with *F. culmorum* medium (**B**).

Two weeks after inoculation, the leaves and roots were collected separately, weighed, and frozen in liquid nitrogen. The subsequent analyses involved: Visual disease rating (DR), chlorophyll *a*, *b*, and carotenoid content (Chl*a*, *b*, and car), total soluble carbohydrate content (TSC), the content of total phenolic compounds (TPC), and cell wall-bound phenolics (CWP), antioxidant enzyme activities, hydrogen peroxide content (H_2O_2), and fresh weight (FW) of leaves and roots.

4.1.3. Experiment II—Open Foil Tunnel

The experiment was carried out in semi-controlled conditions in an open foil tunnel. The seeds were sown into plastic pots (20 × 20 × 25 cm; nine seeds per pot), in six replicates (six pots) for each accession/treatment (control and inoculated plants) combination. The plants were cultivated in universal garden soil substrate pH = 5.8 (Ekoziem, Jurkow, Poland) mixed with sand (1:1, *v*/*v*). Before sowing, the seeds were sterilized in 70% ethanol for one minute and rinsed with sterile water three times for two minutes. Once a week, the plants were fertilized with Hoagland medium [83]. The plants were cultivated until the full anthesis stage—65 BBCH scale [40]. Their spikes were sprayed with the inoculum containing *F. culmorum* spores, while control spikes were sprayed with distilled water. Disease symptoms were evaluated seven and fourteen days after inoculation (DAI 7 and DAI 14). Ripe seeds were collected, and the following yield parameters were evaluated: Number and weight of seeds per spike, the weight of a single seed, and weight of one thousand seeds. Moreover, the content of the following mycotoxins was determined: deoxynivalenol (DON) and its derivatives 3–acetyldeoxynivalenol (3AcDON), 15–acetyldeoxynivalenol (15AcDON), nivalenol (NIV), zearalenone (ZEN) and its derivatives alpha–Zearalanol (α–ZAL), beta–Zearalanol (β–ZAL), alpha–Zearalanol (α–ZEL), beta–Zearalenol (β–ZEL), and T–2 toxin.

4.2. Analyses

4.2.1. Disease Rating (DR) and Loss of Fresh Weight (FW)

Direct assessment based on disease rating (DR) was calculated with the formula described by Warzecha et al. [84] to determine the effect of infection on seedling and root development.

$$DR\% = 100 \times (n_i \times D_i)/ND_{max}$$

where: n_i—number of plants of *i*th category, D_i—numerical value of *i*th category, N—total number of plants in the sample, and D_{max}—maximum scale value (0–5) [42].

To assess the impact of the infection, we also determined the fresh weight (FW) of leaves and roots. All measurements were done in thirty replicates for each cultivar/line.

4.2.2. Chlorophyll (a, b) and Carotenoid (Car) Content

Chlorophylls and carotenoids were estimated spectrophotometrically, according to Czyczyło-Mysza et al. [85]. Plant Leaves samples were dried at 65 °C for 48 h, weighed, and then extracted in 96% ethanol (5 mg/1.5 cm^3), and centrifuged (21,000× *g*, 5 min at 15 °C). The extract was transferred to 96 well plate, and the absorbance was read at 470, 648, and 664 nm (Synergy II, Biotek, Winooski, VT, USA). The concentrations of Chl*a*, Chl*b*, and total carotenoids (car) were calculated using Lichtenthaler and Buschman [86] equations:

$$\text{Chl}a\ (\mu g/cm^3) = 13.36\ A_{664} - 5.19\ A_{648}$$

$$\text{Chl}b\ (\mu g/cm^3) = 27.43\ A_{648} - 8.12\ A_{664}$$

$$\text{Car}\ (\mu g/cm^3) = (1000\ A_{470} - 2.13\ \text{Chl}a - 97.64\ \text{Chl}b)/209$$

where: Chl*a* = chlorophyll *a*, Chl*b* = chlorophyll *b*, A_{470} = absorbance at 470 nm, A_{664} = absorbance at 664 nm, A_{648} = absorbance at 648 nm.

4.2.3. Determination of Total Water-Soluble Carbohydrates (TSC)

Sugars were analyzed by the phenol-sulfuric method of Dubois et al. [87], with modifications reported by Bach et al. [88]. The samples extracted, as for pigment estimation (10 µL) were diluted to 200 µL with water, and 200 µL of 5% phenol (*w*/*w*) solution was added. Then, 1 cm^3 of concentrated sulfuric acid was dispensed, the samples were mixed, and after 20 min incubation at ambient temperature and transferred to 96-well plates and absorbance was read at 490 nm (Synergy II, Biotek, Winooski, VT, USA). Sugar content was expressed as glucose equivalents—using the calibration curve obtained with a standard solution of glucose.

4.2.4. Determination of Total Phenolic Compounds (TPC)

Estimation of total phenolic content was done according to the Singleton method with modifications [88]. The extracts (prepared as described for pigments) were mixed with water diluted Folin–Ciocalteu phenol reagent (5:2, *v*/*v*) and after 10 min saturated Na_2CO_3 (c.a. 25% *w*/*w*) was added (100/400/400 µL). The samples were then incubated for 2 h in darkness, at room temperature. After centrifugation (21,000× *g*, for 15 min at 15 °C), they were transferred to 96-well plates. Their absorbance was recorded at 760 nm (Synergy II, Biotek, Winooski, VT, USA). The pool of phenolic compounds was expressed as mg of gallic acid—using the calibration curve obtained with a standard solution of gallic acid.

4.2.5. Determination of Cell Wall-Bound Phenolics (CWP)

The pellets remaining after extraction of pigments were rinsed with ethanol and hydrolyzed with 3 M NaOH [89] overnight, at room temperature. Then the samples were neutralized with concentrated HCl, then ethanol was added (1 cm^3 per sample), and the resulting solution was analyzed for released phenolics as for soluble forms described in Section 4.2.4.

4.2.6. Activity of Enzymatic Antioxidants

Plant material was homogenized at 4 °C in 50 mM (pH 7) phosphate-potassium buffer containing 0.1 mM EDTA (100 mg of FW plant material per 1 cm^3 of buffer). The activity of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, E.C. 1.11.1.6), and peroxidase (POX, EC 1.11.1.7) were determined. After centrifugation (10,000× *g*, 15 min at 4 °C, 32R, Hettich, Germany), clear supernatant was sub-sampled and assayed for SOD, CAT, and POX activity in 96-well plate format (Synergy II, Biotek, Winooski, VT). SOD activity was determined by the cytochrome reduction method of McCord and Fridovic [90]. CAT activity was measured at 240 nm according to Aebi [91], with H_2O_2 as a substrate. The

activity of POX was assessed using the Lück [92] method with *p*–phenylenediamine as substrate, the absorbance was monitored at 485 nm. The analyses were conducted as described by Gudys et al. and references are cited therein [93–95]. Enzyme activities were presented on a protein basis. Protein content was assayed with the standard Bradford method [96].

4.2.7. Hydrogen Peroxide Content

Plant material was homogenized at 4 °C in 50 mM (pH 7) phosphate-potassium buffer containing 0.1 mM EDTA, as described for enzyme activity analyses. Hydrogen peroxide content was estimated with a commercial Amplex Red (10–acetyl–3,7–dihydroxyphenoxa zine) [97] reagent kit (Invitrogen, Waltham, MA, USA), according to the manufacturer's manual [98]. Briefly, the plant sample was diluted with the reaction buffer, and the working solution containing fluorescence probe precursor (Amplex Red), and 0.2 U·cm^{-3} horseradish peroxidase was added. After 30 min incubation, fluorescence was read at Ex/Em 530/590 nm in 96-well plate format (Synergy II, Biotek, Winooski, VT, USA). The results were quantitated based on a calibration curve made for H_2O_2.

4.2.8. Spore Suspension Preparation and Spike Inoculation Procedure

The spikes were inoculated with the spore suspension of *F. culmorum* prepared as described by Góral et al. [12]. The grain inoculated with *F. culmorum* mycelium and conidia were soaked in distilled water for 1 h and then filtered over two layers of sterile cheesecloth. The spore concentration of the suspension was adjusted to 5×10^5 spores · cm^{-3} using Thoma's chamber. The inoculation was performed according to the methodology described by Warzecha et al. [99], with slight modifications. The spikes from each line were sprayed separately with a hand sprayer, using 2 cm^3 of the conidia suspension per spike, and covered for 48 h with plastic bags. Control plants were sprayed with distilled water, and covered with plastics bags to provide the same experimental conditions. Inoculation was done early in the morning, when the air humidity was relatively high (70–80 %) and the temperature was low (10–14 °C).

The spike inoculation procedure was performed twice. The first inoculation was done three days before the full anthesis stage and repeated seven days later. Seven days after each inoculation, *Fusarium* head blight index (FHBi) was visually evaluated for each accession and calculated using the formula described by Góral et al. [12]:

$$\text{FHBi} = \%\text{ of head infection} \times \%\text{ of head infection per accession}/100$$

Forty-five spikes from each accession at the full ripening stage were harvested, evaluated for yield reduction after *Fusarium* inoculation, and compared with non-inoculated plants. The following yield parameters were calculated: Amount of grain per spike, grain mass per spike, mass of a single grain, and mass of one thousand grains (MTS). After the evaluation of the yield parameters, seed material was collected and stored at −20 °C until mycotoxin analyzes.

4.2.9. UHPLC-MS/MS Estimation of Mycotoxin Accumulation

The samples were analyzed for the content of 10 different mycotoxins by using UHPLC–MS/MS (ultrahigh-performance liquid chromatography coupled with tandem mass spectrometer) as reported by Dziurka et al. [100], with modifications. Plant materials were extracted according to the procedure described by Klötzel and Lauber [101]. The ground samples (0.1 g) were extracted three times in 1 cm^3 of acetonitrile and water (80:20, *v*/*v*) solution (5 min, 30 Hz, 400 MM, Retch, Haan, Germany). Fifty nanograms of heavy-labeled internal standard ([$^{13}C_{18}$]–ZEN, and [$^{13}C_{15}$]–DON) were added to each sample. After centrifugation, the samples were cleaned up on Bond Elut Mycotoxin cartridges (3 cm^3 500 mg, Agilent Technologies, Germany). The column eluate was evaporated under N_2, and the residue was resuspended in 100 µL of acetonitrile/water (50:50, *v*/*v*), and analyzed. The mycotoxins (DON, NIV, sum of 3–acetyl–DON and 15–acetyl–DON, T–2,

sum of zearalenone and its derivatives: a–, b–ZEL, a–, b–ZAN and ZEN, and OTA) were determined using the UHPLC system (Infinity 1260, Agilent Technologies, Germany) with a tandem quadrupole mass spectrometer (QQQ 6410, Agilent Technologies, USA). The samples were separated on a Poroshell 120 Phenyl–Hexyl 2.1 × 5 mm, 2.7 µM column with a gradient of water (A) and methanol (B) both with 0.1% formic acid, from 5% to 75% methanol in 7.5 min, at a flow rate of 0.5 cm^3 min^{-1}. Multiple reaction monitoring (MRM) transitions after positive ESI ionization were used for identification and quantification (details are given in Table S1). Quantitation was based on calibration curves obtained with authentic standards taking account of the recovery rates of the internal standard used. The standards were supplied by Romer (Tulin, Austria), except for zearalenone and its derivatives which were supplied by Sigma-Aldrich (Poznań, Poland)

4.2.10. Yield Components

Ripe seeds were collected, and the yield parameters were evaluated. Number and weight of seeds per spike were calculated in 45 replicates, the weight of a single seed was measured in 45 replicates, while the mass of one thousand seeds (MTS) was measured in three replicates, for each accession/treatment combination.

4.3. Statistical Analyses

The experiments were arranged and performed with the application of a completely randomized design. The normal distribution of data was analyzed using Shapiro–Wilk test. Two-way analysis of variance (ANOVA) and Duncan's multiple range test (at $p < 0.05$) were performed using the statistical package Statistica 13.3 (Stat–Soft, Inc., Tulsa, OK, USA). The data were presented as means ± SE (standard error). Pearson's correlation coefficients were assumed as statistically significant at $p < 0.05$.

5. Conclusions

1. *Fusarium culmorum* infection significantly reduces the content of active photosynthetic pigments and the weight of leaves and roots.
2. The infected cv. 'Tamaroi' and BC_5Nax_2 plants recognized as more resistant to *F. culmorum* than SMH87, accumulated increased amounts of sugar in the leaves, which correlated with an increased number of phenolic compounds.
3. Phenolic compounds participate in H_2O_2 decomposition in durum wheat plants infected by *F. culmorum*.
4. The study confirmed the important role of H_2O_2 in increasing the content of phenolic compounds that are then incorporated into cell walls of plants infected with *F. culmorum.*
5. Nivalenol and deoxynivalenol secreted by *F. cumlorum* significantly reduce the yield of durum wheat.
6. Early evaluation of durum wheat spikes infection done seven days after inoculation with *F. culmorum* spores may help predict the potential degree of DON and NIV accumulation in the grain.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22147433/s1, Table S1: Multiple reactions monitoring (MRM) transitions for the analyzed mycotoxins at positive ion mode (+ESI), capillary voltage 4 kV, gas temperature 300 °C, gas flow 12 L/min and nebulizer pressure 35 psi. MassHunter software was used to control the UHPLC–MS/MS system and in data analysis. For MRM parameters optimization MassHunter Optimizer was used.

Author Contributions: The experiments were conceived and designed by J.P. and A.P.; the experiments were performed by J.P.; biochemical analyses were performed by J.P., A.S., M.D., M.H., P.K. and M.S.; UHPLC-MS/MS analyses was performed by M.D.; the data were statistically analyzed by J.P., A.P.; the original draft paper was written by J.P.; the review and editing were done by J.P. and A.P. All authors have read and agreed to the published version of the manuscript.

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12. Oświadczenia współautorów publikacji wchodzących w skład rozprawy doktorskiej

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Jakub Pastuszak
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 20.09.2022

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Kopeć, P., Płażek, A., Gondek, K., Szczerba, A., Hornyák, M., & Dubert, F. (2020). Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree. *Plant, Soil and Environment*, *66*(6), 257-263.

brałem udział w:

- przeprowadzeniu doświadczenia w warunkach szklarniowych
- opracowaniu metodologii doświadczenia
- wykonaniu pomiarów fluorescencji chlorofilu i wymiany gazowej
- zebraniu materiału roślinnego do analiz parametrów plonu
- wykonaniu pomiarów parametrów plonu
- przygotowaniu materiału do analiz zawartości metali ciężkich
- wykonaniu analizy statystycznej uzyskanych wyników
- wykonaniu graficznej wizualizacji uzyskanych wyników
- analizie i interpretacji uzyskanych wyników
- pisaniu pierwszej i finalnej wersji manuskryptu

Jakub Pastuszak

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz.

Kraków, 2022.09.07

dr inż. Przemysław Kopeć
Zakład Biologii Komórki

OŚWIADCZENIE

Oświadczam, że w pracy:

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brałem udział w:

- zebraniu materiału roślinnego po przeprowadzonym doświadczeniu
- pomoc w wykonaniu do pomiarów parametrów plonu
- analizie uzyskanych wyników

Przemysław Kopeć

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
Dyrekcja/Management: tel./fax + 48 124251844 (102)
Księgowość/Chief Accountant: tel. +48 124251834 (104)
Lab. Fitotron/Phytotron Lab.: tel. +48 124253301

Regon: 356730850
NIP: PL 677-22-12-521
e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

prof. dr hab. inż. Agnieszka Płażek
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 20.09.2022

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brałam udział w:

- opracowaniu schematu doświadczenia
- analizie uzyskanych wyników
- pisaniu wstępnej i finalnej wersji manuskryptu

A. Płaż

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

prof. dr hab. inż. Krzysztof Gondek
Katedra Chemii Rolnej i Środowiskowej
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 22.09.2022

OŚWIADCZENIE

Oświadczam, że w pracy:

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brałem udział w:

- wykonaniu analiz zawartości kadmu w materiale roślinnym

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Anna Szczerba
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 07.09.2022

OŚWIADCZENIE

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brałam udział w:

- zebraniu materiału roślinnego do analiz parametrów plonu
- pomoc w wykonaniu pomiarów parametrów plonu

Szczerba Anna

dr Marta Hornyák
Instytut Botaniki *im. Władysława Szafera*
Polska Akademia Nauk
Grupa Ekologii Funkcjonalnej i Ewolucyjnej

Kraków, 08.09.2022

OŚWIADCZENIE

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brałam udział w:

- zebraniu materiału roślinnego
- wykonaniu pomiarów parametrów plonu
- pomiarach fluorescencji chlorofilu *a*

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz.

Kraków, 2022.09.08

prof. dr hab. Franciszek Dubert

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Kopeć, P., Płażek, A., Gondek, K., Szczerba, A., Hornyák, M., & Dubert, F. (2020). Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree. *Plant, Soil and Environment*, *66*(6), 257-263.

brałem udział w:

- sprowadzeniu materiału badawczego (odmiana Tamaroi i linia BC_5Nax_2)
- edycji merytorycznej manuskryptu

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
Dyrekcja/Management: tel./fax + 48 124251844 (102)
Księgowość/Chief Accountant: tel. +48 124251834 (104)
Lab. Fitotron/Phytotron Lab.: tel. +48 124253301

Regon: 356730850
NIP: PL 677-22-12-521
e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Jakub Pastuszak
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 20.09.2022

OŚWIADCZENIE

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brałem udział w:

- przeprowadzeniu doświadczenia w warunkach szklarniowych
- opracowaniu metodologii doświadczenia
- zebraniu materiału roślinnego do analiz biochemicznych i parametrów plonu
- wykonaniu analiz biochemicznych aktywności enzymów
- wykonaniu pomiarów parametrów plonu
- przygotowaniu materiału do analiz zawartości metali ciężkich
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- analizie i interpretacji uzyskanych wyników
- pisaniu wstępnej i ostatecznej wersje manuskryptu

Jakub Pastuszak

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz.

Kraków, 2022.09.07

dr inż. Przemysław Kopeć

Zakład Biologii Komórki

OŚWIADCZENIE

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brałem udział w:

- opracowaniu metodyki do pomiarów aktywności enzymów
- zebraniu materiału roślinnego do analiz biochemicznych
- przeprowadzeniu doświadczenia w warunkach szklarniowych
- analizie uzyskanych wyników

Przemysław Kopeć

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
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Księgowość/Chief Accountant: tel. +48 124251834 (104)
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e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

prof. dr hab. inż. Agnieszka Płażek
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

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- opracowaniu schematu doświadczenia
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A. Płaż

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

prof. dr hab. inż. Krzysztof Gondek
Katedra Chemii Rolnej i Środowiskowej
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

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UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Anna Szczerba
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 07.09.2022

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brałam udział w:

- zebraniu materiału roślinnego do analiz biochemicznych
- wykonaniu analiz biochemicznych aktywności enzymów

Szczerba Anna

dr Marta Hornyák
Instytut Botaniki *im. Władysława Szafera*
Polska Akademia Nauk
Grupa Ekologii Funkcjonalnej i Ewolucyjnej

Kraków, 08.09.2022

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brałam udział w:

- przeprowadzeniu doświadczenia w warunkach szklarniowych
- zebraniu materiału roślinnego do analiz biochemicznych

Marta Hyżak

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz. **Kraków,** 2022.09.08

prof. dr hab. Franciszek Dubert

OŚWIADCZENIE

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30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
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Księgowość/Chief Accountant: tel. +48 124251834 (104)
Lab. Fitotron/Phytotron Lab.: tel. +48 124253301

Regon: 356730850
NIP: PL 677-22-12-521
e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Jakub Pastuszak
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 20.09.2022

OŚWIADCZENIE

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brałem udział w:

- przeprowadzeniu doświadczenia w warunkach szklarniowych i laboratoryjnych
- opracowaniu schematu doświadczenia
- opracowaniu metodologii doświadczenia
- zbieraniu materiału roślinnego do analiz biochemicznych i fizjologicznych
- wykonaniu pomiarów fizjologicznych (fluorescencja chlorofilu i wymiana gazowa)
- wykonaniu analiz biochemicznych
- wykonaniu analizy statystycznej uzyskanych wyników
- wykonaniu graficznej wizualizacji uzyskanych wyników
- pisaniu wstępnej i finalnej wersji manuskryptu

Jakub Pastuszak

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz.

Kraków, 2022.09.13

dr Michał Dziurka

Zakład Biologii Rozwoju

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Dziurka, M., Hornyák, M., Szczerba, A., Kopeć, P., Płażek, A. (2022). Physiological and biochemical parameters of salinity resistance of three durum wheat genotypes. *International Journal of Molecular Sciences*, 23(15):8397.

brałem udział w:

- wykonaniu analiz biochemicznych

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
Dyrekcja/Management: tel./fax + 48 124251844 (102)
Księgowość/Chief Accountant: tel. +48 124251834 (104)
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Regon: 356730850
NIP: PL 677-22-12-521
e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

dr Marta Hornyák
Instytut Botaniki *im. Władysława Szafera*
Polska Akademia Nauk
Grupa Ekologii Funkcjonalnej i Ewolucyjnej

Kraków, 08.09.2022

OŚWIADCZENIE

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brałam udział w:

- zebraniu materiału roślinnego do analiz biochemicznych
- interpretacja uzyskanych wyników badań

Marta Hornyák

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Anna Szczerba
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 07.09.2022

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Szczerba Anna

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz. **Kraków,** 2022.09.07

dr inż. Przemysław Kopeć
Zakład Biologii Komórki

OŚWIADCZENIE

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Przemysław Kopeć

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
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im. Hugona Kołłątaja w Krakowie

prof. dr hab. inż. Agnieszka Płażek
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
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brałam udział w:

- opracowaniu schematu doświadczenia
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- przeglądzie i edycji merytorycznej manuskryptu

A. Płaż

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Jakub Pastuszak
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 20.09.2022

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Szczerba, A., Dziurka, M., Hornyák, M., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, *22*(14), 7433..

brałem udział w:

- przeprowadzeniu doświadczenia w warunkach szklarniowych i kulturach *in vitro*
- opracowaniu schematu doświadczenia
- opracowaniu metodologii doświadczenia
- zbieraniu materiału roślinnego do analiz biochemicznych i analiz plonu
- wykonaniu pomiarów fizjologicznych (ocena porażenia, parametry plonu)
- wykonaniu analiz biochemicznych
- przygotowaniu próbek do analiz HPLC
- wykonaniu analizy statystycznej uzyskanych wyników
- wykonaniu graficznej wizualizacji uzyskanych wyników
- pisaniu wstępnej i finalnej wersji manuskryptu

Jakub Pastuszak

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

mgr inż. Anna Szczerba
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
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brałam udział w:

- zbieraniu materiału roślinnego do analiz
- wykonaniu analiz biochemicznych

Szczerba Anna

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz. **Kraków,** 2022.09.13

dr Michał Dziurka

Zakład Biologii Rozwoju

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Szczerba, A., Dziurka, M., Hornyák, M., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, 22(14), 7433.

brałem udział w:

- analizach biochemicznych
- wykonaniu analiz zawartości mykotoksyn w ziarnie przy użyciu UHPLC-MS/MS

M Dziurka

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
Dyrekcja/Management: tel./fax + 48 124251844 (102)
Księgowość/Chief Accountant: tel. +48 124251834 (104)
Lab. Fitotron/Phytotron Lab.: tel. +48 124253301

Regon: 356730850
NIP: PL 677-22-12-521
e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

dr Marta Hornyák
Instytut Botaniki *im. Władysława Szafera*
Polska Akademia Nauk
Grupa Ekologii Funkcjonalnej i Ewolucyjnej

Kraków, 08.09.2022

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Szczerba, A., Dziurka, M., Hornyák, M., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, *22*(14), 7433.

brałam udział w:

- zbieraniu materiału roślinnego do analiz
- wykonaniu analiz biochemicznych

Marta Hornyák

INSTYTUT FIZJOLOGII ROŚLIN
im. Franciszka Górskiego
POLSKIEJ AKADEMII NAUK
POLISH ACADEMY OF SCIENCES
The Franciszek Górski
INSTITUTE OF PLANT PHYSIOLOGY

L. dz. **Kraków,** 2022.09.07

dr inż. Przemysław Kopeć
Zakład Biologii Komórki

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Szczerba, A., Dziurka, M., Hornyák, M., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, 22(14), 7433.

brałem udział w:

- zebraniu materiału roślinnego do analiz biochemicznych
- wykonaniu analiz biochemicznych
- edycji pierwszej wersji manuskryptu

Przemysław Kopeć

Adres/Address:
Niezapominajek 21
30-239 Kraków, Polska/Poland
Bank: 31 1130 1150 0012 1266 3720 0001

Centrala/Phone: tel. +48 124251833
Dyrekcja/Management: tel./fax + 48 124251844 (102)
Księgowość/Chief Accountant: tel. +48 124251834 (104)
Lab. Fitotron/Phytotron Lab.: tel. +48 124253301

Regon: 356730850
NIP: PL 677-22-12-521
e-mail: ifr@ifr-pan.edu.pl
www.ifr-pan.edu.pl

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

dr hab. Marek Szklarczyk, prof. URK
Katedra Biologii Roślin i Biotechnologii
Wydział Biotechnologii i Ogrodnictwa
Uniwersytet Rolniczy w Krakowie

Kraków, 15.09.2022

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Szczerba, A., Dziurka, M., Hornyák, M., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, *22*(14), 7433.

brałam udział w:

- wykonaniu analiz biochemicznych

Marek Szklarczyk

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

prof. dr hab. inż. Agnieszka Płażek
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 20.09.2022

OŚWIADCZENIE

Oświadczam, że w pracy:

Pastuszak, J., Szczerba, A., Dziurka, M., Hornyák, M., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, *22*(14), 7433.

brałam udział w:

- opracowaniu metodologii doświadczenia
- opracowaniu schematu doświadczenia
- analizie uzyskanych wyników
- przeglądzie i edycji merytorycznej manuskryptu

A. Płażek

13. Dorobek naukowy

Wykaz publikacji naukowych niewchodzących w skład rozprawy doktorskiej:

1. Płażek, A., Dubert, F., Kopeć, P., Dziurka, M., Kalandyk, A., **Pastuszak, J**., & Wolko, B. (2018). Seed hydropriming and smoke water significantly improve low-temperature germination of *Lupinus angustifolius* L. *International Journal of Molecular Sciences*, *19*(4), 992.

IF_{2018}: 4,183

5-letni IF: 4,331

Punktacja MEiN: 140 pkt

2. Płażek, A., Dubert, F., Kopeć, P., Dziurka, M., Kalandyk, A., **Pastuszak, J.,** ... & Wolko, B. (2018). Long-term effects of cold on growth, development and yield of narrow-leaf lupine may be alleviated by seed hydropriming or butenolide. *International Journal of Molecular Sciences*, *19*(8), 2416.

IF_{2018}: 4,183

5-letni IF: 4,331

Punktacja MEiN: 140 pkt

3. Płażek, A., Słomka, A., Kopeć, P., Dziurka, M., Hornyák, M., Sychta, K., **Pastuszak, J.**, Dubert, F. (2019). Effects of high temperature on embryological development and hormone profile in flowers and leaves of common buckwheat (*Fagopyrum esculentum* Moench). *International Journal of Molecular Sciences*, 20(7), 1705.

IF_{2019}: 4,556

5-letni IF: 4,653

Punktacja MEiN: 140 pkt

4. Hornyák, M., Płazek, A., Kopeć, P., Dziurka, M., **Pastuszak, J**., Szczerba, A., & Hura, T. (2020). Photosynthetic activity of common buckwheat (*Fagopyrum esculentum* Moench) exposed to thermal stress. *Photosynthetica*, *58*(1), 45–53.

IF_{2020}: 3,189

5-letni IF: 3,380

Punktacja MEiN: 70 pkt

5. Hornyák, M., Słomka, A., Sychta, K., Dziurka, M., Kopeć, P., **Pastuszak, J**., … & Płażek, A. (2020). Reducing Flower Competition for Assimilates by Half Results in Higher Yield of *Fagopyrum esculentum*. *International Journal of Molecular Sciences*, *21*(23), 8953.

IF_{2020}: 5.924

5-letni IF: 6.132

Punktacja MEiN: 140 pkt

6. Kopeć, P., Hornyák, M., **Pastuszak, J**., Szczerba, A., Rapacz, M., Waga, J., & Płażek, A. (2021). Changes in the flower and leaf proteome of common buckwheat (*Fagopyrum esculentum* Moench) under high temperature. *International Journal of Molecular Sciences*, *22*(5), 2678.

IF_{2021}: 6.208

5-letni IF: 6.628

Punktacja MEiN: 140 pkt

7. Szczerba, A., Płażek, A., **Pastuszak, J**., Kopeć, P., Hornyák, M., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybean (*Glycine max* L.) cultivars. *Agronomy*, *11*(4), 800.

IF_{2021}: 3.949

5-letni IF: 4,117

Punktacja MEiN: 100 pkt

8. Hornyák, M., Dziurka, M., Kula-Maximenko, M., **Pastuszak, J**., Szczerba, A., Szklarczyk, M., & Płażek, A. (2022). Photosynthetic efficiency, growth and secondary metabolism of common buckwheat (*Fagopyrum esculentum* Moench) in different controlled-environment production systems. *Scientific Reports*, *12*(1), 1–13.

IF_{2022}: 4,996

5-letni IF: 5,516

Punktacja MEiN: 140 pkt

Podsumowanie dla 12 publikacji:

IF: 52,957

5-letni IF: 56,313

Punkty MEiN: 1430

Doniesienia konferencyjne:

1. 6th International Conference for Young Researchers Multidirectional Research in Agriculture, Forestry and Technology, 24–25 April 2017, Krakow, Poster: **Pastuszak, J**., Płażek, A. The impact of the natural stimulator butenolide on the germination process of chosen plant crops.

2. 11th International Conference "Plant Functioning under environmental stress", 12–15 września 2018, Kraków. Referat: Hornyák M., Słomka A., Kopeć P., Dziurka M., Sychta K., Dubert F., **Pastuszak J**., Płażek A. Influence of environmental factors on seed yield of common buckwheat (*Fagopyrum esculentum* Moench.).

3. 11th International Conference "Plant Functioning under environmental stress", 12–15 września 2018, Kraków. Poster: **Pastuszak J.**, Hornyák M., Płażek A., Gondek K., Dubert F. The influence of different Cd concentrations in soil on photosynthetic efficiency of three genotypes of *Triticum durum* Desf.

4. XV Ogólnopolska Konferencja Kultur In Vitro i Biotechnologii Roślin – Biotechnologiczne wykorzystanie zmienności w warunkach kultur in vitro", Rogów, 17–20 września 2018. Referat: Płażek A., Słomka A., Dubert F., Kopeć P., Hornyák M., Dziurka M., **Pastuszak J**. „Wpływ stresu troficznego na rozwój woreczków zalążkowych w słupkach gryki zwyczajnej w warunkach *in vitro*".

5. Konferencja „Bioróżnorodność funkcjonalna gleb Polski". Puławy, 18–19 października 2018 r. Poster: Hornyák M., **Pastuszak J.,** Płażek A. „Ocena wybranych parametrów fluorescencji chlorofilu a u gryki zwyczajnej (*Fagopyrum esculentum* Moench) pod wpływem działania wysokiej temperatury".

6. Konferencja „Bioróżnorodność funkcjonalna gleb Polski". Puławy, 18–19 października 2018 r. Poster: **Pastuszak J.,** Szczerba A., Hornyák M., Kopeć P., Dubert F., Płażek A.: Ocena stopnia wrażliwości wybranych form pszenicy twardej (*Triticum durum* Desf.) na porażenie *Fusarium culmorum*.

7. 58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019. Referat: Hornyák M., Kopeć P., **Pastuszak J**., Płażek A. „Zmiany proteomu liści i kwiatów gryki zwyczajnej (*Fagopyrum esculentum* Moench) pod wpływem działania wysokiej temperatury.

8. 58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019. Poster: Kopeć P., Hornyák M., Płażek A., Dubert F., **Pastuszak J.,** Dziurka M., Słomka A., Hura K. „Różnice w profilu białkowym pomiędzy pąkami kwiatowymi, rozwiniętymi i przekwitłymi kwiatami gryki zwyczajnej (*Fagopyrum esculenthum* Moench).

9. 58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019. Poster: **Pastuszak J.**, Kopeć P., Płażek A., Szczerba A., Hornyák M., Dubert F. „Wpływ jonów kadmu na aktywność enzymów cyklu kwasu glutationowo–askorbinianowego w liściach pszenicy twardej (*Triticum durum* Desf.)".

10. 58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019 Poster: Szczerba A., Dubert F., **Pastuszak J**., Hornyák M., Kopeć P. „Wpływ biostymulatorów na plonowanie wybranych gatunków z rodziny bobowatych.

11. XI Conference "In Vitro Cultures in Biotechnology and Plant Physiology" Kraków, 4–6 grudnia 2019 r. Poster: Hornyák M., Płażek A., Słomka A, Szczerba A., **Pastuszak J.,** Kopeć P., Dubert F. „Does the trophic stress cause defective female gametophyte development and weak seed set of common buckwheat? – *in vitro* and *in vivo* model of experiments".

12. XXXIV Conference on Embriology, Plants – Animals – Humans. Zakopane, 20–23 maj 2020, Poster: Słomka, A., Hornyák M., Sychta, K., Dziurka, M., Kopeć, P., **Pastuszak, J.**, Dubert, F., Płażek, A. „The influence of inflorescence removal treatment on yield of common buckwheat (*Fagopyrum esculentum* Moench)".

Udział w projektach naukowych:

1. Określenie fizjologicznych i genetycznych podstaw odporności pszenicy i jęczmienia na rozhartowywanie. Projekt realizowany w latach 2021–2026. Kierownik projektu: dr inż. Magdalena Wójcik-Jagła, UR Kraków

2. Zwiększenie wykorzystania krajowego białka paszowego dla produkcji wysokiej jakości produktów zwierzęcych w warunkach zrównoważonego rozwoju w ramach programu wieloletniego MRiRW trwający w latach 2016-2020 ustanowiony na podstawie uchwały nr

222/2015 Rady Ministrów z dnia 15 grudnia 2015 r. Kierownik projektu: prof. dr hab. Franciszek Dubert, IFR PAN

3. Badanie mechanizmu degeneracji woreczków zalążkowych i aborcji kwiatów jako przyczyny słabego zawiązywania nasion gryki zwyczajnej (*Fagopyrum esculentum* Moench.) Projekt OPUS 13, UMO-2017/25/B/NZ9/00148, realizowany w latach 2018–2020. Kierownik projektu: prof. dr hab. inż. Agnieszka Płażek, UR Kraków

4. Sekwencjonowanie nowej generacji i mapowanie asocjacyjne jako metody generowania markerów molekularnych cech użytkowych łubinu wąskolistnego. Projekt realizowany w latach 2015–2017 SEGENMAS, PBS3/A8/28/2015. Kierownik zadania: prof. dr hab. inż. Agnieszka Płażek, UR Kraków

Staże naukowe:

W roku 2017 odbyłem miesięczny staż naukowy w Instytucie Fizjologii Roślin *im. Franciszka Górskiego* Polskiej Akademii Nauk w Krakowie. Opiekun naukowy stażu: dr Michał Dziurka z Zakładu Biologii Rozwoju.