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A delay in senescence during rehydration following soil drought is a precondition for limiting yield loss in triticale**

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Abstract. The first aim of this study was to evaluate and compare the response to soil drought in 20 doubled haploid lines of triticale. Its second aim was to evaluate and compare plant regeneration after drought in relation to the senescence process during rehydration. The measurements performed focused on water content, photosynthetic apparatus activity, chlorophyll levels, and the content of phenolic compounds and soluble carbohydrates. Measurements were performed on flag leaves, and also on leaves located below a subflag leaf. Doubled haploid lines with a high and low yield capacity which had been subjected to drought during their generative development stage were selected for the research. Despite varying levels of flag leaf hydration under drought conditions, the chlorophyll content values found in the flag leaves were at a similar level in the individual doubled haploid lines. In both the high- and low-yield doubled haploid lines, soil drought induced changes in the level of photosynthetic pigments, soluble carbohydrates, and phenols below a subflag leaf. Furthermore, the reduction in, or even the inhibition of senescence during the rehydration period was identified as an important factor for plant productivity after exposure to soil drought. Therefore, the selection of phenotypes with a higher tolerance to soil drought should also include a rehydration period in order to evaluate plant regenerative potential after drought.

Keywords: water stress, rehydration, triticale, senescence

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INTRODUCTION

Soil droughts are becoming increasingly extreme and unpredictable, this is the result of more frequent and longer periods with precipitation deficits. These are a consequence of global warming and its resultant changes in climate (Barrs, 1968; Eckstein *et al*., 2019; Howard *et al*., 2010). This situation represents a serious threat to global agriculture, as soil droughts significantly reduce the yield obtained from crops, including cereals (Daryanto *et al*., 2015).

A visible manifestation of drought is accelerated plant senescence associated with the yellowing of leaves due to chlorophyll degradation (Schlemmer *et al*., 2005). This leads to a significant reduction in plant assimilation surface and photosynthetic capacity, which, as a consequence, leads to a reduction in yields (Hura *et al*., 2019; Ostrowska *et al*., 2019a; Riasat *et al*., 2019). Moreover, leaf chlorophyll content is a fundamental variable for the understanding of plant responses to the environment as it is a potential indicator of the degree of stress, this is due to the fact that it has a direct role in the photosynthetic process of light capture and electron transport (Schlemmer *et al*., 2005). Drought induced senescence also affects the levels of phenolic compounds (Hura *et al*., 2011; Massolo *et al*., 2011) and carbohydrates (Masclaux *et al*., 2000; Quirino *et al*., 2001). It has been demonstrated that the accumulation of phenolic compounds

in stressful conditions may reduce chlorophyll activation, and thus reduce the photochemical activity of photosystems (Burchard *et al*., 2000). As carbohydrates are consumed in the synthesis of phenolic compounds, the increase in cellular levels of phenols is frequently accompanied by a reduction in carbohydrate levels (Arnold *et al*., 2004). For these reasons, chlorophyll degradation, a reduction in carbohydrate levels, and an increase in phenolic compound levels may be considered as biochemical indicators of senescence in plant organs (Ostrowska *et al*., 2019b; Hura *et al*., 2019).

Rivero *et al*. (2007) are of the opinion that plant tolerance to drought can be increased by delaying senescence. However, such studies should consider not only the immediate effect of soil drought, but also the rehydration period after the exposure to a period of moisture deficit ends (Chen *et al*., 2016). Rehydration after soil drought is a period of plant regeneration during which the optimum level of plant metabolism should be restored, this includes the inhibition or reduction of the plant senescence process. However, it has been demonstrated that during rehydration, flag leaf senescence may continue, or even accelerate (Baczek-Kwinta *et al*., 2006; Hura *et al*., 2015; Hura *et al*., 2019). This is an effect of the intensified metabolic processes which occur in hydrated cells, and cause, amongst other effects, PSII electron transport chain dysfunction and the transfer of electrons from primary acceptors Q_A/Q_B onto oxygen molecules. This process results in the excessive production of reactive oxygen species (superoxide anion, and hydrogen peroxide) that accelerates the plant senescence process (Jajic *et al*., 2015). A similar situation is observed during rehydration in mosses and lichens, which undergo a significant degree of desiccation during drought. In this case, extracellular and intracellular peroxidases, high light intensity or specifically localized plasma membrane NADPH and NADH are indicated to be possible sources generating reactive oxygen species (Mayaba *et al*., 2002; Minibayeva and Beckett, 2001).

In the case of winter cereals, soil drought at the generative growth stage causes the greatest yield loss. The rehydration period following such a drought is too short and therefore insufficient for complete plant regeneration (Barnabas *et al*., 2008; Hura *et al*., 2015). Therefore, the inhibition of the senescence process after drought at the generative developmental stage of cereal growth is of crucial importance in limiting yield loss. It should be emphasized that senescence in cereal plants is initiated in the bottom leaves of the main stem and progresses towards the flag leaf, which is typically the last one to exhibit the signs of senescence (yellowing) (Guo and Gan, 2005).

The first aim of this study was to evaluate and compare the reaction of doubled haploid (DH) lines of winter triticale to soil drought conditions. In that part of the experiment, water content and the activity of the photosynthetic apparatus were measured in selected flag leaves. Furthermore, the levels of chlorophyll, phenolic compounds, and carbohydrates in the leaves in the lower part of the plants, under the subflag leaf were analysed. The second aim of the study was to evaluate and compare plant regeneration after drought in relation to senescence processes during rehydration. In that section, the chlorophyll content in selected flag leaves was analysed. DH lines with high and low yield capacity subjected to soil drought during the generative development stage were selected for research.

MATERIALS AND METHODS

The research involved 20 DH lines of winter triticale from the mapping population 'Hewo' x 'Magnat' (Hura *et al*., 2017): 10 lines with a high yield potential (DH 2007-7/4(115), DH 2006-HM17, DH 2007-9/3(151), DH 2007-7/4(140), DH 2007-4/4(150), DH 2006-HM27, DH 2006-HM7, DH 2007-7/3(44), DH 2007-7/4(120), and Magnat) and 10 lines with a low yield potential (DH 2006-HHM7(184), DH 2007-11/2(126), DH 2006-HM48, DH 2006-HM31, DH 2007-3/3(181), DH 2007-9/3(107), DH 2007-10/2(1), DH 2007-12/3(19), DH 2006-HHM8, DH 2007-3/2(149)) which were exposed to soil drought at the generative growth stage.

The seeds of the tested DH lines were sown in plastic pots (3.7 L; 9 plants per pot) that were filled with a mixture of soil ("Eko-ziem universal soil", Jurków, Poland) and sand (1:3; v/v). Two-leaf seedlings were subjected to 8 weeks of vernalization in a cool chamber at +4°C, with a photoperiod of 10/14 h (day/night) and also with an illumination of PPFD (photosynthetic photon flux density) set at 150 µmol m^{-2} s^{-1}. After this time period, at four-leaf stage the plants were transferred into a greenhouse chamber. Plant growth was continued during a 5-month period (January to May). In the greenhouse, the air temperature was about 26/18°C (±2°C) day/night, and air humidity reached 40%. PPFD at the level of the top leaves was about 150-200 µmol $m^{-2}s^{-1}$. Once per week, the plants were irrigated with a full-strength Hoagland's solution (Hoagland, 1948).

During the stage at which the flag leaf blade is completely visible (Zadoks, GS39), soil drought was individually applied to each DH line. Watering was halted when the soil moisture level reached 35% (7 days), and this level was maintained for the next 14 days (35% means about 674 g of available water per pot; 90-95 ml of water was added daily to maintain the pots at 35%). Soil moisture was controlled gravimetrically every day (Hura *et al*., 2017). After this time, the leaf water content (LWC), chlorophyll fluorescence and SPAD chlorophyll content of the flag leaf were measured. In order to secure the materials for future chemical analysis (photosynthetic pigments, soluble carbohydrates, soluble phenols), the leaves located below the subflag leaf (the first two adjacent leaves from each plant) were collected (Fig. 1). Then, a 14-day rehydration phase was started through optimal watering (the soil moisture level was kept at 75%) of the plants. After this time, the

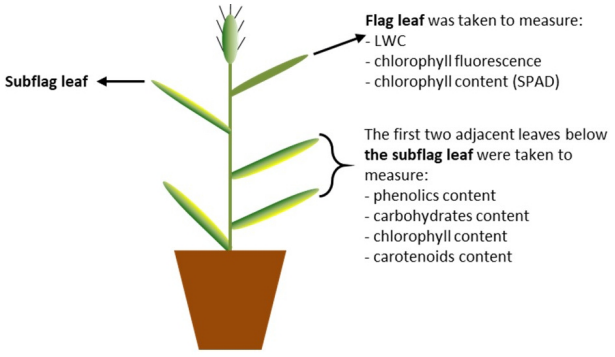

Fig. 1. Scheme of leaf sampling for measurements.

chlorophyll content of the flag leaf was measured using the SPAD meter. At the end of the growing season, the yield of the main shoot was determined.

Lyophilized flag leaves (Freeze Dry System/Freezone® 4.5, LABCONCO Kansas City, MO, USA) were ground to a fine powder in a mixer mill homogenizer (MM400, Retsch, Haan, Germany). The powdered plant material was then subjected to certain analyses (chlorophyll, carotenoid, soluble phenolic and soluble carbohydrate contents) according to (Hura *et al*., 2019). Each of these metabolites was analysed spectrophotometrically using a microplate reader (Synergy II; BioTek, Winooski, VT, USA). Biochemical analyses were performed in 9 biological replicates of each DH line.

The leaf water content (LWC) was analysed by quantitatively sampling the leaf fresh mass (LFW), followed by drying at 75°C for 48 h, and weighing the resulting dry mass (LDW). LWC was calculated according to the following formula: LWC = (LFW−LDW)/LDW (Du *et al*., 2010). The measurements for each line were taken using 10 replicates.

The measurements were carried out using a fluorometer Handy PEA (Hansatech Instruments Ltd. Kings Lynn, UK). They were taken after 30 min of leaf adaptation to darkness. The light intensity reaching the leaf was 3 000 µmol (quantum) m^{-2} s^{-1} (peak at 650 nm). Fluorescence was recorded during irradiation between 10 µs and 1 s. During the initial 2 ms, data were collected every 10 µs with a 12 bit resolution. After this period, the measurement frequency was dropped automatically (Hura *et al*., 2015; Ostrowska *et al*., 2019a). The collected data were analysed using a JIP test, based on the theory of energy flow in PSII (Appenroth *et al*., 2001; Strasser *et al*., 2010; Strasser *et al*., 2004). The following parameters were calculated per excited leaf cross-section (CSm): ABS/CS_m (light energy absorption), TR_o/CS_m (amount of excitation energy trapped in PSII reaction centres), ET_o/CS_m (amount of energy used for electron transport), DI_o/CS_m (amount of energy dissipated from PSII), and RC/CS_m (number of active reaction centres). Furthermore, F_v/F_m (quantum yield of PSII), and PI (overall performance index of PSII photochemistry) were determined. Additionally, the following parameters for photosystem I were determined: the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (ψR_o), the efficiency with which an electron can move from the reduced intersystem of electron acceptors to the PSI end electron acceptors (δR_o), and the quantum yield of electron transport from Q_A to the PSI end electron acceptors (φR_o). The measurements for each line were taken using 20 replicates.

Measurements were performed on flag leaves with a CL-01 Chlorophyll Meter (Hansatech Instruments Ltd., Kings Lynn, UK).

On average 5 mg of powdered plant material was extracted in 95% ethanol (1.5 mL) for 15 min and centrifuged at 2,000 g (Universal 32R, Hettich, Germany) for 10 min. The ethanolic extract (100 µL) was added to a 96-well microplate, and the absorbance was measured at 470, 648 and 664 nm. The concentration of the chlorophyll and carotenoids was then calculated according to Lichtenthaler and Wellburn (1983).

The soluble carbohydrate content was analysed spectrophotometrically as described by Marcińska *et al*. (2013). On average 5 mg of powdered plant material were extracted with 1.5 mL of 96% ethanol for 15 min. Then the samples were centrifuged at 21 000 g for 15 min and 40 µL of the supernatant were transferred into test tubes (10 mL) containing 400 µL of deionized water. After that, 400 µL of 5% phenol and 2 mL of concentrated sulphuric acid were added. The reaction mixtures were incubated for 20 min and transferred to 96-well plates. The absorbance was read at 490 nm.

The total soluble phenolic content was measured according to the modified method of Singleton *et al*. (1999). An aliquot of the extract (50 µl) was diluted in 0.5 ml of deionized water and 0.2 ml of Folin-Ciocalteu reagent and after 10 min, 0.7 ml of saturated Na_2CO_3 was added. The samples were then mixed after 2 h of incubation and transferred to 96-well plates. The absorbance was read at 765 nm. Chlorogenic acid was used as a standard.

Grain yield (from the main shoot) is presented as the average grain yield in grams per plant. The yield was determined using 10 repetitions. For all measurements, one replicate means one plant.

After examining the parameters, their mean was calculated and the standard error was determined. All data were analysed using Statistica 13.1 software (Statsoft Inc., USA). Correlations between the measured parameters were tested at a probability of $p<0.05$.

RESULTS

The hydration of the studied flag leaves varied to a notable extent, from very low in DH lines 2007-10/2(1), DH2006-HM48, DH2006-HM31, DH2007-12/3(19), and DH2007-9/3(107) (0.56-1.17 g g^{-1}), to very high in lines DH2006-HHM8, DH2007-11/2(126), Magnat, DH2007-3/2(149), and DH2007-3/3(181) (1.94-2.36 g g^{-1}) (Table 1).

Table 1. Soil drought influence on leaf water content (LWC) (n=10), chlorophyll levels (n=10), and chlorophyll fluorescence parameters (n=20) in the flag leaves of winter triticale on day 21 of the soil drought

Line DH	LWC (g g^{-1})	F_v/F_m	ABS/CS_m	TR_o/CS_m	ET_o/CS_m	DI_o/CS_m	PI	δR_o	φR_o	ψR_o	CHL (SPAD)
2006-HHM7(184)	1.76±0.11	0.822±0.005	3174±49	2611±51	1411±49	563±5	1.98±0.13	0.343±0.008	0.153±0.007	0.185±0.008	13.93±1.02
2007-11/2(126)	1.95±0.07	0.823±0.003	3188±42	2624±41	1423±38	564±6	1.94±0.11	0.319±0.006	0.143±0.005	0.173±0.006	14.05±0.90
2006-HM48	0.75±0.14	0.766±0.011	2820±67	2169±73	965±52	651±19	0.88±0.07	0.238±0.008	0.081±0.004	0.105±0.005	13.69±0.92
2006-HM31	0.88±0.16	0.759±0.014	2942±57	2236±68	1072±47	705±43	1.04±0.11	0.308±0.011	0.113±0.007	0.147±0.007	14.82±1.07
2007-3/3(181)	2.36±0.23	0.815±0.005	3124±59	2549±57	1314±64	575±15	1.9±0.21	0.316±0.010	0.134±0.008	0.164±0.010	12.83±0.85
2007-9/3(107)	1.17±0.08	0.797±0.009	2946±1 19	2366±114	1011±88	580±10	1.03±0.13	0.296±0.004	0.098±0.006	0.122±0.007	12.69±0.99
2007-10/2(1)	0.56±0.12	0.749±0.020	2589±88	1956±99	907±58	632±36	0.96±0.10	0.319±0.014	0.109±0.005	0.146±0.007	13.69±1.09
2007-12/3(19)	1.16±0.14	0.772±0.017	2434±122	1905±126	819±79	529±19	0.99±0.13	0.344±0.024	0.108±0.006	0.141±0.009	13.91±1.05
2006-HHM8	1.94±0.12	0.820±0.001	3231±20	2648±18	1445±22	583±6	1.91±0.09	0.384±0.011	0.173±0.007	0.211±0.008	14.23±0.85
2007-3/2(149)	2.11±0.05	0.821±0.003	3013±52	2477±49	1322±40	537±4	1.88±0.09	0.304±0.004	0.133±0.003	0.162±0.004	13.93±1.02
Magnat	1.98±0.13	0.830±0.002	3288±18	2728±16	1458±25	560±6	2.18±0.11	0.343±0.012	0.154±0.007	0.185±0.009	15.21±0.87
2007-7/4(115)	1.48±0.09	0.806±0.007	3209±76	2594±76	1146±46	614±4	1.04±0.06	0.302±0.005	0.107±0.003	0.132±0.003	14.62±0.99
2006-HM17	1.82±0.11	0.825±0.002	3402±44	2806±38	1452±23	596±8	1.83±0.08	0.330±0.006	0.142±0.004	0.172±0.005	13.66±0.98
2007-9/3(151)	1.45±0.08	0.790±0.010	2840±103	2260±104	988±84	580±9	1.01±0.13	0.317±0.005	0.106±0.006	0.134±0.006	14.45±0.84
2007-7/4(140)	1.85±0.12	0.795±0.010	2992±105	2396±104	1082±79	596±6	1.06±0.12	0.301±0.004	0.106±0.006	0.132±0.006	13.46±1.04
2007-4/4(150)	1.68±0.07	0.820±0.004	2974±62	2443±61	1288±61	531±4	1.84±0.14	0.331±0.009	0.144±0.008	0.175±0.009	13.76±0.84
2006-HM27	1.64±0.09	0.822±0.002	3160±38	2598±36	1352±31	562±4	1.82±0.08	0.368±0.010	0.157±0.006	0.192±0.007	14.37±0.86
2006-HM7	1.78±0.11	0.827±0.002	3192±33	2642±31	1342±24	550±7	1.82±0.08	0.303±0.007	0.128±0.004	0.154±0.005	14.16±1.00
2007-7/3(44)	1.65±0.05	0.786±0.011	3043±88	2407±94	1086±69	635±13	1.00±0.10	0.300±0.007	0.106±0.005	0.133±0.006	13.97±0.62
2007-7/4(120)	1.45±0.05	0.812±0.003	3205±52	2604±49	1160±34	602±8	1.10±0.07	0.280±0.004	0.101±0.003	0.125±0.003	12.89±1.05

Chlorophyll fluorescence parameters (n=20) including the quantum yield of PSII (F_v/F_m), light energy absorption (ABS/CS_m), amount of excitation energy trapped in PSII reaction centres (TR_o/CS_m), amount of energy used for electron transport (ET_o/CS_m), amount of energy dissipated from PSII (DI_o/CS_m), the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (ψRo), the efficiency with which an electron can move from the reduced inter system of electron acceptors to the PSI end electron acceptors (δR_o) and the quantum yield of electron transport from Q_A to the PSI end of electron acceptors (φR_o). White rows – low-yield triticale DH lines, gray rows – high-yield triticale DH lines.

Changes in the photochemical activity of the flag leaves (Table 1) corresponded to their varied water content. Lines with lower LWC values demonstrated a limited PSII quantum yield (F_v/F_m), lower light absorption (ABS/CS_m), a drop in the energy use (TR_o/CS_m, ET_o/CS_m), and a disrupted performance in both photosystems, PSII and PSI (PI). The opposite effect, *i.e.*, a higher activity of the photosynthetic apparatus in drought conditions, was observed in lines with a higher level of flag leaf hydration, with the exception of the DH2007-7/3(44) and DH2007-7/4(140) lines, which despite a medium (DH2007-7/3(44): LWC = 1.65 g g^{-1}) and a high (DH2007-7/4(140): LWC = 1.85 g g^{-1}) water content demonstrated the relatively low activity of the photosynthetic apparatus. Generally, in drought conditions the DH lines with a higher level of flag leaf hydration were characterized by a higher probability that a trapped exciton would move an electron into the electron transport chain beyond Q_A (ψR_o), with an increase in the efficiency with which an electron can move from the reduced intersystem of electron acceptors to the PSI end of electron acceptors (δR_o), and with a higher quantum yield of electron transport from Q_A to the PSI end electron acceptors (φR_o). Despite the varying level of flag leaf hydration in drought conditions, the chlorophyll content was at a similar level in individual DH lines (Table 1).

Both in the high- and low-yield DH lines, the soil drought affected the level of photosynthetic pigments, dissolved carbohydrates, and phenols below the subflag leaf (Table 2). The content of chlorophyll *a* and chlorophyll *b*, as well as the carotenoid levels were highest in DH2006-HM48, the parent line Magnat, DH2006-HM17, DH2007-4/4(150), and DH2006-HM27, while it was lowest in the DH2007-3/3(181), DH2006-HHM8, DH2007-3/2(149), DH2007-9/3(151), and DH2007-7/3(44) lines. In the same conditions of soil water deficit, the high-yield parent line Magnat was clearly characterized by the highest level of photosynthetic pigments of all the studied DH lines (chlorophyll *a* – chl *a*: 4.60 μg mg^{-1} d.w., chlorophyll *b* – chl *b*: 3.25 μg mg^{-1} d.w., carotenoids - car:

Table 2. Soil drought influence on photosynthetic pigment content (n=9), soluble carbohydrates (SC) (n=9), and soluble phenolic compounds (SPh) (n=9) in the leaves below the subflag leaf of winter triticale on Day 21 of the soil drought

Line DH	CHL *a*+*b*	CHL *a*	CHL *b*	CAR	SC	SPh
	(μg mg^{-1})					
2006-HHM7(184)	1.21±0.09	0.86±0.06	0.36±0.03	0.48±0.01	22.81±3.01	9.29±0.25
2007-11/2(126)	1.36±0.06	0.94±0.04	0.42±0.02	0.44±0.01	26.06±0.93	8.96±0.10
2006-HM48	3.91±0.17	2.81±0.12	1.11±0.05	1.01±0.03	64.04±3.69	6.92±0.21
2006-HM31	3.49±0.18	2.49±0.14	1.00±0.05	0.89±0.06	50.44±1.68	6.70±0.18
2007-3/3(181)	0.89±0.16	0.61±0.11	0.28±0.05	0.25±0.02	37.97±1.81	9.30±0.32
2007-9/3(107)	2.58±0.36	1.85±0.27	0.73±0.09	0.57±0.06	46.57±1.20	6.84±0.21
2007-10/2(1)	2.79±0.03	2.00±0.02	0.79±0.01	0.79±0.01	70.08±1.45	6.25±0.39
2007-12/3(19)	2.64±0.12	1.83±0.08	0.81±0.04	0.62±0.01	75.05±1.42	7.22±0.30
2006-HHM8	1.07±0.14	0.76±0.10	0.31±0.04	0.48±0.03	27.62±2.06	9.01±0.12
2007-3/2(149)	0.61±0.08	0.41±0.05	0.20±0.02	0.23±0.03	36.41±2.03	8.71±0.15
Magnat	4.60±0.41	3.25±0.28	1.36±0.13	1.00±0.07	59.42±2.60	5.39±0.29
2007-7/4(115)	1.16±0.06	0.79±0.04	0.37±0.02	0.33±0.02	35.12±1.43	10.04±0.10
2006-HM17	3.91±0.10	2.75±0.07	1.16±0.03	1.05±0.01	49.13±1.23	7.09±0.28
2007-9/3(151)	0.83±0.05	0.59±0.03	0.25±0.02	0.27±0.01	46.40±0.73	8.35±0.07
2007-7/4(140)	1.23±0.04	0.85±0.03	0.38±0.01	0.31±0.01	36.73±0.63	9.02±0.21
2007-4/4(150)	4.13±0.16	2.93±0.11	1.20±0.04	0.76±0.03	60.19±2.24	7.03±0.07
2006-HM27	3.92±0.29	2.78±0.20	1.14±0.09	1.16±0.03	55.04±1.35	7.46±0.10
2006-HM7	3.13±0.23	2.23±0.16	0.90±0.07	0.83±0.03	48.63±1.90	6.87±0.34
2007-7/3(44)	0.86±0.06	0.57±0.04	0.28±0.02	0.25±0.01	37.84±1.21	8.39±0.07
2007-7/4(120)	1.17±0.14	0.79±0.10	0.38±0.04	0.36±0.03	33.95±0.87	9.16±0.12

Photosynthetic pigment content including chlorophyll *a* – CHL *a*, chlorophyll *b* – CHL *b*, carotenoids – CAR. White rows – low-yield triticale DH lines, gray rows – high-yield triticale DH lines.

1.00 μg mg^{-1} d.w.). The low-yield line 2007-3/2(149) had the lowest level of chl *a* (0.61 μg mg^{-1} d.w.), chl *b* (0.41 μg mg^{-1} d.w.), and car (0.23 μg mg^{-1} d.w.) in the pool of studied lines (Table 2).

Soil drought stress caused a varied reaction in the triticale DH lines in terms of soluble carbohydrates and phenol content in the leaves below the subflag leaf (Table 2). The carbohydrate levels ranged from 22.81 to 75.05 (μg mg^{-1} d.w.) in the low-yield, and from 33.95 to 60.19 (μg mg^{-1} d.w.) in the high-yield lines. A high carbohydrate level was observed in lines DH2006-HM48 (64.04 μg mg^{-1} d.w.), DH2007-10/2(1) (70.08 μg mg^{-1} d.w.), DH2007-12/3(19) (75.05 μg mg^{-1} d.w.), Magnat (59.42 μg mg^{-1} d.w.), and DH2007-4/4(150) (60.19 μg mg^{-1} d.w.), while the lowest level was noted in DH2006-HHM7(184) (22.81 μg mg^{-1} d.w.), DH2007-11/2(126) (26.06 μg mg^{-1} d.w.), DH2006-HHM8 (27.62 μg mg^{-1} d.w.), DH2007-7/4(115) (35.12 μg mg^{-1} d.w.), and DH2007-7/4(120) (33.95 μg mg^{-1} d.w.). The soluble phenol content during soil drought conditions was less varied in individual DH lines. In low-yield lines the level of phenolic compounds in leaves below the subflag leaf ranged from 6.25 μg mg^{-1} d.w. (DH2007-10/2(1)) to 9.30 μg mg^{-1} d.w. (DH2007-3/3(181)). In high-yield lines, the content of these compounds varied from 5.39 μg mg^{-1} d.w. (Magnat) to 10.04 μg mg^{-1} d.w. (DH2007-7/4(115)) (Table 2). In general, it was observed that a high level of phenolic compounds is accompanied by a lower carbohydrate content in leaves below the subflag leaf, and indeed this was documented by a statistically significant correlation between these two parameters (Fig. 2).

Table 3 shows results for the chlorophyll content in the flag leaf on day 14 of rehydration, and also the yield of the main stem for individual DH lines of winter triticale. A higher chlorophyll level (20.9-22.6) in the high-yield DH lines was found to correspond to a higher grain yield (0.36-0.49 g). In the low-yield DH lines (0.14-0.18 g), a significantly lower chlorophyll level was observed (11.7-13.4). This relationship was confirmed by a statistically significant correlation between the yield and the chlorophyll content in the flag leaves, as shown in Figure 3.

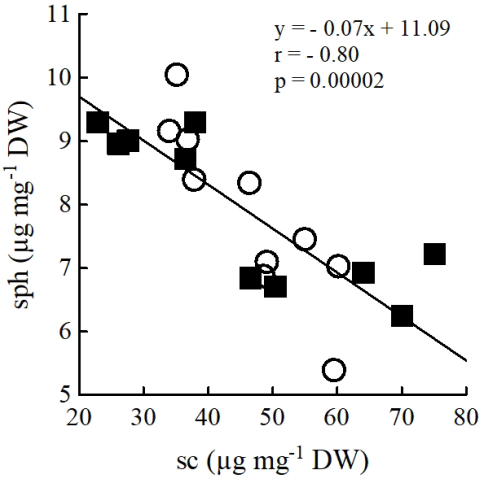

Fig. 2. The relationship between the phenolic compounds content (sph) and soluble carbohydrate levels (sc) in the leaves below the subflag leaf, in 20 DH lines of winter triticale. White circles – high-yield triticale DH lines, black squares – low-yield triticale DH lines.

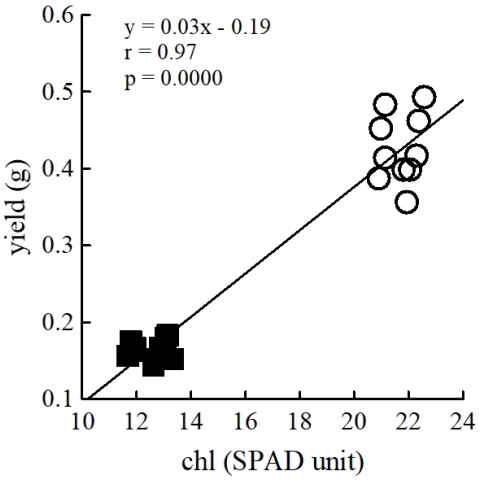

Fig. 3. The relationship between the main stem yield (yield) and the chlorophyll level (chl) in the flag leaves analysed on day 14 of rehydration. White circles – high-yield triticale DH lines, black squares – low-yield triticale DH lines.

Table 3. Chlorophyll content (n=10) in the flag leaves of 20 DH lines of winter triticale on day 14 of rehydration post soil drought, and the main stem yield (n=10).

Line DH	CHL (SPAD)	Main stem yield (g)
2006-HHM7(184)	11.69±1.01	0.156±0.019
2007-11/2(126)	11.79±0.99	0.174±0.015
2006-HM48	11.93±0.85	0.166±0.018
2006-HM31	11.93±0.91	0.163±0.017
2007-3/3(181)	12.61±0.87	0.144±0.015
2007-9/3(107)	12.72±0.96	0.152±0.017
2007-10/2(1)	12.86±0.84	0.166±0.017
2007-12/3(19)	13.07±0.62	0.179±0.015
2006-HHM8	13.16±1.04	0.183±0.019
2007-3/2(149)	13.35±0.87	0.152±0.017
Magnat	20.89±1.01	0.388±0.050
2007-7/4(115)	20.99±0.99	0.452±0.018
2006-HM17	21.13±0.85	0.414±0.020
2007-9/3(151)	21.13±0.91	0.483±0.054
2007-7/4(140)	21.81±0.87	0.399±0.027
2007-4/4(150)	21.92±0.96	0.356±0.018
2006-HM27	22.06±0.84	0.399±0.036
2006-HM7	22.27±0.62	0.417±0.025
2007-7/3(44)	22.36±1.04	0.462±0.054
2007-7/4(120)	22.55±0.87	0.493±0.042

White rows – low-yield triticale DH lines, gray rows – high-yield triticale DH lines.

DISCUSSION

The studied triticale DH lines were characterized by a varied response to the soil water deficit. This was demonstrated through the determination of the relationships between water levels, the photosynthetic apparatus activity, and the chlorophyll levels (Table 1). The parameters analysed are among those most frequently used to select plants with an increased tolerance to drought, as they may be directly compared to the leaf water content (LWC), the extent of the damage to the photosynthetic apparatus (chlorophyll fluorescence), or progress in the senescence process, which may be evaluated through the measurement of the chlorophyll content (Yu *et al*., 2016).

One visible manifestation of drought is plant senescence which is associated with chlorophyll degradation that is detectable in terms of the visible yellowing of the leaves (Ougham *et al*., 2008). Plant senescence caused by soil drought leads to reduced photosynthetic activity, and, as a consequence, contributes to a reduced grain yield in cereals (Munne-Bosch and Alegre, 2004; Pandey *et al*., 2017; Riasat *et al*., 2019). Additionally, Hura *et al*. (2011) demonstrated that the level of chlorophyll content during a drought depends on plant age. Matile *et al*. (1996), on the other hand, stated that during foliar senescence, plants catabolize chlorophyll into water-soluble porphyrin derivatives that merely accumulate in the mesophyll. In our study, the chlorophyll content in the flag leaves was at a similar level across the different DH lines. This may result from the fact that in cereals the senescence which is triggered by soil drought progresses from the bottom up to the flag leaf, therefore, its signs may not be visible or detectable in the flag leaf at a given point in time (Ostrowska *et al*., 2019b).

There is a possibility that it was the level of LWC and not chlorophyll degradation that had a decisive influence on the photosynthetic apparatus activity in the flag leaves.

In the studied DH lines, the flag leaves with the highest hydration level were characterized by a higher level of photosynthetic activity, whereas those with a lower water content had a lower photosynthetic activity (Table 1). The water deficit in the leaves increases the sensitivity of the photosynthetic apparatus to light and therefore the risk of photoinhibition damage (Guidi *et al*., 2019; Ma *et al*., 2006). It has been shown that overloading the electron transport chain (ETC) in PSI and PSII generates reactive oxygen species (ROS) (Gill and Tuteja, 2010). If ETC is overloaded, then PSII may leak electrons from plastoquinones Q_A and Q_B, this may result in the reduction of molecular oxygen to a superoxide anion and its further dismutation to H_2O_2 (Borisova-Mubarakshina *et al*., 2018). Therefore, an undisturbed electron flow in the thylakoid membranes may minimize the risk of photoinhibition damage. A visible differentiation in the senescence of individual DH lines was observed in leaves in the lower part of the plants, under the subflag leaf (Table 2). In lines with the highest chlorophyll content, the senescence process associated with chlorophyll degradation was the slowest one. It has been shown in Arabidopsis that during dehydration, a senescing leaf loses water more rapidly than a non-senescing one (Zhang *et al*., 2012). On the other hand, the plant senescence associated with the yellowing followed by the drying of the leaves below the flag leaf may be one of the mechanisms of adaptation to soil drought, because both reduced leaf area and reduced transpiration to limit water loss and in this way plant survival is improved (Munne-Bosch and Alegre, 2004).

Another biochemical indicator for senescent plant organs is the accumulation of phenolic compounds (Massolo *et al*., 2011; Torras-Claveria *et al*., 2012). In our study, a high level of phenolic compounds correlated with a low carbohydrate content in individual triticale DH lines (Fig. 1). The level of phenolic compounds depends on the availability of carbohydrates because the metabolic pathways of carbohydrates and of phenolic compounds are connected by the shikimic acid pathway (Averesch and Kroemer, 2018; Weaver and Herrmann, 1997). The metabolic pathways of both substances are interconnected: the phenolic (and also amino acid) pathways use the products of carbohydrate metabolism as their precursors (Ndoumou *et al*., 1996). In vitro tests have also confirmed the use of carbohydrates as a source of carbon for the synthesis of secondary metabolites including phenolic compounds (Khan *et al*., 2018; Wu *et al*., 2006). Lindroth *et al*. (2002) have proved that the concentrations of simple sugars declined during senescence, and that patterns of change differed between the genotype and nutrient treatments.

A reduction in the carbohydrate content in the leaves below the subflag leaf (Table 2) may also result from carbohydrate allocation to younger leaves. Young leaves become "sink leaves" because on the one hand, they accumulate free amino acids, while on the other, they effectively use carbohydrates to build cellular structures and for energy production. Older, senescent leaves become "source leaves" accumulating mineral nitrogen and progressively losing organic compounds such as carbohydrates and amino acids (Masclaux *et al*., 2000). The opposite effect, *i.e.*, an increase in the carbohydrate content accompanied by a decrease in the phenolic compounds content was observed following the exogenous application of 6-benzylaminopurine, which prolonged post-harvest life in *Anthurium andraeanum* (Favero *et al*., 2020). Quirino *et al*. (2001), on the other hand showed that certain monosaccharides, particularly galactose, fructose and glucose, accumulate in the senescent leaves of *Arabidopsis*. Wingler *et al*. (1998) noted that the glucose and fructose contents increased with leaf age in tobacco. However, in the studied triticale DH lines in conditions of the soil drought, it was found that the reduction in the level of soluble carbohydrates, which were of paramount importance for plant productivity (Ainsworth and Long, 2005; Leakey *et al*., 2009), occurred with a simultaneous increase in phenolic compounds content which could be considered as a biochemical indicator/sign of progressing plant senescence.

In crop plants, a relationship between the measured (physiological, biochemical, or molecular) parameters and their yield potential is frequently sought (Lecoeur *et al*., 2011). However, our results for the soil drought period did not correlate with the yield from individual DH lines, while such a statistically significant relationship was obtained for the yield and chlorophyll level during the rehydration period (Table 3, Fig. 2). Based on these outcomes, we suggest that the inhibition of drought-induced senescence in the rehydration period following the drought may be crucial for restoring optimum productivity in DH lines of winter triticale. Rulcova and Pospisilova (2001) demonstrated the significant influence of the photosynthetic pigment levels on plant regeneration which was determined during the rehydration period after soil drought. It should be emphasized that the occurrence of a drought during the reproductive stage of cereal development is particularly detrimental, as it is combined with a very short period of possible regeneration after the exposure period to the stress agent ends (Altenbach *et al*., 2003; Barnabas *et al*., 2008; Hura *et al*., 2015). Therefore, the plant senescence induced during a soil drought may limit the possible restoration of the maximum yield potential (Andrianasolo *et al*., 2016; Ramirez *et al*., 2014). Hura *et al*. (2019) observed a reduced content of chlorophylls *a*/*b* and of carotenoids in a not-fully recovered DH line when compared to a fully recovered DH line. Furthermore, they demonstrated that a long-term effect of the reduced activity of the photosynthetic apparatus and accelerated aging during

rehydration was a significant decrease in yield potential in the not-fully recovered DH line. On the other hand, lowering the chlorophyll level may be one of the ways of avoiding photoinhibition damage to the photosynthetic apparatus (Munne-Bosch and Alegre, 2000), but it appears more likely to occur under water stress than under rehydration.

In our opinion, the observed higher yield potential in individual DH lines (Table 3) is associated, *e.g.* with the inhibited/limited senescence process during the rehydration period through the increased chlorophyll level in the flag leaves. Generally, the content of photosynthetic pigments is considered to be a good indicator of plant vigour and photosynthetic capacity under both optimal and adverse environmental conditions (Carter and Spiering, 2002).

CONCLUSIONS

1. Our study demonstrated that a reduction in, or even an inhibition of the senescence process during the rehydration period is an important factor for plant productivity after exposure to soil drought.

2. The selection of phenotypes with a higher tolerance to soil drought should also include the rehydration period, in order to evaluate plant regenerative potential after the cessation of stress.

3. The rehydration period is of particular importance for winter triticale, in which the soil drought which occurs during reproductive growth (*e.g.* heading, flowering) is translated into the greatest loss in yield due to a short period of possible plant regeneration in optimal conditions of soil water content.

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