



SZENT ISTVÁN UNIVERSITY

**STRESS AND ECOPHYSIOLOGICAL INVESTIGATIONS
ON THE SPECIES AND GENOTYPES CLOSELY RELATED
OF WHEAT**

DÓRA SZOPKÓ

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PhD school

name: PhD School of Biological Sciences

field: Biology

head: Zoltán Nagy
professor, DSc
Szent István University
Faculty of Agricultural and Environmental Sciences

supervisor: Sándor Dulai
college professor, PhD
Eszterházy Károly University,
Department of Botany and Plant Physiology

.....
Head of PhD School

.....
Supervisor

1. BACKGROUND AND AIMS

1.1. Background

The effects of global climate change can greatly promote the development of abiotic stress factors that endanger the productivity of bread wheat (*Triticum aestivum* L.). The most prominent problem is the extreme rainfall pattern and the high average temperature (TRENBERTH *et al.* 2007), which may result agricultural damages by drought, salt and/or heat stress. Drought is the most important abiotic stress factor (ARAUS *et al.* 2002) but also around 30% of the agricultural lands are affected by salt stress (MUNNS 2005). The extent of negative effects in plants is determined by the NaCl concentration in the soil, the duration of stress and the genotype of the plant (ZHU 2001). Artificial selection also contributed to the decreased genetic diversity of wheat leading to a decline in stress tolerance. At the same time, cultivated or wild species closely related to bread wheat may represent a suitable gene donor to produce new wheat lines having a promising adaptation capacity (FAROOQ *et al.* 1995, FAROOQ 2002, COLMER *et al.* 2006). Some varieties of cultivated barley (*Hordeum vulgare* L.) have many useful agronomic traits (e.g., earliness, winter-hardy), therefore they represent a prominent candidate for breeding programmes. Some genomic regions on the 4H and 7H chromosomes of barley can also contribute to improve salt tolerance of wheat (GORHAM 1990, SHAVRUKOV *et al.* 2010, LONG *et al.* 2013).

A positive relationship of photosynthetic capability and crop production has been well documented. Accordingly, those barley chromosomes ensuring the maintained photosynthetic capacity under stress condition even in the wheat genetic background could play prominent role. Reduction of the relative water content (RWC) of the shoot may negatively affect the different photosynthetic processes (CHAVES 1991, BAJJI *et al.* 2000). For this reason, 4H barley chromosome could be advantageous under salinity induced osmotic stress due to carrying RWC controlling genes (FARSHADFAR *et al.* 2008). Salt-induced water deficiency can be prevented through the accumulation of osmotically active compounds associated with better salt tolerance (ASHRAF and FOOLAD 2007). Earlier results have showed that a genomic region on 7H barley chromosome involved in osmotic adjustment (TEULAT *et al.* 1998) may also related to a greater biomass production (GONZÁLEZ *et al.* 1999). In addition, 7H chromosome could also contribute to the avoidance of unfavourable periods by inducing earliness (ARANYI *et al.* 2014).

Under natural conditions water deficit and salt stress occur often in combination with high light intensity and heat stress. These stress factors may limit the processes of the carbon metabolism simultaneously (DULAI *et al.* 2005). During climate change, it may become increasingly common for cultivated wheat to tolerate the combined effects of these stress factors (SUZUKI *et al.* 2014). Consequently, the survival of wheat can be determined by its ability to coordinate mechanisms protecting against the above-mentioned

stress factors. The synchronization of regulating/protecting processes largely determines the flexibility of plants' tolerance under the effects of the combined factors at a given time and space. Accordingly, the measure of phenotypic plasticity in the changing environment may be essential for the survival (LARCHER 1995) and it can significantly influence the prevention of the effects of global climate change (GRATANI 2014). Simultaneous environmental factors may elicit a response different from that given to a single factor, resulting in intensification, overlapping or antagonistic effects (OSMOND *et al.* 1986). Although the rate of photosynthesis is significantly reduced by high temperature (BERRY and BJÖRKMAN 1980) however, the thermal tolerance of the photosynthetic apparatus may be improved by drought and salt preconditioning (LU and ZHANG 1999, DULAI *et al.* 2006, YAN *et al.* 2012).

The vegetation period in the habitats of several *Aegilops* species could be characterised by hot summers with a low amount of seasonal or annual rainfall (DULAI *et al.* 2006). Relating to this, *Aegilops* species might adapt to the unfavourable environmental conditions thus their superior ability to tolerate several abiotic stresses has already been described (HOLUBEC *et al.* 1992, ZAHARIEVA *et al.* 2001, MOLNÁR *et al.* 2004). The basis for the survival of wild wheat can be assured by an effective coordination of defensive processes which can make these plants a potential gene source for improving wheat stress tolerance.

1.2. Aims

According to the above-mentioned facts, in the first part of the Ph.D. dissertation, our aim was the investigation of the salt tolerance of two wheat×barley addition lines on the basis of the responses of their growth, water status and photosynthetic parameters. During the experiments, we were looking for the answer whether the presence of the 4H and 7H chromosomes of barley had a positive influence on the salt tolerance of the wheat *cv.* Asakaze, and also these derivatives could serve as a suitable genetic material to increase the salt tolerance of wheat. During the selection, we would like to recognize which protecting/regulating mechanisms can participate in the formation of better salt tolerance in the addition lines and what extent they could be attributed to barley chromosomes.

In the second part of the dissertation, we were aimed at the study of *Aegilops* lines that could function as a gene source for increasing the salt and/or drought tolerance of wheat. The basis for the selection was the growth, water status, osmoregulation and photosynthetic parameters under increasing drought and salt stress. We also sought to recognize whether the salt and drought pre-treatments could influence and what extent the high temperature tolerance of the photosynthetic apparatus of the wheat and *Aegilops* lines depends on the intensity of light. Our aim was also to compare the water-deficient and salt-stressed samples partly according to the degree of phenotypic plasticity. By the

experiments, we would like to identify wild wheat lines which could coordinate processes against the combined effect of water-shortage/salt stress, high light and high temperature more efficiently than wheat.

2. MATERIALS AND METHODS

2.1. Plant materials

In our experiments the 4H and 7H wheat (Asakaze)/barley (Manas) addition lines (MOLNÁR-LÁNG *et al.* 2000, 2007, 2012), together with the parental lines and *Aegilops* species (*Ae. comosa* TA2760, *Ae. umbellulata* MvGB420, *Ae. umbellulata* AE740/03) and Mv9kr1 (winter wheat) were investigated. The seeds of Asakaze, Manas and the addition lines were provided by Márta Molnár-Láng, Agricultural Institute of the Hungarian Academy of Science (Martonvásár, Hungary). The seeds of Mv9kr1 and *Aegilops* species were also provided by the gene bank of the Agricultural Research Institute of the Hungarian Academy of Sciences.

2.1. Plant growth and treatments

The seeds were germinated on filter paper moistened with distilled water in Petri dishes for 2 days after 24 hours wetting. The germinated seeds were grown in half-strength modified Hoagland nutrient solution (NAGY and GALIBA 1995) in 1,500 ml pots or were planted in soil (5 seeds/pot). Plants grow at 25/20°C in a growth chamber with a photosynthetic photon flux density of 200 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 14/10 hours of light/dark illumination. Salt stress was induced in *Aegilops* and Mv9kr1 line by applying 150 and 300 mM concentration of NaCl (Sigma, St. Louis, USA) while the salt stress in the wheat×barley addition and the parental lines was induced by 100, 200 and 300 mM concentration of NaCl in seven-day cycles. After reaching the highest salt concentration, the salt was eliminated from the medium. Measurements were made before the treatment (5-week old plant), after each seven-day treatment and after two and seven days of regeneration without NaCl. The watering of the *Aegilops* lines and Mv9kr1 wheat line grown in soil was abolished after the age of 5 weeks. In the case of water-deficient plants, the measurements were performed on the 4th, 7th and 10th day of water shortage. The ability to regenerate was investigated by the total humidification of the soil.

2.3. Growth and germination tests

The shoot and root dry mass (g/plant) was determined at the end of the whole experimental period using 10 plants per treatment. To determine the dry mass, the leaves and roots were dried at 105°C for 12 hours. In the case of wheat×barley addition lines, we examined their germination ability under saline condition. Seeds (3×30 seeds of each genotype/treatment) were surface-sterilized in 10 % sodium hypochlorite for 15 minutes, rinsed in distilled water

and germinated on wet filter paper containing 0, 100, 200 and 300 mM NaCl in Petri dishes for 3 days at room temperature.

2.4. Determination of relative water content and osmotic potential of the leaves

The water status of the plants was traced by determining the relative water content (RWC) according to the following equation: $RWC [\%] = [(FM - DM)/(SM - DM)] \times 100$, where FM is the fresh mass, SM is the water-saturated mass and DM is the oven dry mass. Fresh mass of the leaves was measured, after which they were dried at 105°C for 4 hours. To determine the water-saturated mass, the leaves were incubated in distilled water in a Petri dish for 24 h at room temperature. The osmolality of the leaves was measured using an OSMOMAT 010 (Gonotec, Berlin, Germany) and osmotic potential values were calculated as described by Bajji *et al.* (2001) using the following formula: $OP \text{ (MPa)} = -c \text{ (mosmol kg}^{-1}) \times 2,58 \times 10^{-3}$. 0.1 g fresh mass was measured from the leaves which were homogenized with 2 ml of distilled water to obtain the appropriate volume of sample required for the measurements. The homogenates were centrifuged 10,000 RPM for 10 minutes then 50 µl of the supernatant was used to determine the osmotic potential value.

2.5. Determination of proline content

0.1 g (FW) of leaves was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered. Two ml of the filtrate was reacted with 2 ml acid-ninhydrin and 2 ml of acetic acid for 1 hour at 100°C. The reaction mixture was extracted with 4 ml toluene and mixed for 11-15 sec. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm using toluene for a blank. The proline concentration (µg g⁻¹ FW) was determined from a standard curve and calculated on a fresh weight basis according to Bates *et al.* (1973) and Ringel *et al.* (2003).

2.6. Determination of Na and K content

The amounts of sodium (589 nm) and potassium (766,5 nm) in the leaves and roots were determined from air-dried samples (0.5 g) using an atomic absorption instrument (Varian SpectrAA-50/55) after microwave digestion (Chem Mars, MARS240/50) with cc. HNO₃ and HCl (ANTON *et al.* 2012).

2.7. Gas exchange measurements

The CO₂ assimilation of intact leaves was measured with an infrared gas analyser (GFS-3000FL, Walz, Effeltrich, Germany). The net assimilation rate (P_N), stomatal conductance (g_s) and intercellular CO₂ concentration (C_i) were calculated in the light-saturated state of photosynthesis (1,000 µmol m⁻² s⁻¹) using the equations reported by von Caemmerer and Farquhar (1981). The gas exchange chamber parameters were 25°C, 20% relative humidity. The CO₂

concentration of the reference air was 360 ppm. The maximal assimilation rate (P_{Nmax}) was determined at saturating light intensity ($1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and CO_2 concentration (1,200 ppm). Then the CO_2 concentration was decreased in several steps (1,200, 600, 360, 120 ppm) to zero ppm for 10 minutes intervals. The stomatal (L_s) and nonstomatal (L_{ns}) limitation were determined on the basis of C_i v. P_N curves, as described by Lawlor (2002). The carboxylation efficiency ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was calculated as the initial slope of C_i v. P_N curves according to Pfanz *et al.* (2007).

2.8. Chlorophyll fluorescence and P700 measurements

The *in vivo* chlorophyll *a* fluorescence was measured in dark-adapted intact leaves using a dual channel P700 and chlorophyll fluorescence measuring system (Dual PAM-100, Walz, Effeltrich, Germany). The initial level of fluorescence (F_0) was excited by a weak 460-nm light beam ($>1 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) after 15 min dark adaptation. The maximal fluorescence level of the dark- (F_m) and light- (F_m') adapted leaves were determined by applying saturating flashes ($15,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) lasting 0.8 s. Photosynthesis was induced by continuous illumination of the leaf at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (650 nm, actinic light) for 10 min. In the next step the intensity of actinic light was increased ($1,000 \mu\text{E m}^{-2} \text{s}^{-1}$) in the presence of far-red light. After 10 minutes far-red was switched off and parameters were detected. The fluorescence parameters were calculated as described by van Kooten and Snel (1990) and Klughammer and Schreiber (2008a) on the basis of the following equations: maximal quantum yield of PSII, $F_v/F_m = (F_m - F_0)/F_m$; effective quantum yield of PSII, $\phi_{PSII} = (F_m' - F_s)/F_m'$; quantum yield of regulated energy dissipation, $\phi_{NPQ} = (F_s/F_m') - (F_s/F_m)$; quantum yield of nonregulated energy dissipation, $\phi_{NO} = F_s/F_m$ (BILGER and SCHREIBER 1986, GENTY *et al.* 1989, KRAMER *et al.* 2004).

P700 was measured simultaneously with chlorophyll fluorescence via changes in absorbance in the near infrared spectrum (difference signal measured at 875-830 nm) as described by Klughammer and Schreiber (1994, 2008b). Complete P700 oxidation (P_m) is induced by a saturation pulse in the presence of far-red light (730 nm). Complete reduction is induced after the saturation pulse and cessation of far-red light, with the zero P700 signal denoted by P_0 . In the presence of actinic light, a fraction a (donor-side limited closed centres) is oxidized by the actinic light resulting in an intermediate P700 signal (P). In this state the saturation pulse-induced signal change corresponds to the oxidation of the active fraction b (open centres), with the maximal P700 signal (P_m') (KLUGHAMMER and SCHREIBER 2008b). The complementary PSI quantum yields were calculated on the basis of the following equations: photochemical quantum yield of PSI, $\phi_{PSI} = (P_m' - P)/P_m$; nonphotochemical quantum yield of PSI, related to limitation on the donor side, $\phi_{ND} = P/P_m$; nonphotochemical quantum yield of PSI, related to limitation on the acceptor side, $\phi_{NA} = (P_m -$

P_m'/P_m . The yield of the cyclic electron flow around PSI was estimated from the difference between ϕ_{PSI} and ϕ_{PSII} , $\phi_{\text{CEF}} = \phi_{\text{PSI}} - \phi_{\text{PSII}}$ (HUANG *et al.* 2010).

2.9. Heat-induced chlorophyll fluorescence

The responses of the *in vivo* chlorophyll *a* fluorescence to heat were measured in dark-adapted leaves with a pulse amplitude modulation fluorometer (PAM 101-103, Walz, Effeltrich, Germany) as described by Dulai *et al.* (1998). The initial level of fluorescence (F_0) was detected after 15-min dark adaptation and excited by a weak 650-nm light beam modulated at 1.6 kHz ($0.01 \mu\text{mol m}^{-2} \text{s}^{-1}$). The maximal fluorescence level of the dark-adapted (F_m) and light-adapted (F_m') leaves was induced by a white saturating flash ($15,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 0.8 s duration. Photosynthesis was induced for 15 min by continuous actinic light of 200 or 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. To determine the breakpoint (T_c) of the F_0 vs. T or the steady-state fluorescence (F_s) vs. T curves the heat induction of fluorescence method was applied as described by Schreiber and Berry (1977). The phenotypic plasticity ($T_{c1}-T_{c0}$) was determined by the difference between the T_c of the drought and salt treated sample (T_{c1}) and the T_c of the control sample (T_{c0}).

The minimum chlorophyll fluorescence (F_0) was monitored in leaf discs exposed to elevated temperature from 25°C to 49-55°C at a rate of 1°C min⁻¹. F_s vs. T curves were started when the photosynthesis was steady state condition at 200 and 1,000 $\mu\text{E m}^{-2} \text{s}^{-1}$ actinic light intensity. The saturation light impulse was applied at 25, 30, 35, 38, 41, 43 and then at any further 2°C temperature rise to detect F_m' . T_c was determined as the interception of regression lines fitted to F_0 and F_s data. The fluorescence parameters were calculated as described by van Kooten and Snel (1990) on the basis of the following equations: maximal quantum yield of PSII, $F_v/F_m = (F_m - F_0)/F_m$; effective quantum yield of PSII, $\phi_{\text{PSII}} = (F_m' - F_s)/F_m'$ and non-photochemical quenching of chlorophyll fluorescence, $\text{NPQ} = (F_m - F_m')/F_m'$ as described by Bilger and Björkman (1990).

2.10. Statistical analysis

The results were obtained in three independent series of experiments. The results are the means \pm standard deviations (SD) of five measurements. Differences between treatments or genotypes within each treatment were determined by means of Tukey's post hoc test ($P \leq 0.05$) using the SPSS 16.0 software. The determination of critical breakpoints (T_c) and the response of C_i v. P_N curves were carried out by Microsoft Excel v. 14.0.

3. RESULTS

3.1. Study of the salt tolerance of 4H and 7H Asakaze×Manas addition lines

The successful germination ability and the effective root growth of the barley *cv.* Manas were manifested in the 7H addition line (7H add) under salt stress, while the 4H chromosome did not contribute to the improved growth parameters of the wheat *cv.* Asakaze. The most successful capacity to preserve the water content of the shoot was observed in the case of 4H addition line (4H add) to 100 mM NaCl, confirming the role of 4H chromosome in maintaining RWC under osmotic stress (FARSHADFAR *et al.* 2008). When salt stress became severe 4H add could retain its water status with intense stomatal closure like that of Asakaze while 7H add and the barley line could avoid drastic water losses, as well as exhibiting only a moderate decrease in stomatal conductance. During the moderate salt stress (200 mM NaCl), the potassium content of the leaves in 7H add and Manas showed a decrease by 43-46% compared to the control, while the reduction in wheat was considerably lower. Exposure to 200 mM NaCl, the potassium content in the roots decreased by 50% and the sodium content was almost five times higher compare to the control in the case of 7H add and wheat. By contrast, Na⁺ content in the root of Manas increased from 5.1 mg to 67.2 mg g⁻¹ dry mass and K⁺ content was only 5.3 mg lower than that of the non-stressed sample. The proline content in the root of Manas significantly exceeded the value measured in wheat at moderate salt stress, while in the case of the shoot opposite changes were observed. The role of the 7H chromosome in osmotic adaptation (TEULAT *et al.* 1998) is confirmed by the fact that the proline content in the root of 7H add although not reaching the barley level, but showed a significantly higher value than that of wheat. However, in the case of the shoot, the proline content in 7H add exceeded the level of the barley and showed almost the same value detected in the parental wheat genotype.

Salt stress induced by 200 mM NaCl did not cause significant reduction in the net CO₂ assimilation rate in the case of Manas and 7H add, while the nonstomatal limitation (L_{ns}) have already contributed to the down-regulated photosynthesis in 4H add and Asakaze. During the stronger salt load (300 mM NaCl), L_{ns} was the highest in Manas while the extent of L_{ns} was more favourable in the 7H add similar to that in wheat. The 200 mM NaCl treatment did not affect the electron transport processes in Manas and the 7H add line, but under the strong salt stress the inhibited carbon assimilation was already manifested in the down-regulation of photochemical processes. Parallel with the Φ_{PSII} reduction, Φ_{NPQ} increased in Manas and 7H add. The higher NPQ might provide the preservation of the maximal quantum yield of PSII (F_v/F_m) (BJÖRKMAN and POWLES 1984). This phenomenon was more pronounced in the case of the 4H add treated with 200 mM NaCl thus its primary photochemical processes were not influenced even under strong salinity also indicated by the unchanged F_v/F_m (HANACHI *et al.* 2014). In parallel with the down-regulated electron transport around PSII, a rise in cyclic electron transport (CEF) around the PSI was observed in the 7H add and barley at 300 mM NaCl

while CEF in the 4H add line was significantly induced already by the 200 mM salt treatment. By contrast, the fluorescence induction parameters in wheat cv. Asakaze were practically unaffected by salt stress. In the case of 7H add, insufficient recovery of photosynthesis and the acceleration of senescence could be observed, which might be closely related to the salt-induced earliness.

3.2. Study of the salt and drought tolerance of the wheat cv. Mv9kr1 and the *Aegilops* lines

The 10-day drought induced practically a same reduction in the root and shoot in the case of wheat cv. Mv9kr1 and TA2760 line, so the ratio of shoot/root (S/R) did not change significantly. When the MvGB420 line was studied, a 26% increase in root weight was observed because of 10 days of water scarcity, thus the S/R ratio significantly decreased ($P \leq 0.05$), which was also shown in the other *Ae. umbellulata* line (AE740/03). Compared to the 300 mM NaCl treated samples, the shoot growth of MvGB420 exceeded the values of wheat line ($P \leq 0.05$), moreover, the salt sensitivity of MvGB420 was less pronounced than the other lines according to the degree of biomass reduction. The S/R ratio remained unchanged in MvGB420 and TA2760, while the wheat and AE740/03 reacted with significantly less S/R ratio to the salt stress, which was caused by a drastic reduction in the shoot mass. In our experiments, 10-day drought reduced the water content of the shoot to 40% in most lines, while only 10-25% of the control was lost when the strongest salt treatment was applied. In the shoot of the wheat, the strongest drought (10 day) and salt treatment (300 mM NaCl) induced a similar osmotic potential (-2 MPa), while in the salt-treated *Aegilops* lines showed significantly lower osmotic potential than the samples stressed by severe water scarcity. Under non-irrigated condition (10 day) the increment of proline content in the shoot of wheat was more pronounced than in the wild wheat, while salinization had a greater effect on the proline accumulation in both the roots and shoots of wild wheat than that of wheat cv. Mv9kr1. The K content in TA2760 was effectively maintained even after exposure to severe drought stress, but 300 mM NaCl already resulted a significant reduction in TA2760 thus this line showed similar value to that measured in the other *Aegilops* lines. In the case of the other examined lines the reduction in potassium content of the shoots and mainly the roots, was more pronounced during salt treatment than under water deficit.

Increasing salt stress, similar to water scarcity, resulted in a gradual decrease in stomatal conductance in most lines but almost complete closure of the stomata was observed only during the 10-day water stress. Parallel with the low stomatal conductance, the activity of CO₂ assimilation was strongly inhibited. During the 300 mM NaCl treatment, the carbon assimilation of the lines showed some activity but the measure of this inhibition was proportional to that of the water-deficient plants. Besides the closure of stomata, the L_{ns} factors also contributed to the limited photosynthesis. While L_{ns} was almost identical in

all the lines after 10 days of drought, the differences between salt-treated samples were more pronounced. In *Aegilops* lines the L_{ns} factors was already intensified by the weaker salt stress (150 mM), but in the wheat even the stronger salt treatment did not induce such a large increase in L_{ns} as in the case of wild wheat lines. Moreover, L_{ns} in *Ae. umbellulata* lines did not recover completely even by the 7th day of the regeneration period. The 10-day drought induced photoprotective NPQ mechanisms in all lines and the same extent, but the increase of this parameter was detected only in *Aegilops* lines when salt stress was induced. Relating to this, all the drought-treated samples responded a rise in CEF, but a significant operation of CEF was showed by only the salt stressed wild wheat lines. NPQ was effective in the avoiding photodamage of PSII during both treatments, because of no increment was noticed in the quantum yield of nonregulated energy dissipation.

3.3. Effect of the drought and salt stress on the thermal tolerance of photosynthetic apparatus in wheat *cv.* Mv9kr1 and *Aegilops* lines

According to the T_c points of the F_0/T curves, the thermal stability of PSII was not improved by water deficiency in the absence of light since no significant shift in T_c points was detected. By contrast, 150 mM NaCl could already increase the heat stability in all the examined lines. The temperature dependence of F_v/F_m also confirmed that an increase in thermal tolerance was induced by salt preconditioning. Although wheat *cv.* Mv9kr1 after exposure to 10-day water stress showed a slight improvement at the same time, this shift has been more strongly induced due to the severe salt treatment (300 mM) in all lines, but especially in wild wheat lines. In the presence of light ($200 \mu\text{E m}^{-2}\text{s}^{-1}$), increased thermal stability was observed already in the case of control plants by comparison with dark-adapted samples. When water shortage or salt treatment was applied besides the light, the heat resistance in some of the tested lines was even more intense than in dark condition. On the basis of the temperature dependence of effective quantum yield of PSII, the thermal tolerance in wheat line was more significantly improved by 10-day drought than in wild wheat at 200 and at $1,000 \mu\text{E m}^{-2}\text{s}^{-1}$ light intensity. However, the improvement in the resistance against high temperature was more remarkable in wild wheat lines compared to wheat *cv.* Mv9kr1 under salt conditions. During the 7-day re-watering, T_c values of salt-adapted *Aegilops* lines did not decrease to the control level in contrast to the T_c values observed in wheat line at $200 \mu\text{E m}^{-2}\text{s}^{-1}$ light intensity. Similarly, the T_c values of the wheat and TA2760 reacting with a significant shift to withholding watering for 10 days were restored to the control level when drought stress was eliminated. Heat-induced and rapid conformational change in the pigment-protein complexes of PSII favours the development of NPQ (HAVAUX 1994). This process showed a growing tendency in a certain temperature range relative to the values set at 25°C in the non-stressed and dehydrated plants at $200 \mu\text{E m}^{-2}\text{s}^{-1}$ light intensity. The maximal

values of NPQ were closed to the T_c points in the case of control and drought stressed samples at the applied light intensities (200 and 1,000 $\mu\text{E m}^{-2}\text{s}^{-1}$). Accordingly, the maximal values of the NPQ curves in the wheat and TA2760 line were higher due to the 10-day water deficiency than those of the control curves. 300 mM NaCl treatment also triggered the shift of NPQ to a higher temperature range except for MvGB420.

Significant shifts in T_c values could be generated by the obligate presence of light already in non-stressed plants in comparison with the T_c values measured in the dark on the basis of the absolute phenotypic plasticity. At the same time, the treatments (drought and salinity) applied in parallel with light have already resulted in more significant differences between the individual lines and within a line depend on the treatment. The phenotypic plasticity of water-deficient wheat was not exceeded by any of the wild wheat lines. However, the plasticity of the light-adapted MvGB420 line treated with 300 mM NaCl was more flexible than that of wheat, as it could increase the heat resistance of its photosynthetic apparatus by 6-7°C compared to its absolute control.

3.4. New scientific results

1. In the genetic background of the wheat *cv.* Asakaze, the presence of 7H barley chromosome positively modified the carbon assimilation and osmotic adaptation ability of wheat at 100 and 200 mM NaCl, resulting in the expression of the favourable properties of parental wheat and barley varieties in the 7H addition line.
2. The moderate stomatal closure, the capacity for osmotic adaptation and the prevention of the non-stomatal limitation presented by salt-treated 7H addition line contributed largely to the maintenance of a more efficient carbon assimilation rate than that of the parental wheat up to 200 mM NaCl.
3. The control of the Na^+ transport of the shoot under salt stress cannot be modified by the presence of the 7H barley chromosome in the examined wheat variety, which refers to the characterization of the control of Na^+ uptake by more barley chromosomes.
4. The 4H chromosome of barley can be successfully used to enhance the salt tolerance of wheat *cv.* Asakaze under weak (100 mM NaCl) salt stress due to its property for efficient water retention.
5. The genes found on barley 7H chromosomes in the genetic background of wheat *cv.* Asakaze, contribute to the induction of salt-stress-induced earliness even under laboratory conditions.
6. The light-induced improvement of thermal tolerance of photosynthetic apparatus is affected by drought and salt treatment differently in the case of same line.

7. As a result of the 150 mM NaCl pre-treatments, the thermal stability of PSII was increased in the dark in the examined lines in contrast to water-deficient experiments where no significant improvement was observed.
8. The salt-induced improvement in thermal stability is partly due to light-independent processes connected mainly with the ionic effect of salt-stress. The improved heat resistance observed in the dark- and salt-adapted wild wheat lines, could be indirectly caused also by the remarkable reduction in osmotic potential induced by 300 mM NaCl.
9. By increasing the temperature, the nonphotochemical quenching parameter reaches its maximum values around the critical temperature points determined for the applied treatment. Accordingly, in light-adapted state, the processes in the background of nonphotochemical quenching can provide protection against the combined effect of salt and heat stress.
10. MvGB420 (*Aegilops umbellulata*) line could contribute to improve the stress tolerance of bread wheat, due to its capability to coordinate processes against the combined effect of salt stress, high temperature and high light more efficiently than wheat associated with its original habitat.

4. CONCLUSIONS AND SUGGESTIONS

Artificial selection (plant improving) has resulted in a reduction in the level of genetic diversity of cultivated wheat which could also be manifested by a decreased resistance to several environmental stress factors (e.g., drought, high temperature). At the same time, wheat lines with developed genetic variability can be used to select those varieties having better stress tolerance compare to that of the currently cultivated. Some barley varieties and *Aegilops* lines may be a suitable gene source for improvement of the genetic diversity of wheat.

The genomic regions in the 4H and 7H chromosomes of the cultivated barley can facilitate the cultivated wheat to an enhanced salt tolerance in accordance to several studies (FORSTER *et al.* 1990, GORHAM 1990, SHAVRUKOV *et al.* 2010, MUNNS *et al.* 2012, LONG *et al.* 2013). Accordingly, wheat *cv.* Asakaze lines carrying the 4H and 7H chromosome of barley *cv.* Manas (4H add and 7H add) were tested in our experiments. The 4H chromosome plays a prominent role in avoiding significant water loss during osmotic stress (TEULAT *et al.* 2003, FARSHADFAR *et al.* 2008). These results are supported by our findings as the obligate presence of the 4H barley chromosome in the genome of wheat *cv.* Asakaze has proved sufficient to retain high RWC values under mild salt treatment (100 mM NaCl) parallel with an adequate maintenance of the carbon assimilation capability (CHAVES 1991, BAJJI *et al.* 2000). Although, above the concentration of 100 mM NaCl, the 4H barley chromosome had a particularly negative effect on the carbon assimilation ability of Asakaze, but if the goal was to increase the osmotic stress tolerance of wheat, it could be a successful gene source based on its gas exchange

parameters and water retention capacity under mild salt stress. Accordingly, the 4H add line may be advantageous in selection studies aiming to increase the water utilization capacity of wheat (MOLNÁR *et al.* 2007), therefore 4H add should be involved in a more detailed study to characterize its responses to drought.

The CO₂ fixation rate in the 7H add substantially exceeded the values of wheat to 200 mM NaCl treatment, since at this stage of the stress the carbon assimilation capability in this addition line was not significantly impaired similarly to the parental barley line. Moreover, when salt stress became stronger, the most pronounced increment in L_{ns} was observed in barley while this parameter showed a significantly less rise in the 7H add similarly to that of wheat. Consequently, the positive properties of the parental lines were associated in the case of 7H add. Photochemical and electron transport processes may also affect photosynthetic CO₂ fixation during salt stress both in wheat and barley, but the contribution of these processes to the limitation of CO₂ assimilation usually depends on the duration/intensity of the salt treatment and the sensibility of plant (KALAJI *et al.* 2011). However, no significant change in the fluorescence induction parameters of the wheat *cv.* Asakaze was detected during salt stress, even under a significant limited P_N . Since the salt-induced decrease of the F_v/F_m parameter and the inhibition of the acceptor side of PSI were not detected in wheat, possibly an alternative electron sink might be operated in Asakaze thus preventing photoinhibition (BIEHLER and FOCK 1996, WINGLER *et al.* 2000). The electron transport processes were also unlimited in the barley *cv.* Manas and 7H add up to 200 mM NaCl treatment, but during the strongest salt load the inhibited carbon assimilation has been manifested in the down-regulation of photochemical processes. The decrease in Φ_{PSII} was due to an increase in Φ_{NPQ} , which provided the prevention of photoinhibition in PSII by the dissipation of excess excitation energy when carbon assimilation is limited (BJÖRKMAN and POWLES 1984, QIU *et al.* 2003). In parallel with the down-regulation of electron transport around PSII, a more intensive CEF was observed in the barley and 7H add line exposed to 300 mM NaCl. The higher CEF also favours lumen acidity resulting an increase in energy-dependent NPQ and thus protecting the acceptor side of PSI and inhibiting the photodamage of PSII (RUMEAU *et al.* 2007). As a consequence of the 7H barley chromosome, the wheat *cv.* Asakaze can be more effectively protected against strong intensity of light during salt-stressed condition due to the intensive development of photoprotective processes.

The stomatal regulation in 7H add was similar to that of barley under salinity stress, in which the significant decrease in the K⁺ content of the shoot could also play a determinative role (BENLLOCH-GONZALEZ *et al.* 2010). It was observed only moderate stomatal closure in the case of 7H add and barley during the treatments, which allows not only CO₂ diffusion into the leaves, but also the cooling of the assimilation surface by escape of water as a vapour. Accordingly, higher stomatal conductance in 7H add may contribute to the

survival during a period characterized by a high salt concentration not only with a sufficient dry matter production but also a particular protection against heat stress due to the transpiration cooling. Despite the high level of g_s , 7H add and barley did not suffer any significant water loss during moderate salt stress (200 mM NaCl) compared to wheat reacting with stronger stomatal closure. This means that effective osmoregulatory processes were induced by 200 mM NaCl in the 7H add and barley (DARKÓ *et al.* 2015), through which effective water absorption could be achieved by optimizing the osmotic potential. Osmotic adaptation could also be attributed to the considerable proline accumulation observed in roots of 7H add and barley, which was more pronounced than in the case of wheat. This is also supported by previous observations revealing that 7H barley chromosome may play a role in osmotic adaptation (TEULAT *et al.* 1998). Accordingly, it is likely that higher g_s with an adequate osmotic adaptation will be an effective strategy for maintaining the plant's photosynthetic activity up to moderate salt stress and it is a useful tool for characterizing salt tolerance (SZOPKÓ *et al.* 2017). Under stress conditions, moderate stomatal closure and osmotic adjustment that ensures water absorption are characteristic of earliness in plants (GONZÁLEZ *et al.* 1999). Salt-induced earliness was also observed in the case of 7H add. This property may also be beneficial if the grain-filling process is accompanied by high temperature thus the unfavourable period can be avoided. Earliness in 7H add is probably attributable to the gene/genes found on the 7H barley chromosome (YAN *et al.* 2006, FAURE *et al.* 2007), which were also successfully expressed in the genome of Asakaze.

In addition to earliness, genetic regions detected on the 7H barley chromosome can also help avoid Na^+ toxicity, as they control the mechanism of exclusion of Na^+ from the shoot (SHAVRUKOV *et al.* 2010). In our experiments, the Na^+ content of the shoot was lower while Na^+ content of the root was higher in barley *cv.* Manas at 200 mM NaCl treatment than those of wheat and 7H add. Accordingly, the presence of the 7H chromosome did not affect the salt accumulation capability of the wheat in the root and did not result in effective Na^+ exclusion from the shoot which was observed in the case of barley *cv.* Manas. At the same time, the compartmentalization of Na^+ into the vacuole is also an integral part of the adaptation processes under salt stress (WIDODO *et al.* 2009). In the shoot of 7H add, the major amount of the Na^+ probably accumulated in the vacuole, since this line could maintain its PSII activity and carbon assimilation rate at a satisfactory level during treatment with 200 mM NaCl. However, in the case of barley the major amount of Na^+ was accumulated in the root. Since the root biomass in barley was the least limited under salinity, therefore, it was assumed that Na^+ was transported to the vacuole such as in 7H add. The accumulated Na^+ in the leaves could also contribute to the effective water conservation as an inorganic osmolyte (GORHAM *et al.* 1985). Avoidance of the salt-inhibited root growth (MUNNS *et al.* 2006) was more efficient in 7H add than in wheat, which may facilitate an improved water

absorption ability (HAYS *et al.* 1991, MARCUM *et al.* 1995). The S/R ratio in Manas was not significantly modified by the rising concentration of NaCl and 7H add line also did not show any significant increase in this ratio. Accordingly, the changes in the biomass production of 7H add induced by salt treatment were very similar to those of parental barley. Summarizing the results, we can conclude that the beneficial properties of the parental lines have been associated in the 7H add. For this reason, this line may serve as suitable plant material for studies on the quantity and quality of the crop yield. According to this the more detailed stress physiological study of this line is suggested supplementing the effects of different environmental factors to heat stability. Furthermore, the use of this line in the pre-breeding programmes is also recommended.

Wild wheat (*Aegilops*) species can be tolerated more successfully the extreme climatic conditions of their habitats than certain wheat varieties by preserving their natural variability (FAROOQ *et al.* 1995, MUJEEB-KAZI *et al.* 1996, FAROOQ 2002). Taking into consideration the occurrence of the examined *Aegilops* species (KILIAN *et al.* 2011), they have to tolerate the effects of several stress factors (e.g., high temperature, salt stress, high light intensity) at a given time, which may be based on the coordination of their protecting/regulating mechanisms (DULAI *et al.* 2005).

The selection of *Aegilops* lines was based on changes in photosynthetic parameters under drought and salinity, as well as on water regulation and related parameters influencing the individual processes of photosynthesis (CHAVES 1991, BAJJI *et al.* 2000). Although the net carbon assimilation capability of the Mv9kr1 wheat and *Aegilops* lines was almost equally affected by increasing water deficit and salt stress in the background of the decline there were partly different phenomena that could be associated with different degree of osmotic stress. Based on the effects of water scarcity and increasing salt concentration on RWC the 10-day drought had a greater osmotic stress on the examined lines than 300 mM NaCl. Thus, besides the osmotic effect of salt treatment, its ionic components also played a determinative role in the inhibition of carbon assimilation (MUNNS 2002) and in the induction of adaptation processes. In the wheat shoot, strong drought and salt treatment resulted in a similar osmotic potential (OP), whereas the 300 mM NaCl treatment resulted in significantly lower OP than 10-day drought in *Aegilops* lines. Accordingly, ionic stress appearing in the salt treated *Aegilops* lines could also have had a major effect on the accumulation of osmotically active compounds (CHAUDHARY *et al.* 1997) and/or the Na⁺/Cl⁻ ions were osmotically active (GORHAM *et al.* 1985). On the basis of low OP, parallel with the significant increase of proline at 300 mM salt concentration, other osmoprotective agents such as betain or glycine betaine could be accumulated in the *Aegilops* lines (CHEN and MURATA 2008). In addition to organic osmolits, the accumulated Na⁺ and Cl⁻ ions could also contribute to the low OP (ZHANG *et al.* 2010). The ionic stress found in *Aegilops* can be confirmed by the fact that carbon assimilation was more considerably limited by non-stomatal factors than those in wheat under salt

stress. In parallel, the presence of ion toxicity also had a harmful effect on photochemical processes as non-stomatal inhibitor (MURANAKA *et al.* 2002, KALAJI *et al.* 2011). For this reason, during the strong saline treatment, the downregulation of the PSII was required for the wild wheat species to prevent photoinhibition by enhancing the Φ_{NPQ} and Φ_{CEF} . At the same time, in the Mv9kr1, with the smallest OP change and probably low Na^+ and/or Cl^- accumulation, salt treatment did not induce a significant change in photochemical processes, similarly to the Asakaze wheat variety, thus the alternative electron sinks operated efficiently (BIEHLER and FOCK 1996, WINGLER *et al.* 2000). This was confirmed by the fact that no photodamage was observed in this line. Compared to the effect of water scarcity and increasing salt concentration, the 10-day drought treatment has had a greater osmotic stress on the examined lines than the 300 mM NaCl but the photosynthetic CO_2 fixation was similarly affected by drought and salt treatment. Thus, the the temperature effects were studied on plants characterized by a similar stress pressure, both in terms of water deficiency and salt treatment.

In darkness, salt stress strongly increased thermal stability of PSII already at 150 mM NaCl, as opposed to water deficit where no significant improvement was observed. As dehydration did not occur in the salt treated shoots, therefore, the decrease in heat sensitivity was probably due to the ionic effects of NaCl. This finding can be especially true for *Aegilops* lines, since osmotic stress did not cause an improvement of the thermal stability. At the same time, a significant increasing was induced by salt stress as demonstrated by their phenotypic plasticity and the temperature dependence of F_v/F_m . In this context, it has been known that PSII heat sensitivity is greatly associated to the inactivation of OEC (NASH *et al.* 1985, WANG *et al.* 2010) and the salt treatment enhances OEC or PSII heat stability and membrane rigidity in several ways (MÜLLER and SANTARIUS 1978, KRISHNAN and MOHANTY 1984, CHEN *et al.* 2004, WEN *et al.* 2005, YAN *et al.* 2012). Thus, these changes may trigger the formation of higher thermal stability even in dark adapted state. Accordingly, the examination of the salt-induced changes in the structure of the photosynthetic membranes, the Cl^- content and the activity of the OEC may be useful for the detection of the processes operating in the background of the thermostability.

According to the xanthophyll hypothesis (HAVAUX and TARDY 1996), the light has increased the PSII thermostability, which was further enhanced by water and salt stresses. But this heat sensitivity decreasing occurred in different measure in the case of given lines. Based on the temperature dependence of the NPQ, it can be seen that the secondary effects of low lumen pH (MÜLLER *et al.* 2001) also play an important role in the protection against both the heat and the water/salt stresses. Therefore, it might be possible that the protective processes (in the early stages) against the effects of excess light, high temperature, and water/salt stresses share certain characteristics on common bases. The synchronization of protecting/regulating mechanisms may be of a great benefit

from the fact that the different ecological factors (temperature, light, water or salt conditions) vary in interdependence with each other in the field (DULAI *et al.* 2006). According to the above-mentioned facts, the thermal stability changes of photosynthetic apparatus observed through the inhibition of NPQ may even suggest whether certain NPQ components play a role and to what extent in the protection against the combined effects of these stress factors.

In the light adapted state, the drought-induced thermal stability increasing was moderate in the *Ae. umbellulata* lines, however, the phenotypic plasticity of these plants varied quite flexibly with the combined light and salt treatment, and their thermal stability increased considerably compared to the absolute control (dark, good water supply). In spite of this, in the Mv9kr1 the heat-tolerance change by water deficit was pronounced. Consequently, the temperature sensitivity is generally enhanced by both salt treatment and water deficit in light adapted state, but in each line to varying degrees. In this respect, it is also suggested that osmotic stress/drought and salt tolerance are not necessarily linked with regard to regulating mechanisms (NAGY and GALIBA 1995). As a consequence, the thermal tolerance of each line can be influenced to altered degrees. The largest difference was detected in the MvGB420 (*Ae. umbellulata*) line thus the light-induced mechanisms in salt treated state have greatly increased its thermal tolerance. However, in this respect light had not play a prominent role during water deficit.

Since, the salt-induced improvement in T_c point and L_{ns} parameter were not recovered to the control level by the 7-day re-watering in the case of MvGB420, therefore the higher thermal resistance of this line was probably due to the salt ionic effects. On the basis of absolute phenotypic plasticity, the processes protecting against the combined effect of salt stress, high light and heat stress were more effectively operated in *Aegilops* lines than in wheat. This phenomenon is probably to be correlated also with the ecological conditions of the original habitat of wild wheat species. The greatest degree of tolerance increase was observed in *Ae. umbellulata* lines (MvGB420 and AE740/03), at the same time a difference was also noticed between the lines. Accordingly, *Ae. umbellulata* lines may function as a suitable plant material in the study of the processes operating in the background of the improved thermal tolerance at salt-adapted state whereas wheat line could be used for the determination of the mechanisms relating the development of drought-induced higher thermal stability. Although, the examined *Aegilops* lines showed similar photosynthetic behavior to both water scarcity and salt stress, however, when salt treatments were combined with heat stress, the MvGB420 line has appeared to be a suitable candidate for increasing the salt and thermal tolerance of wheat.

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5. LIST OF PUBLICATION RELATED TO THE DISSERTATION

Journals with impact factor:

SZOPKÓ, D., DARKÓ, É., MOLNÁR, I., KRUPPA, K., HÁLÓ, B., VOJTKÓ, A., MOLNÁR-LÁNG, M., DULAI, S. (2017): Photosynthetic responses of a wheat (Asakaze)-barley (Manas) 7H addition line to salt stress. *Photosynthetica*, 55, 317-328. (IF: 1.507, ISSN: 1573-9058).

DARKÓ, É., JANDA, T., MAJLÁTH, I., **SZOPKÓ, D.**, DULAI, S., MOLNÁR, I., TÜRKÖSI, E., MOLNÁR-LÁNG, M. (2015): Salt stress response of wheat-barley addition lines carrying chromosomes from the winter barley "Manas". *Euphytica*, 203, 491-504. (IF: 1.39, ISSN: 0014-2336)

DULAI, S., MOLNÁR, I., **SZOPKÓ, D.**, DARKÓ, É., VOJTKÓ, A., SASS-GYARMATI, A., MOLNÁR-LÁNG, M. (2014): Wheat-*Aegilops* biuncialis amphiploids have efficient photosynthesis and biomass production during osmotic stress. *Journal of Plant Physiology*, 171, 509-517. (IF: 2.77, ISSN: 0176-1617)

International conference proceeding, abstracts:

SZOPKÓ, D., MOLNÁR-LÁNG, M., MOLNÁR, I., DARKÓ, É., VOJTKÓ, A., SASS-GYARMATI, A., DULAI, S. (2012): Stress physiological screening of salt tolerance of photosynthetic parameters in wheat/barley introgression lines. Plant Biology Congress, 29 July-3 August 2012, Freiburg, Germany.

DULAI, S., MOLNÁR, I., **SZOPKÓ, D.**, DARKÓ, É., VOJTKÓ, A., SASS-GYARMATI, A., MOLNÁR-LÁNG, M. (2012): Photosynthesis in wheat-*Aegilops* amphiploids during water deficit. Plant Biology Congress 29 July-3 August 2012, Freiburg, Germany.

SZOPKÓ, D., MOLNÁR-LÁNG, M., MOLNÁR, I., DARKÓ, É., VOJTKÓ, A., SASS-GYARMATI, A., DULAI, S. (2013): Effects of NaCl on the photosynthetic parameters of wheat-barley genotypes. 16th International Congress on Photosynthesis Research, 11-16 August 2013, St. Louis, USA.

DULAI, S., MOLNÁR, I., **SZOPKÓ, D.**, VOJTKÓ, A., SASS-GYARMATI, A., MOLNÁR-LÁNG, M. (2013): Photosynthesis and biomass production in wheat-*Aegilops* amphiploids during osmotic stress. 16th International Congress on Photosynthesis Research, 11-16 August 2013, St. Louis, USA.

Hungarian conference proceeding, abstracts:

SZOPKÓ, D., MOLNÁR, I., LÁNGNÉ MOLNÁR, M., VOJTKÓ, A., SASS-GYARMATI, A., DULAI, S. (2012): A fotoszintetikus folyamatok vízhiány és só toleranciája búza-árpa introgressziós vonalakban. IX. Magyar Ökológus Kongresszus, 2012. szeptember 5-7., Keszthely, Magyarország.

DULAI, S., MOLNÁR, I., **SZOPKÓ, D.**, VOJTKÓ, A., SASS-GYARMATI, A., LÁNGNÉ MOLNÁR, M. (2012): Száraz termőhelyekről származó kecskebúza fajok és búza-kecskebúza amfiploidok fotoszintézise vízhiány alatt. IX. Magyar Ökológus Kongresszus, 2012. szeptember 5-7., Keszthely, Magyarország.

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