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Structural changes of the photosynthetic apparatus under osmotic stress in different *Triticum aestivum* and *Aegilops biuncialis* genotypes

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ABSTRACT The photosynthetic apparatus of three *Aegilops biuncialis* genotypes differing in the annual rainfall of their habitat (1050 mm, 550 mm and 225 mm), two drought resistant *Triticum aestivum* genotypes and one with high crossing efficiency (Mv9Kr1) were examined under the effect of osmotic stress brought about by PEG in the nutrient solution. Two *Aegilops* genotypes *Ae.b.*225 and *Ae.b.*550 proved to be relatively stable under osmotic stress showing only slight changes of Chl concentration of the leaves and relative amount of chlorophyll proteins (CPs) in the thylakoids. In spite of the structural stability, PEG treatment considerably lowered the yield of low temperature fluorescence emission. The relative increase of emission around 700 nm can be attributed to LHCII aggregation related quenching processes. More pronounced changes in the relative amount of CPs were observed in *Triticum* genotypes and in *Ae.b.*1050. These changes, which included the relative decrease of PSII core complex and the relative increase of LHCII, reversed during the recovery period. Low temperature fluorescence yield decreased evenly throughout the spectrum and the lack of relative increase around 700 nm points to a quenching process other than LHCII aggregation. The *Ae.b.*1050 and Mv9Kr1 wheat genotypes proved to be the most susceptible to osmotic stress with more pronounced decrease of PSIICC and increase of LHCII, less fluorescence decline in the emission spectra, and slower recovery of these parameters.

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KEY WORDS

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wheat

Under Hungarian climatic conditions drought stress of wheat is a permanent problem often causing yield losses of cereal production. Therefore the improvement of drought resistant cultivars is one of the main aims of wheat breeding.

Photosynthesis, which is the most significant process influencing crop production, is also inhibited by drought stress (Bradford and Hsiao 1982). It was shown to inhibit PSII functioning both by down-regulation of PSII electron transport and by direct damage of the D1 protein (Giardi et al. 1996). Little is known, however, about other structural changes in thylakoids under water stress conditions. Water deficiency causing the closure of stomata leads to a lowered internal concentration of CO_2, which in turn inhibits the Calvin-cycle, and the consequent shortage of reducible coenzymes facilitates the arising of photoinhibitory conditions (Horton et al. 1996).

Structural parameters of thylakoids were compared in different *Triticum aestivum* and *Aegilops biuncialis* genotypes to characterize the water deficiency tolerance of their photosynthetic apparatus, and evaluate the applicability of *Aegilops* genotypes in future wheat breeding.

Materials and Methods

Three *Aegilops biuncialis* genotypes (MvGB642, MvGB382 and MvGB1094, ICARDA Gene Bank, Syria) differing in the annual rainfall of their habitat (1050 mm, 550 mm and 225 mm, respectively), two drought resistant *Triticum aestivum* genotypes (Kobomugi, Sakha) and one with high crossing efficiency (Mv9Kr1) were examined.

Plants were grown in modified Hoagland solution in growth chamber (Conviron, Ontario, Canada) under 12/12 h light (200 $\mu Em^{-2}s^{-1}$)/dark (20/18 °C and 70/75% RH) period. Osmotic stress treatment started after introducing 12 m/m% PEG into the nutrient solution of three week old seedlings. PEG concentration was weekly increased stepwise to 15, 18, and 21 m/m%, after which the treated plants had a recovery period of one week in the absence of PEG.

Chlorophyll content of leaves and plastid suspensions were determined according to Porra et al. (1989). Plastids and thylakoids were isolated, and chlorophyll proteins (CP), solubilised with glucosidic detergents, were separated with Deriphat-PAGE as in Sárvári and Nyitrai (1994).

77K fluorescence emission spectra of thylakoid suspensions (1 µg chlorophyll ml^{-1} in isolating buffer containing 50% of glycerol) were measured by a Jobin Yvon FluoroMax-2 spectrofluorimeter. The excitation wavelength was 440 nm. The slit widths of the excitation and emission monochromators were 2 and 1 nm, respectively. Measurements were taken by 0.5 nm with integration time of 0.1 s. Spectra of samples are averages of three successive measurements. Spectra were corrected for the photomultiplier sensitivity.

Results and Discussion

Chl content and Chl *a/b* ratio of leaves are widespread used to characterize the general state of the photosynthetic

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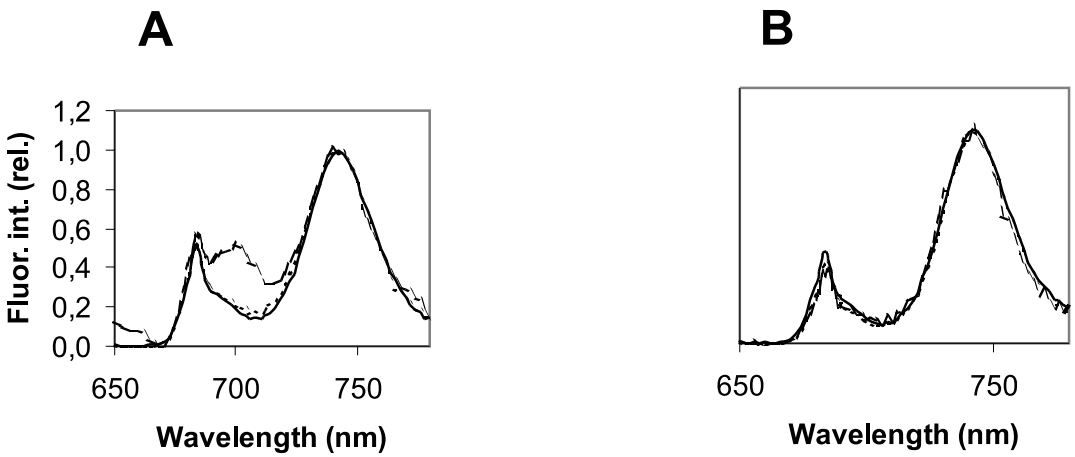

Figure 1. 77K fluorescence emission spectra of *Ae.b.*1050 (A) and Kobomugi (B). Control - solid line, 21 m/m% PEG treated - dashed, and treated plants after recovery - dotted. Spectra were normalised to the long wavelength emission maximum.

apparatus. Although Chl content per leaf area changed during the growth period probably due to differences in growth rates, but the changes related to the control were moderate. In the case of *Ae.b.*1050 and Mv9Kr1 21 m/m% PEG treatment increased the Chl content per leaf area, but it decreased to the level of the control during the subsequent recovery period. This may be explained by the restricted ability of the cells to elongate when PEG inhibited water uptake, which was followed by an increased water uptake when the osmotic stress treatment ceased.

The change in Chl *a/b* ratio provides further information about modification processes taking place in the photosynthetic apparatus under osmotic stress. There are notable differences in the variations of the Chl *a/b* ratio under PEG treatment in the examined genotypes. The Chl *a/b* ratio started to decrease from 15% PEG treatment in the *Ae.b.*1050 and Mv9Kr1 genotypes and this decrease continued in the recovery period as well. In the case of the other genotypes there was only a slight decrease during the PEG treatment. The response during the recovery period was, however, quite different. While Chl *a/b* ratio significantly decreased in *Ae.b.*550, it increased in Kobomugi, but remained about the same in *Ae.b.*225 and Sakha. It should be mentioned that after the recovery period PEG treated leaves of *Aegilops* genotypes had significantly lower Chl *a/b* ratio than the isolated thylakoids. This possibly point to the existence of a population of plastids with altered structure (aging, fractured), which were not pelleted during the isolation process. Elucidation of this phenomenon requires the examination of the ultrastructure of chloroplasts.

Changes in the Chl *a/b* ratio could be explained by the changes in the CP composition. Either there was no significant change during the PEG treatment (*Ae.b.*550 and *Ae.b.*225, Sakha) or there was a decrease in the relative proportion of PSIICC, and PSI to a lesser extent, while the proportion of LHCII increased (*Ae.b.*1050, Mv9Kr1 and Kobomugi). Decrease of D1 at transcript and template level was found also in water stressed maize (Hao et al. 1999). The altered CP composition either remained during the recovery period as in *Ae.b.*1050 or the changes reversed to restore the CP composition.

Compared to the relatively small changes in the CP composition decline of the 77K fluorescence yield was more pronounced under osmotic stress. It refers to structural changes in the thylakoids, which cause increased quenching (Horton et al. 1996). It was most pronounced in the

Table 1. Changes of the photosynthetic parameters under osmotic stress. Data are presented as percentage of the control values.

				Relative amount of CPs		
		Chl *a+b*	Chl *a/b*	PSI	PSIICC+CA	LHCII
*Ae.b.*1050	21% PEG	139,9	93,0	99,3	94,5	113,2
	Recovery	90,1	91,8	102,1	82,0	114,8
Ae.b. 225	21% PEG	103,3	102,5	100,3	103,7	98,2
	Recovery	96,9	102,6	100,3	99,3	99,9
Mv9kr1	21% PEG	117,3	94,9	100,0	84,9	114,0
	Recovery	99,4	88,0	97,4	91,9	102,7
Kobomugi	21% PEG	91,9	87,3	98,3	55,6	111,5
	Recovery	86,8	97,6	113,5	75,4	96,7

*Ae.b.*1050 and *Ae.b.*225 genotypes (around 80% of the control), but in *Ae.b.*550 it was only 20%. While in *Triticum* only the quenching of fluorescence could be observed (around 50%), in *Aegilops* the fluorescence quench was accompanied by changes in the shape of the emission spectra. Namely, there was an increase in forms emitting around 700 nm. Earlier studies have indicated that the appearance these spectral forms are in close connection with the LHCII aggregation related to the quenching processes (Siffel and Braunova 1999). Therefore, it could be supposed that LHCII aggregation did play an essential role in the quenching of Chl fluorescence in *Aegilops*, while other quenching mechanisms were more important in *Triticum*.

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