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Photosynthetic responses of sun- and shade-grown chlorophyll b deficient mutant of wheat

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Abstract

In this study, were compared the photosynthetic performance of chlorophyll b (Chl b)-deficient mutant lines (ANK-32A and ANK-32B) and wild type (WT) of spring wheat (*Triticum aestivum* L.) grown under sun and shade light regimes, at fourth fully developed leaf, grown in pots under natural climatic conditions. Analyses were based on measurements of pigment composition and fast chlorophyll (Chl) a fluorescence kinetics. The content of Chl *a+b* sun (WT 372, ANK-32A 144 and ANK-32B 128 mg*m^{-2}) versus shade (WT 293, ANK-32A 150 and ANK-32B 151 mg*m^{-2}) was statistically significant and the difference between WT and mutant ANK. Unlike the sun-grown, shade grown plants of ANK mutants did not express a severe chlorina phenotype.

Keywords: photosynthetic pigments, sun and shade leaves, wheat

Introduction

The photosynthetic apparatus of plants responds to changing light quantity and quality with coordinated changes in both the light-harvesting antennae of the photosystems and the amounts of electron transport components and ATP synthase. These compositional modulations are accompanied by changes in thylakoid membrane organization and photosynthetic capacity. It is now clear that there is a dynamic continuum of organization and function of the photosynthetic apparatus from the appressed granal and non-appressed stroma thylakoids within a chloroplast, to different chloroplasts within a leaf, to leaves within and between species. While it is very unlikely that there is a unique solution to photosynthesis in the sun or shade, substantial changes in composition, and hence thylakoid membrane organization and function, are elicited as part of sun/shade responses (Anderson et al., 1988). Acclimation results in the development of ‘sun’ and ‘shade’ leaves that typically differ in a suite of structural, biochemical and physiological traits (e.g. leaf size and thickness, stomatal densities, nitrogen and maximum photosynthetic rates, dark respiration rates and others Anderson et al., 1995; Boardman 1977; Demmig-Adams 1998), which are thought to reflect adaptive trade-offs in the investment of resources for light harvesting and carbon assimilation (Evans 2004; Givnish 1988). It is well known that the pigment composition is also affected by sun/shade acclimation. The variations in pigment content as a response to sun/shade acclimation reflect differences in the complement of specific chlorophyll-proteins. (Aro et al., 1986; Barnes et al., 2013).

Studies based on photosynthetic mutants help know better the flexibility and complementarities of individual photosynthetic mechanisms.The mutants with reductions in chlorophyll b of a typical yellow-green phenotype, called also ‘chlorina’ mutants. In addition to modification in antenna and pigment composition, it cannot be forgotten that the depletions of LHC II in chlorina mutants usually result in severe imbalances in the relative rates of excitation of photosystem I (PSI) and photosystem II (PSII) (Andrews et al., 1995). This frequently results in a lower proportion of PSI compared to PSII. Changes in the distribution of both photosystems are considered to be a response of the plant to reduce the imbalance in light absorption between PSI and PSII (Terao et al., 1996). An elevated PSII to PSI ratio creates, however, conditions in which another imbalance can be expected between the two photosystems with potentially harmful consequences. There are many contentious issues in the field of photoprotective responses, and further research in this area is still needed. The major progress in the last decades has been achieved using different photosynthetic mutants and transgenic plants. Therefore, the experiments performed on the chlorina mutants with an expected imbalance in the PSI/PSII function may also contribute to progress in understanding the regulation of electron and proton transport as well as photoprotection of both photosystems. In fact, there exists an enormous number of chlorophyll-b deficient or chlorophyll b-less mutants of different plant species. Many of them, such as chlorina f2, have been extensively studied and their responses to various conditions are well described (Brestic et al., 2008; Gilmore et al., 1996).

Materials and methods

Plant material and cultivation

The plants of spring wheat (*Triticum aestivum* L.) were used for the experiments. The Chl-b-deficient ‘‘chlorina’’ mutants ANK-32A and ANK-32B (hereinafter denoted as ANK mutants) are near-isogenic hexaploid lines containing a chlorina mutation of the cn-A1 locus introduced from the Chlorina-1 (ANK-32A) or from a chlorina mutant line of AN-215 (ANK-32B). As a ‘‘wild type’’ (WT), were used the non-mutant hexaploid wheat genotype Corso with a similar developmental pattern as both ANK mutant lines.

Plants grown in 10 liter plastic pots filled with humus soil substrate (2 pots for each genotype, 10 plants per pot.). Six pots were exposed to full sunlight during their entire growth period, whereas 6 pots were placed in the shade, provided with a non-woven textile cover over them; this reduced the photosynthetic active radiation (PAR) to ~13% of the sunlight.

Determination of photosynthetic pigments

The segments from the mature, fully expanded leaves were homogenized using sea sand, $MgCO_3$, and 100%acetone and then extracted with 80% acetone. After 2-min centrifugation at 2,500 rpm, absorbance of the solution was measured by a UV–vis spectrophotometer (Jenway, UK), at 470, 647, and 663 nm, with a correction for scattering measured at 750 nm. The concentrations of Chl *a*, Chl *b*, and carotenoids (Car) per leaf area unit were determined, using the equations of Lichtenthaler (1987), as described elsewhere (Zivcak et al., 2014). Eight leaves of each genotype were analyzed.

Chl *a* fluorescence measurements were performed using the Handy-PEA Continuous Excitation Plant Efficiency Analyser (Hansatech Instruments Ltd, UK). The leaf samples were illuminated with continuous red light (wavelength in peak 650 nm; spectral line half-width 22 nm). The light was provided by an array of 3 light-emitting diodes. The light pulse intensity used was 3500 $\mu mol*m^{-2}*s^{-1}$ and the duration of the light pulse was 1 s. The fluorescence signal was recorded with a maximum frequency of 105 points s^{-1} (each 10 µs) within 0–0.3 ms, after which the frequency of recording gradually decreased collecting a total of 118 points within 1 s. Leaf segment measurements were performed in the middle part of a leaf blade, away from the main leaf vein, after 30 min of dark adaptation, using leaf clips (Brestic et al., 2012).

Data processing and analysis

The measurements were carried out in at least 10 repetitions. The mean values ± standard errors (a = 0.05) are presented here. The statistical significance of differences was assessed using ANOVA followed by Duncan’s multiple range tests to identify statistically homogenous groups.

Results and Discussion

Photosynthetic parameters measured in ANK wheat mutants were compared with WT on a 4th fully developed leaf. Shade leaves showed a lower photosynthetic pigment concentration than those grown under the sun (Table 1). However, no significant changes were observed in the Chl *a*/*b* ratio in shade leaves. It means that in shade-acclimated plants the chlorina phenotype was not manifested. In sun-exposed plants, ANK mutants had significantly lower Chl content compared to WT; the difference was much higher than in shade leaves. As expected, the Chl *a*/*b* ratio was much higher in Chl b deficient mutants. Similarly, the Chlorophyll to carotenoid ration was lower in mutants compared to WT.

Is well known that under conditions of high light, for example, under a sunny habitat, plants have usually smaller PSII antenna size (Evans 1996; Lichtenthaler 1985). In turn, under low-light conditions (in a shady habitats) plants have larger PSII antenna size. The reason is that the amount of the outer PSII antenna proteins (the major peripheral antenna proteins) changes in response to light conditions, while the inner PSII antenna proteins (the core antenna proteins and the inner peripheral antenna proteins) remain unchanged (Anderson et al., 1997; Tanaka and Tanaka 2000). Hence, the lower value of the Chl *a*/*b* ratio is expected in shade leaves, as has been documented in many studies, e.g., in the sun and the shade leaves of forest trees (Lichtenthaler et al., 2007).

Table 1. Values of photosynthetic pigments in leaves

Parametres*	Sun			Shade		
	WT	ANK-32A	ANK-32B	WT	ANK-32A	ANK-32B
Chl *a* (mg*m^{-2})	275±13^{a}	116±7^{c}	105±5^{c}	195±10^{b}	112±6^{c}	108±5^{c}
Chl *b* (mg*m^{-2})	97.4±4^{a}	27.7±2^{c}	23.7±3.5^{c}	97.5±8.3^{a}	37.6±3.9b^{c}	43.6±3.9^{b}
Chl *a*+*b* (mg*m^{-2})	372±16^{a}	144±9^{c}	128±8^{c}	293±12^{b}	150±7^{c}	151±4^{c}
Car (mg*m^{-2})	89.6±2.9^{a}	46.2±2.5^{c}	42.9±1.6^{c}	55.6±3^{b}	31.8±1.3^{d}	27.5±1.3^{d}
Chl *a*/*b* ratio	2.8±0.1^{c}	4.2±0.1^{ab}	4.8±0.6^{a}	2.1±0.2^{c}	3.2±0.4^{bc}	2.8±0.5^{c}
Chl/Car ratio	4.2+0.1^{c}	3.1+0^{d}	3+0.2^{d}	5.3+0.3^{ab}	4.7+0.2^{bc}	5.6+0.3^{a}

*Sun-full light; shade-light level ~13% of full light. Mean values ± SE from 8 replicates are presented. Letters indicate significant differences at P<0,05 according to Duncan's multiple range tests.

The basic fluorescence parameters measured in the dark-adapted state (Figure 1) indicate much lower F_0 (minimum fluorescence from the dark-adapted leaf; PSII centers open) and F_m (maximum fluorescence from the dark) values in ANK mutants. This can be caused by a higher leaf absorbance due to higher Chl content in WTand more abundant PSI and PSII (Brestic et al., 2016). Interestingly, there was a significant difference in the values of the maximum quantum yield of PSII photochemistry (Fv/Fm) PSII and PSI quantum efficiencies between WT and ANK mutants. This might be mainly due to changes in the PSI/PSII ratio and hence, changes in the distribution of absorbed light between PSII and PSI, as described Zivcak et al., (2015).

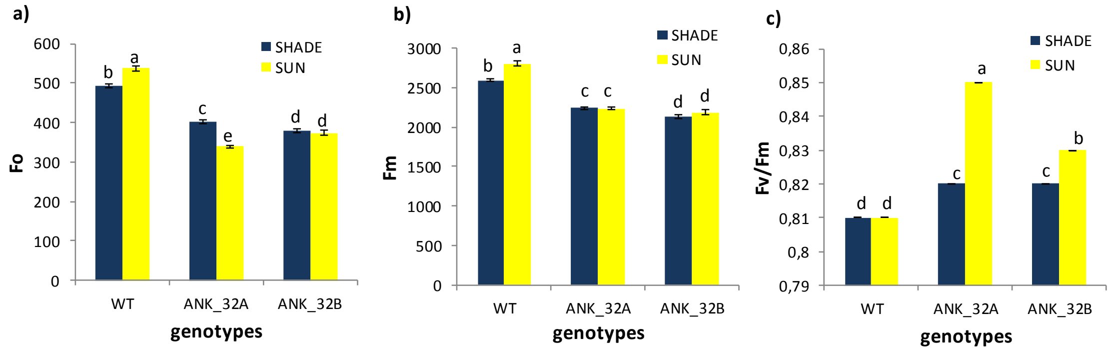

Figure 1. Selected parameters derived from fast fluorescence kinetic measurements in the sun and the shade wheat. a) minimum fluorescence from dark-adapted leaf - F_0, b) maximum fluorescence from dark - F_m, c) related to maximum photochemical efficiency of PSII - $F_V/F_{m.}$ Letters indicate significant differences at P<0,05 according to Duncan's multiple range tests.

Significantly, higher values of F_v/F_m in sun grown mutants compared to the shade variants indicates the higher PSI signal in shade plants, which corresponds to diminished chlorina phenotype in shade compared to sun variants of ANK mutants. In sun-exposed chlorina mutants, the low PSII antenna size is balanced by the low PSI content (Andrews et al., 1995), and therefore, any increase in PSII antenna size has to be associated with an increase in PSI content, both leading to an increase in F_0 and F_m and tiny decrease of Fv/Fm parameter.

Conclusions

Dynamic acclimation of the photosynthetic apparatus in response to environmental cues, particularly light quantity and quality, is a widely observed and important phenomenon which contributes to the tolerance of plants against stress and helps to maintain, as far as possible, the optimal photosynthetic efficiency and resource utilization. ANK mutants expressed the higher vulnerability in plants exposed to direct sunlight, leading to decrease in PSI content and enhanced chlorinous phenotype. On the other hand, the more stable shaded conditions led to more suitable PSI to PSII ratio and the phenotype of the mutants was approaching towards the wild type. The results emphasize the importance of photosystem I content for maintaining the dynamic flexibility of photosynthetic apparatus at the sub-cellular level.

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