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Investigations

A State of PSI and PSII Photochemistry of Sunflower Yellow-Green Plastome Mutant

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Abstract: Chlorophyll deficient mutants are appropriate model objects for a large number of investigations in the field of photosynthesis. In this study, we identified a state of Photosystem I (PSI) and Photosystem II (PSII) photochemistry and light responses of the sunflower yellow-green mutant line - *en:chlorina*-7, wherein mutations in chloroplast genes have been previously localized. The conducted research revealed low content of photo-oxidizable PSI and an impaired quantum yield of PSII photochemistry in the *en:chlorina*-7 line as compared with a wild type. Disturbances of PSI and PSII in yellow-green plastome mutant line of sunflower (*en:chlorina*-7) are associated with mutations in *psaA* and *psbB*, respectively.

Keywords: Yellow-Green Mutants, Sunflower Plastome Mutant, PSI and PSII Reduction, *psaA*, *psbB*

Introduction

Chlorophyll (Chl) deficient mutants are the classic model objects for exploring the molecular mechanisms of photosynthetic apparatus biogenesis as well as the chloroplast development on the whole (Rassadina *et al*., 2005; Wang *et al*., 2014). Most of such kind researches are carried out on mutants with yellow-green leaf color, so called «chlorina phenotype» (Li *et al*., 2013; Brestic *et al*., 2015). Chlorina mutants have been identified in many higher plants, such as Arabidopsis (Harper *et al*., 2004), rice (Chen *et al*., 2013), wheat (Li *et al*., 2013), barley (Preiss and Thornber, 1995), maize (Asakura *et al*., 2008), tea (Wang *et al*., 2014), sunflower (Markin *et al*., 2016) and others. The investigation of genes associated with mutant phenotype is an actual issue for genetics of photosynthesis.

Chlorophyll mutations are of two types - nuclear or extranuclear. Virtually all chlorina lines used in the studies have nuclear origin of mutant phenotype. However chlorophyll deficient mutants with extranuclear genetic origin are of particular interest. This is primarily because mutations in plastid or mitochondrial DNA could not directly influence on Chl biosynthesis and the decrease of Chl occurs by retrograde signaling pathways involvement. Therefore, extranuclear chlorophyll mutants are also an appropriate model for investigating the interactions between organelles and nuclear genomes.

In the Southern Federal University, there have been obtained series of chlorophyll mutant lines using chemical mutagenesis (Beletskii *et al*., 1969). In current study we decided to use the yellow-green plastome mutant line–*en:chlorina*-7. The *en:chlorina*-7 line has chlorophyll b-deficient phenotype and characterized with about 35% decrease of chlorophyll (a+b) content and approximate 20% carotenoid reduction (Usatov *et al*., 2004). Previous studies of the *en:chlorina*-7 have revealed extranuclear genetic origin of mutant phenotype (Usatov *et al*., 2004). Further investigation by Markin *et al*. (2016) has shown 7 SNP in plastid genome of the *en:chlorine*-7 line compared to the *3629* line, however only 3 SNP in genes *rpoB*, *psaA*, *psbB* were producing amino acid substitutions (nonsynonymous substitutions). The latter were represented by following substitutions: serine for leucine in 138 position of β-subunit of RNA-polymerase (*rpoB*), threonine for isoleucine in 528 position of photosystem I P700 chlorophyll a apoprotein A1 (*psaA*) and histidine for tyrosine in 157 position of photosystem II CP47 reaction center protein (*psbB*) (Markin *et al*., 2016).

Comparative analysis of the *en:chlorine*-7 and the wild type line mitochondrial genomes has revealed no associations with chlorophyll deficient phenotype. Therefore the most obvious reasons for mutant phenotype could be mutations in *rpoB*, *psaA*, *psbB.* However using the BLAST program (https://www.ncbi.nlm.nih.gov/blast/) we found more than

20 species with a normal Chl content which had nucleotide sequences of *rpoB* 100% similar to the *en:chlorina*-7 allelic variant of *rpoB* gene. So the *rpoB* polymorphism is unlikely to be associated with the chlorina phenotype and here an influence of *psaA*, *psbB* mutations on the phenotype has been considered in this study.

Although a genetic nature of the *en:chlorina*-7 mutant line is clear, there is a lack of information about specific photosynthetic responses of mutant plants. It is also unclear that is a primary reason for Chl deficiency– an impaired synthesis of Reaction Centers (RCs) or a reduction of Light Harvesting Complexes (LHC) molecules. In this study, we compare the state of Photo System I (PSI) and photosystem II (PSII) photochemistry and light responses of the *en:chlorina*-7 mutant compared to a wild type.

Materials and Methods

Plant Material

The study was carried out on the domesticated sunflower (*Helianthus annuus*) line 3629 and the yellow-green plastome mutant sunflower line *en:chlorina*-7, which was obtained by a mutagenesis induced with N-nitroso-N-methylurea from an original inbred line 3629. A technique of the inducible mutagenesis has been described earlier (Usatov *et al*., 2004).

Sunflower plants were gown in regularly irrigated pots in growth chamber KBWF 720 (Binder, Germany). The growing conditions were following: the temperature -26°C, the humidity -70% and dark/light cycles-10/14 h. When plants have reached 4th leaf stage, we conducted the measurements, using the both leaves from a third leaves pair. Seven plants from each line were studied.

Chlorophyll Fluorescence Measurements

To determine the chlorophyll fluorescence induction parameters PAM-fluorometer Dual-PAM-100 (Heinz Walz, Germany) with a chlorophyll fluorescence unit and P700 dual wavelength (830/875 nm) unit was used. Saturation pulses (10,000 µmol photons m^{-2} s^{-1}) were applied for the measurements of chlorophyll fluorescence parameters so for the assessment of the P700 parameters. Prior to measurements, all plants were dark adapted in a dark box for 30 min. After the determination of *Fo*, *Fm* and *Pm*, the 150 µmol photons m^{-2} s^{-1} light intensity was used for starting processes of photosynthesis. When the stable state was reached, a rapid light curve was induced (light intensities 30, 37, 46, 77, 119, 150, 240, 363, 555 and 849 µmol photons m^{-2} s^{-1}; 30 sec at each light intensity) also a saturation pulse and a far-red pulse were used for F′o measurements after 30 sec at each light intensity. Following chlorophyll fluorescence parameters were calculated: $Fv/Fm = (Fm - Fo)/Fm$, $Y(II) = (F'm/F')/F'm$, $qP = (F'm - F)/(F'm - F'o)$, $qN = (Fm - F'm)/(Fm - Fo')$, $NPQ = (Fm - F'm)/F'm$ (Oxborough and Baker, 1997; Kramer *et al*., 2004). *Fo* and is a value of minimum fluorescence in the 30 min dark-adapted state and *F*′o is a calculated value of the minimum fluorescence value in the light-adapted state (Lysenko *et al*., 2014). *F* is a value of light-adapted steady state fluorescence. Fv/Fm is a value of PSII maximum quantum yield after dark adaptation and is an informative criteria to study induced changes in PSII (Sanusi *et al*., 2011). Y(II) is a value of PSII effective quantum yield. qP is a coefficient of photochemical quenching, qN is a coefficient of nonphotochemical quenching. The NPQ parameter reflects the measure of nonphotochemical fluorescence quenching. Y(NPQ) is the quantum yield of regulated energy dissipation in PS II and Y(NO) is the quantum yield of nonregulated energy dissipation in PS II, were calculated according (Kramer *et al*., 2004).

Measurements of P700 Redox State

The P700 redox state was measured by Dual PAM-100 with a dual wavelength (830/875 nm) unit, following the method of Klughammer and Schreiber (1994). Saturation pulses (10,000 µmol photons m^{-2} s^{-1}), were used for assessment of P700 parameters. The $P700^{+}$ signals (P) may vary between a minimal (P700 fully reduced) and a maximal level (P700 fully oxidized). The P700 fully oxidized level-*Pm* value, was determined using a saturation pulse after pre-illumination with a far-red light. P′m was determined similarly to Pm, but using the actinic light background instead of a far-red illumination. Y(I) is the PSI photochemical quantum yield, it was measured by a fraction of overall P700 that in a given state was reduced and not limited by the acceptor side. It was calculated as Y(I) = (P′m-P)/Pm. Y(ND) value represents a fraction of overall P700 that is oxidized in a given state. Y(ND) value may be enhanced by a transthylakoid proton gradient (photosynthetic control at cyt b/f complex as well as down-regulation of PSII) and PSII photodamage. It was calculated as Y(ND) = P/Pm. Y(NA) value represents a fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of oxidized acceptors. It was calculated as $Y(NA) = Pm - P'm/Pm$.

Measurement of PS I and PS II Electron Flows

PSI and PSII electron flows were calculated as follows: $ETR(I) = Y(I) \times PAR \times \alpha I$, $ETR(II) = Y(II) \times PAR \times \alpha II$ (Miyake *et al*., 2005). αI and αII were obtained as $\alpha I = p \times dI$ and $\alpha II = p \times dII$, where *p* is the absorptance (the fraction of the incident light absorbed by leaves) and *d*I and *d*II are fractions of the absorbed light distributed to PSI and PSII, respectively.

Data Processing and Analysis

All measurements were carried out in at least 6 repeats. Here the mean values and standard errors are

presented. The Student's t-test ($\alpha = 0.05$) was used for statistical analysis of significant differences.

Results

The basic fluorescence parameters measured in the *en:chlorina*-7 mutant were compared with the line 3629 (as a wild type (WT)) and are presented in Table 1.

Mean Values ± Standard Error

Most fluorescence parameters differed strongly between *en:chlorine*-7 and line 3629, among them the PSI parameter - ***Pm*.** The mutant plants had much lower value of *Pm* (35%) as compared with a WT. The *Pm* value depends on a maximum amount of the photo-oxidizable P700, which is a parameter representing an amount of efficient PSI complexes (Huang *et al*., 2010). To visualize the P700 signal changes of the original kinetics as well as chlorophyll fluorescence kinetics are presented in Fig. 1.

For comparison, Fm - one of the main PSII fluorescence parameters was reduced only on 10% in mutant plants. These changes indicate a greater reduction of PSI complexes as compared with PSII complexes.

However some changes in values of fluorescence parameters pointed at the PSII diminution. Fo parameter was higher in the chlorina mutant line and, as a consequence, mutant plants had reduced effective quantum yield of PSII photochemistry. An increase of Fo directly have influenced on the maximum quantum yield of PSII after dark adaptation (Fv/Fm). The rate of electron transport was also lower in mutant plants. Especially clearly a decrease of PSII efficiency was seen in the analysis of ETR(II) light curves (Fig. 2).

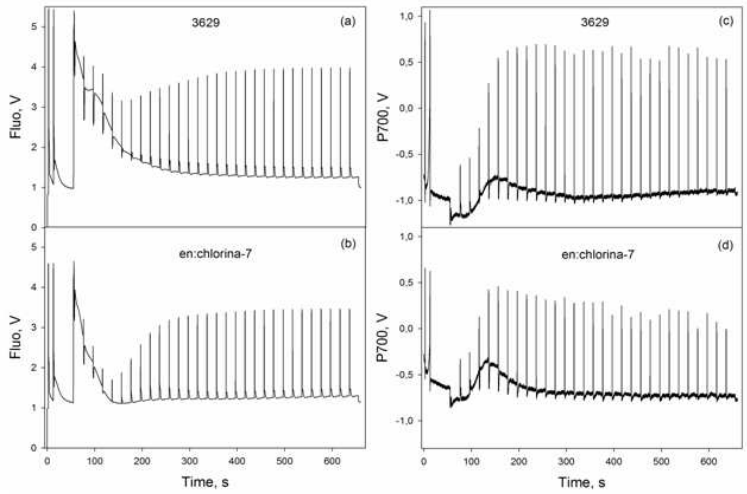

Fig. 1. Slow kinetics of chlorophyll *a* fluorescence induction (a,b) and kinetics of the changes of P700 signal (c, d) for the line 3629 (a, c) and for the mutant line *en:chlorina-7* (b, d)

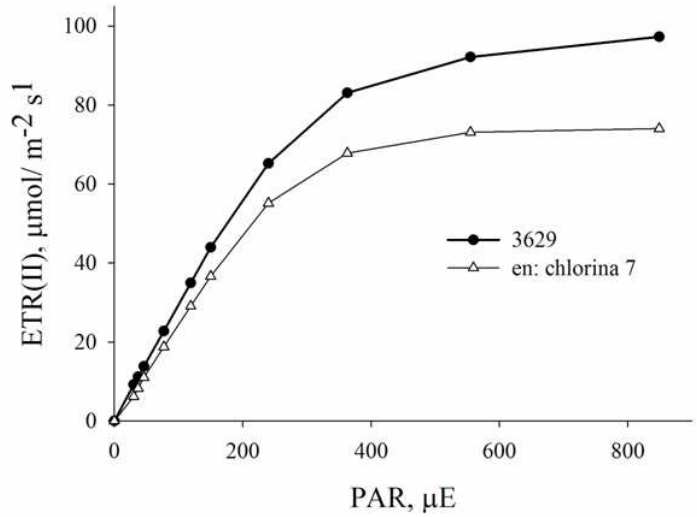

Fig. 2. Light curves, reflecting dependence of ETRII on the intensity of an actinic light in the line 3629 and in the mutant line *en:chlorina*-7

Table 1. Values of chlorophyll fluorescence and P700 parameters measured in the line 3629 (WT) and in the mutant line *en:chlorine*-7

Parameters	Line 3629	*en:chlorine-7*
Fo	0.83±0.03	1.00±0.01
Fm	5.34±0.14	4.69±0.14
Fv/Fm	0.84±0.00	0.79±0.00
Y(II)	0.69±0.01	0.64±0.02
ETR(II)	43.75±0.92	40.10±1.13
qP	0.87±0.02	0.87±0.02
qN	0.31±0.00	0.29±0.00
NPQ	0.36±0.00	0.32±0.00
Y(NPQ)	0.08±0.00	0.08±0.00
Y(NO)	0.22±0.01	0.27±0.01
Pm	2.12±0.01	1.36±0.15
Y(I)	0.78±0.05	0.86±0.00
ETR(I)	48.95±2.90	54.25±0.21
Y(ND)	0.02±0.00	0.03±0.01
Y(NA)	0.21±0.05	0.11±0.00

At low intensities of actinic light the difference between ETR(II) of the *en:chlorina*-7 and the WT was relatively small (5-10%), but at high intensities of actinic light ETR(II) showed a great difference (up to 30%). The analysis of light curves has not revealed so a big difference between the *en:chlorina*-7 and the WT in any other parameter.

It is interesting to note that, there was no significant difference in coefficients of photochemical quenching (qP) and nonphotochemical quenching (qN) between the mutant and the WT. However the value of NPQ ratio was higher in the WT compared to the yellow-green mutant. The NPQ value reflects PS II down-regulation, which is a protective mechanism against excess light intensity and also the NPQ value could be an indicator of transthylakoid proton gradient (Busch *et al*., 2008). We expected some differences in NPQ values between the mutant and the WT at the higher intensity of actinic light, but there were no significant changes in light curves. Values of quantum yields of regulated (Y (NPQ)) and non-regulated (Y (NO)) energy dissipation were slightly increased in mutant plants. Nevertheless, such variations are insufficient for making assumptions about changes in the energy dissipation of PSII.

An opposite pattern is observed in values of complimentary quantum yields of PSI photochemistry (Table 1). Much lower values of Y(NA) in the chlorina mutant indicated a decreased acceptor side limitation of PSI. Consequently, an increased Y(ND) parameter, indicated a higher oxidation status (redox poise) of P700 (donor side limitation of PSI) in the mutant compared to the WT. However, a reduction of Y (NA) in the *en:chlorine*-7 may be caused by an activation of Calvin cycle, which in turn increases the quantum yield of PSI photochemistry (Y(I)) and thus, the more effective rate of electron transport (ETR (I)) is observed.

Discussion

Previous studies have revealed non-synonymous mutations in the *en: chlorine 7* plastid genes - *psaA*, *psbB* (Markin *et al*., 2016). Hence the chlorophyll deficient phenotype may be associated with an impaired function of core subunits of PSI (*psaA*) either PSII (*psbB*) or with the disturbance of both proteins. It is important to note that, if these mutations had led to complete loss of proteins function, a white phenotype in plants would have been detected, similar to that have been observed in tobacco deletion mutants (Leelavathi *et al*., 2011). Lack of published information about chlorina mutants with extranuclear genetic origin and a rarity of studies of mutation in *psaA* and *psbB* in higher plants, it makes it difficult to approve some statements, but the assumption could be made.

According to data obtained from PAM fluorometer measures a few assumptions could be established. The first one is that, there is a depletion of P700 in mutant plants. This is evidenced by a considerable decrease of the *Pm* value, which is the direct indicator of a low content of photo-oxidizable PSI (Grieco *et al*., 2012). A possible reason for such significant reduction of PSI could be *psaA* mutation - Thr528Ile. However to confirm this assumption further investigations, especially proteomic analyses, are required.

Another assumption could be made, that the mutation in *psbB* (His157Tyr) associated with a reduction of PSII. In the *en:chlorina*-7 line the Fm value was not decreased so greatly as the *Pm* value, although mutant plants had worsening in large number of PSII parameters–Fo, Fv/Fm, Y(II), ETR(II).

Thus we assume that both mutations have phenotypic effect, however which genetic changes lead to chlorophyll deficient phenotype is still not entirely clear. On the one hand the lower chlorophyll content may be observed due to the reaction centers reduction. The decrease of *Pm* and Fm values may be associated with RC lessening of PSI and PSII, respectively, but without additional molecular analyses, we are not able to exactly establish RC lessening. It is interesting to note that Brestic *et al*. (2015) also revealed a great decrease of the *Pm* value in wheat yellow-green mutant lines.

On the other hand the reason of chlorophyll deficiency may be due to a diminution of light harvesting complexes. This assumption can be supported by an a\b chlorophyll ratio increase in *en:chlorina*-7 plants. In spite the fact that the Chl-b breakdown is observed at degradation of LHC, nevertheless the a\b chlorophyll ratio is not a sufficient criteria for unambiguous conclusion (Kovács *et al*., 2006; Tanaka and

Tanaka, 2011). More informative for the confirmation of the decreasing LHC content are ETR(II) changes. Mutant plants had the reduced rate of electron transport and without the reduction of PSII antenna there must be an excess of excited electrons. The excess of excited electrons had to impact on parameters of an energy dissipation, but values of energy dissipation were similar in both mutant and WT plants even in high actinic light conditions.

Although we tend to think that a decline of chlorophyll content in the *en:chlorina*-7 is primarily associated with a decrease in LHC, there is an insufficient evidence for the definite approval. We may expect that further studies using yellow-green mutants may resolve this dispute. As well as the conducted research contributes to knowledge in a field of chlorophyll deficient phenotype development reasons.

Conclusion

The conducted research revealed a low content of photo-oxidizable PSI and an impaired quantum yield of PSII photochemistry in the *en:chlorina*-7 line as compared with the wild type. These disturbances of PSI and PSII in the yellow-green plastome mutant line of sunflower (*en:chlorina*-7) are associated with mutations in *psaA* and *psbB*, respectively. The results obtained could be useful for investigations in a field of the genetics of photosynthesis.

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Author's Contributions

M.S. Makarenko: Participated in data analysis and the entire process of the article preparation

N.V. Kozel: Participated in all laboratory trials and writing manuscript chapter - «Materials and Methods»

A.V. Usatov: Designed the research plan and organized the investigation

O.F. Gorbachenko: Participated in plant material preparation and some experiments

N.G. Averina: Coordinated the data-analysis

Ethics

This article is original and contains unpublished material. The authors declare that there is no conflict of interest regarding publication of this paper. The authors declare that no ethical issues are going to arise after the work has been published.

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