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海外特別研究員最終報告書

独立行政法人日本学術振興会 理事長 殿



海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。 なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地(派遣先国名)<u>用務地: フランス国立科学研究センター (国名: フランス</u>

 研究課題名(和文)<u>※研究課題名は申請時のものと違わないように記載すること。</u> 植物における光合成系の発達・衰退を制御する 02 利用戦略に関する研究

3. 派遣期間: 平成 30 年 4 月 30 日 ~ 令和 2 年 3 月 20 日

4. 受入機関名及び部局名

フランス国立科学研究センター, CEA-Saclay, I2BC

5. 所期の目的の遂行状況及び成果…書式任意 **書式任意(A4 判相当3ページ以上、英語で記入も可)** (研究・調査実施状況及びその成果の発表・関係学会への参加状況等) (注)「6. 研究発表」以降については様式10-別紙1~4に記入の上、併せて提出すること。

Changes in photosynthetic electron transport during leaf senescence in two barley varieties grown in contrasting growth regimes

Leaf senescence is important process for remobilization of nutrients and grain filling in plants, but the initiation mechanisms are still poorly understood. One of the most expected candidates triggering leaf senescence is the alteration of photosynthetic electron transport in the thylakoid membrane that has impacts on the redox level and reactive oxygen species (ROS) production in chloroplasts. However, the initiation of leaf senescence can be affected by various environmental variations, which makes it difficult to generalize the sequences of leaf senescence. Recently, the different senescence scenarios in chloroplasts have been shown in two barley varieties grown in field conditions: "Lomerit" shows a decrease in the activity of photosystem (PS) II prior to that of PSI and accumulates two type ROS, 10_2 and 0_2^- , and "Carina" that first loses PSI activity earlier and generates only 02 (Krieger-Liszkay et al. 2015 Planta 241, 1497-1508). Here, we investigated changes of photosynthetic electron transport and ROS production at the early stage of leaf senescence in these two barley varieties to identify the key factor for initiation of leaf senescence in two growth conditions (field and controlled-laboratory). ROS generation was analyzed using spin trapping EPR spectroscopy, and changes in photosynthetic electron transport were detected in vivo by absorption changes in the near infrared spectral region allowing to follow changes in P700 (the reaction center chlorophyll of PSI), plastocyanin, and ferredoxin. Loss of plastocyanin was observed at the early stage of leaf senescence by spectroscopic and immunological analyses. Finally, we discuss these findings in the context of the initiation of leaf senescence.

Changes in chlorophyll content and ROS production during senescence

Primary leaves of the two barley varieties cv. Lomerit and cv. Carina grown under outdoor conditions and periodically exposed to full sunlight were compared with primary leaves of plants grown under weak light in growth chambers. Total chlorophyll content, a general marker of leaf senescence, started to decrease at almost the same time (about 3 weeks after sowing) in both varieties regardless of the growth conditions, and thereafter it declined faster in the outdoor-plants (Fig. 1A). For the following experiments, we defined day 17 as an early leaf senescence stage and day 31, at which the chlorophyll content started to decrease, as an intermediate stage of senescence. The ratio of chlorophyll a to b first decreased during leaf senescence, which started earlier in the growth-chamber-plants (Fig. 1B). At a late stage of senescence (40 days), the a/b ratio increased in the outdoor-plants, implying that the antenna complexes such as PSII light-harvesting complex were degraded.

The increase in the chlorophyll a/b ratio in cv. Lomerit correlates well with an increase in ${}^{1}O_{2}$ production upon senescence (Fig. 2A) which was also only observed in thylakoids isolated from outdoor plants from this variety. The same increase in the yield of ${}^{1}O_{2}$ production had been reported previously when flag leaves of field material were analyzed (Krieger-Liszkay et al. 2015). The signal amplitude of the HO· adduct generated from $O_{2} \cdot -/H_{2}O_{2}$ slightly increased in both varieties when grown outdoors (Fig. 2B), however, this was only a trend since the difference was statistically not significant. An increase of these ROS has also been reported previously during senescence in flag leaves from barley varieties (Krieger-Liszkay et al. 2015). ROS productions did not change during leaf senescence in the primary leaves from low light grown growth-chamber plants. These data show that senescence follows similar patterns in primary leaves and flag leaves when grown outdoor in non-controlled conditions. More intriguingly, the data shown in Fig. 2 show that senescence is not necessarily correlated with an increase in ROS production.



Changes in the photosynthetic electron transport system during senescence

Next, we assessed whether changes in the photosynthetic electron transport system occur prior to chlorophyll loss in barley primary leaves. Using a recently developed near infrared spectrophotometer (Klughammer and Schreiber 2016, Shimakawa et al. 2020), the redox states of P700, Fd and PC were simultaneously analyzed in vivo in intact leaves of the outdoor- and growth-chamber-plants. Shimakawa et al. 2020 showed the typical responses of the redox states of P700, Fd and PC to red actinic and far-red light in the primary leaves of Lomerit 10 days and 17 days (Shimakawa et al. 2020) after sowing. In the dark, both P700 and PC are completely reduced and Fd is oxidized in plant leaves. Under far-red light, preferentially exciting PSI, P700 and PC are mostly kept oxidized (Shimakawa et al. 2020). Thereafter, on applying a saturated pulse light, both P700 and PC are fully oxidized, resulting in the relative values for total oxidizable amounts of P700 and PC in vivo. Further, the maximum reduction level of Fd is observed when the leaves were illuminated with a strong actinic light for a few seconds. This light intensity and illumination time resulted only in a transient oxidation of P700 and PC. In comparison to 10 days old leaves, a loss in the amplitude of the PC signal and a faster oxidation of P700 upon far red illumination was observed in 17 days old leaves (Shimakawa et al. 2020).

The maximum amplitudes of P700, Fd and PC signals were followed in mature leaves (day 10) to senescing leaves (day 30), performing measurements every 7 days (Fig. 3). The P700 signal, reflecting the PSI content of the primary leaves, was stable until day 24 in most samples, only the signal in cv. Carina grown growth-chambers started to decline at this stage (Fig. 3A). The content of Fd was significantly higher in the outdoor-plants than in the growth chamber-plants, in mature leaves of both Lomerit and Carina (Fig. 3B). The higher amount of Fd is a sign of higher capacity of forward electron transport towards carbon assimilation. Loss of the Fd signal followed the same trend as observed for P700, indicating that the losses of these two components are synchronized. Different to P700 and Fd, PC started to decline at a linear rate at the early stage of leaf senescence (day 17) in both varieties regardless of the growth conditions (Fig. 3C). A significant decrease of PC is earlier than the decrease in total chlorophyll content (Fig. 1A).

To investigate whether the observed loss of plastocyanin impacts electron transport activity, oxidation levels of P700 were analyzed using two different intensities of actinic light (Fig. 4). Growth-chamber plants showed a strong limitation of electron donation to PSI detected as an increase in the P700 oxidation level. When measured at low light intensity (100 µmol photons m⁻² s⁻¹) donor side limitation of PSI is already visible in the growth-chamber grown plants, with a stronger limitation in cv. Carina, while outdoor grown plants were able to keep P700 completely reduced even at the latest stage measured (31 days). At high light (820 µmol photons m⁻² s⁻¹), all plants showed donor side limitation, particularly in growth-chamber plants. Since the PC level decreased to the same extent in plants from both growth regimes (Fig. 3C), the difference in PSI donor side limitation cannot be explained by a lower amount of PC. Instead, limitation of electron transport to PSI has to be localized downstream of PC. One possible explanation may be a higher proton gradient in the growth-chamber plants that may induce higher levels of non-photochemical quenching (NPQ) and stronger limitation of electron transport at the level of the cytochrome $b_6 f$ complex, the socalled photosynthetic control.

To assess the capacity of electron transport under the two light regimes used for growth, electron transport rates were measured at the two light intensities. When measured at low light, the electron transport rates decreased slightly in the growthchamber plants during the time course of senescence, while at high light intensity it decreased strongly in the growth-chamber plants, but remained stable until day 24 in the outdoor plants (Fig. 5A and B). In outdoor grown plants electron transport rates measured at high light intensity stayed high and a decrease was only observed at day 31, a senescence stages characterized by decrease in chlorophyll (Fig. 1). NPQ, when measured at low light, was rather low for all plants independent of the growth conditions (Fig. 5C), while at high light intensity, NPQ was much higher for the outdoor than for the growth-chamber plants (Fig. 5D), showing that these plants are acclimated to abiotic stress conditions.

Overall, the two varieties showed only slight differences in properties of the photosynthetic electron transport chain occur during early stages of senescence. This result was rather surprising since the two varieties have clearly distinguishable senescence strategies in later stages with Lomerit preferentially losing PSII activity and grana stacks and Carina losing preferentially PSI activity and stroma lamellae (Krieger-Liszkay et al. 2015, Krupinska et al. 2012). Compared with Lomerit, Carina

plants grown in growth-chambers at low light, showed a more pronounced loss of Fd, a higher level of P700 oxidation caused by PSI donor side limitation and a slightly lower electron transport rate 24 days after sowing (Fig. 3B, 4, 5A and B). This indicates that under the given growth conditions, primary leaves of Carina senesce slightly faster than those of Lomerit.



References

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