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ORIGINAL PAPER

Contribution of ABA metabolism and ROS generation to sugar starvation-induced senescence of rice leaves

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Abstract

To clarify the relationship of sugar starvation with ABA-induced ROS generation during leaf senescence, the genotype-dependent differences in sugar concentration, endogenous ABA content, and ROS generation as well as their temporal patterns during leaf senescence were investigated by using two rice genotypes, namely, Zhehui7954 and its corresponding mutant with the premature senescence of flag leaves (*psf*). Meanwhile, the interplay between sugar starvation and ABA metabolism in the induction of leaf senescence was examined using detached leaves. The results showed that the *psf* mutant differed evidently from its wild type (Zhehui7954) in the temporal pattern of soluble sugar, sucrose and hexose (fructose and glucose) contents during leaf senescence, with the rapidly dropping concentrations of total soluble sugar, and sucrose, and hexose for the senescing leaves of *psf* mutant. Sugar starvation evidently accelerated leaf senescence and significantly enhanced the ABA concentration and malonaldehyde (MDA) accumulation in detached leaves, while exogenous sugar supply severely suppressed the ABA concentration and ROS level in detached leaves, thereby the delayed leaf senescence for the detached leaves treated by exogenous sugar supply. Correspondingly, ABA biosynthesis inhibitor (NDGA) effectively retarded the sugar starvation-induced leaf senescence, while ABA catabolism inhibitor (DNCZ) obviously accelerated leaf senescence by enhancing the endogenous ABA concentration in senescent leaves. Furthermore, sugar starvation severely repressed the transcripts of several key genes related to ABA biosynthesis and its degradation (*NCED1*, *NCED4, NCED5, ABA8ox2* and *ABA8ox3*), with the significantly lower amount of their transcriptional expression in the senescent leaves of *psf* mutant relative to its wild type during leaf senescence. Hence, the disequilibrium between ABA biosynthesis and catabolism was strongly responsible for sugar starvation-induced leaf senescence, which was derived from the suppression of ABA degradation, rather than the enhancement of ABA biosynthesis.

Keywords Rice (*Oryza sativa* L.) · Leaf senescence · Sugar starvation · ABA · ROS

Introduction

Leaf senescence is an important developmental process that eventually leads to leaf death in plant life cycle. During this process, leaf cells undergo the sequential complex changes in plant physiology and cellular metabolism, such as breakdown of chloroplasts, loss of photosynthetic activity, replacement of carbon assimilation by the catabolism of chlorophyll and macromolecules, and programmed cell death (Asad et al. 2019). Leaf senescence occurs in an age-dependent manner, but the initiation and progression of leaf senescence can be greatly modulated by various environmental and endogenous factors, including nutritional and hormonal signals, water and sugar status, light regimes, and temperature change (Woo et al. 2019). Sugars act as the key

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factors in metabolic control of plant growth and development, ranging from energy sources, storage molecules, and structural components for cell carbon skeleton (Ciereszko 2018). Numerous studies have suggested that endogenous sugar concentration in leaf tissues contributes to the regulation of leaf senescence and nutrient remobilization from senescent leaves to storage grains (Wingler et al. 2009). In a densely growing crop population, sugar starvation-induced senescence often occur at the increased plant densities for leaves below a canopy (Weaver and Amasino 2001; Borras et al. 2003). Nitrogen deficiency-induced leaf senescence have also been demonstrated to be closely associated with the varying sugar metabolism in leaf tissues among different nitrogen levels (Zakari et al. 2020). The participation of sugar signaling in nitrogen deficiency-induced leaf senescence is mainly characterized by the feedback inhibition of the elevated sugar accumulation on the production capacity of photosynthetic assimilates, both excessive sugar accumulation at early steps and sugar starvation at the following steps can lead to the accelerated leaf senescence under nitrogen deficiency (Agüera et al. 2010). Furthermore, sugars and oxidative damage act synergistically in manipulating the onset and durability of leaf senescence (Wingler et al. 2009). For instance, sugar starvation is easily caused by darkness, with the subsequent occurrence of chlorophyll degradation and oxidative damage (Brouwer et al. 2012; Wang et al. 2016a; Li et al. 2020). On the other hand, the deprivation of sunlight for the part areas of individual leaves in rice plants retards leaf senescence and reduces ROS generation under the elimination of sugar starvation in shaded areas, in which the unshaded areas in same leaf can provide sufficient sugar to the shaded areas along the phloem transport, concurrently with the avoidance of sunlight inducible oxidative damage for the shaded areas (Wang et al. 2016b). Interestingly, excessive ROS generation and oxidative damage under strong solar intensity and water stress conditions was strongly responsible for the induction of leaf senescence in many plant species, which was not caused by sugar starvation (Barber and Andersson 1992; Wingler et al. 2005). Hence, it is a crucially important to elucidate the interaction between sugar accumulation and ROS generation in regulating the initiation and progression of leaf senescence for crop yield improvement.

Just like sugar signaling, abscisic acid (ABA) is an important phytohormone that regulates various developmental processes and the adaptive responses of plants to numerous stresses, including drought, salinity, low temperature, and other biotic factors. The invovlement of ABA in the regulation of leaf senescence have been also reported in many plant species. Exogenous ABA induces the expressions of senescence-associated genes (*SAG*), in conjunction with the alterations of carbohydrate metabolism and sugar concentration in leaf tissues (Bolouri-Moghaddam et al. 2010). Under sunlight-dependent leaf senescence and the exposure of plant to drought-stress condition, ABA coordination with ROS generation induces programmed cell death (PCD) and causes the premature leaf senescence. In *Arabidopsis*, transgenic plants with the over-expression of hexokinase gene exhibit the premature senescence phenotype, with a significant increase in ABA concentration in leaf tissues (Dai et al. 1999; Yoshida et al. 2002). Studies on wheat, rice, maize have also revealed that nitrogen deficiency and drought treatment cause an increase in ABA concentration in leaf tissues and also induces premature leaf senescence due to ROS generation being possibly activated by ABA (Asad et al. 2019; Zakari et al. 2020). Conversely, the markedly altered sugar accumulation and reduced ROS generation are strongly responsible for the delayed leaf senescence under heavy nitrogen application and darkening treatment, which is also closely associated with the lowering ABA concentration in leaf tissues (Zakari et al. 2020). But so far, the metabolic mechanism underlying the relationship of sugar starvation-induced senescence with ROS generation and ABA concentration in senescent leaves remains unclear.

Generally, the endogenous level of ABA in plant tissues was controlled by the equilibrium between ABA biosynthesis and catabolism, and the concentration of ABA in plant leaves is regulated not only by ABA biosynthesis, but also by its catabolism (Nambara and Marion-Poll 2005; Ikegami et al. 2009; Mega et al. 2015). The first biosynthetic step in ABA biosynthesis is the conversion of zeaxanthin to all-trans-violaxanthin, in which the 9-cis-epoxidized carotene dioxygenase (NCED) catalyzes the oxidative cleavage of 9-cis-violaxanthin and/or 9-cis-neoxanthin to produce xanthoxin, this reaction is a limiting step for the rate of ABA biosynthesis pathway, while abscisic acid 8'-hydroxylase (ABA8ox) is the key enzymes responsible for ABA catabolism/degradation (Nambara and Marion-Poll 2005). In rice, 9-*cis*-epoxycarotenoid dioxygenase is encoded by five homologues *OsNCED1*, *OsNCED2*, *OsNCED3*, *OsNCED4* and *OsNCED5* (Zhu et al. 2009), and ABA 8'-hydroxylase is encoded by three homologous genes, namely *OsABA8ox1, OsABA8ox2* and *OsABA8ox3* (Saika et al. 2007; Mega et al. 2015). However, our understandings are still lacking on the effect of sugar starvation on the expression of various isoform genes involving in ABA biosynthesis and degradation events and its relation to leaf ABA concentration and ROS generation during leaf senescence.

In this study, we compared the genotype-dependent difference in the temporal patterns of sugar, sucrose, hexose, and ABA accumulation during leaf senescence by using the early leaf senescence mutant and its wild type. To clarify the triggering effect of sugar starvation on leaf senescence and its relationship with ABA metabolism and ROS generation, the detached leaf segments were treated by in vitro dark exogenous sucrose, ABA anabolism inhibitor

nordihydroguaiaretic acid (NDGA), and ABA catabolism inhibitor diniconazole (DNCZ).

Materials and methods

Plant materials and field experiment

Two rice genotypes, an excellent *indica* restorer line (Zhehui7954) and its corresponding mutant with the premature senescence of flag leaves (*psf*) were used in this study. The *psf* mutant was derived from Zhehui7954 cultivar (*Oryza sativa* L. ssp. *indica*) mature seeds with gamma irradiation as mutant factor, and the stably inherited mutant was obtained through the successive self-pollination for more than eight generations. In a preliminary work, we have reported that the *psf* mutant did not differ visibly from its wild type in plant phenotype and leaf senescence at seedling and tillering stages (Wang et al. 2016a). However, the flag leaves of *psf* mutant exhibited the senescence symptom after anthesis, and the extent of exacerbated senescence in phenotype appeared to be leaf age-dependent under normal field growth (Wang et al. 2016a, b). In our present investigation, we focused primarily on the genotype-dependent differences in sugar concentration, endogenous ABA content, and ROS generation as well as their temporal patterns during leaf senescence for the *psf* mutant and it wild type.

The field experiments were performed in 2017 and 2018 at the experimental station of Zhejiang University (120°04′ E, 30°18′ N), Hangzhou, China. Rice seeds were sown in April 25 and transplanted in May 24. Three replications of each genotype were sowed in RCBD plots with 10 × 12 row size. Plant spacing was maintained to 18 cm × 18 cm for each hill. The field management was performed according to the local practices. The soil type was the periodically waterlogged paddy soil, with 1.71 g kg^{-1} of total N, 23.8 mg kg^{-1} of available P and 114.2 mg kg^{-1} of exchangeable K. At the approximate heading day, a total of 80–90 panicles with uniform appearance and anthesis were selected and subsequently tagged. The samples of flag leaf of the tagged panicles were started to be taken at full heading stage and ended at the mature stage by keeping 7-day interval. The collected samples were frozen in liquid nitrogen and stored in − 80 °C for further experimental use.

Exogenous incubation experiment in detached leaves

Experiment I

In order to investigate the relationship of sugar starvation-induced leaf senescence with endogenous ABA concentration and ROS generation, the flag leaves of the *psf* mutant and its wild type were carefully detached from plants at 14 days after anthesis (DAA). The detached leaves of both genotypes were cut into 3 cm and soaked into petri dishes containing sterilized distilled water for two hours to eliminate wound stress. For sugar starvation treatment, these petri dishes were placed in artificial growth chamber (Model PRX-450D; Safu, Shanghai, P.R China) at 25 °C under complete darkness, and the leave segments were subsequently sampled at 3-day interval (0 day–6 day after the initial incubation). All detached leaf samples were kept frozen and then stored at − 80 °C after an observation for its senescence symptoms. Five replications (petri dishes) were performed for each sampling time.

Experiment II

The effects of exogenous sugar concentration on the senescent symptoms and also the transcriptional expressions of various genes involving in ABA biosynthesis and its catabolism were further examined by using the detached leaf segments of the *psf* mutant at anthesis day. Detached leaf segments were imposed to different concentration of sucrose treatments, and the gradient concentration of exogenous sucrose was 0 mM, 40 mM, 80 mM, 160 mM, 320 mM and 600 mM. For each treatment, 25 mL of sucrose solution were added in petri-dishes, with 4 dishes for each incubating concentration. The similar incubation with a series of mannitol concentration was used as control. Prior to these immersions, the leaf segments were placed in distilled water for 2 h to eliminate the wound stress. After 6 days-incubation at 25 °C under complete darkness, the detached leaf segments were sampled and stored for subsequent analysis after their senescence symptoms being recorded with a digital camera.

Experiment III

To verify the contribution of ABA metabolism in the senescing leaves to the regulation of sugar starvation induced leaf senescence, the detached leaf segments of the *psf* mutant at 14 DAA were employed to conduct an experiment on the artificially induced alterations in endogenous ABA concentration and its metabolism for the senescing leaves. In this experiment, the detached leaf segments were soaked in petri-dishes containing 25 mL solution of 10 mM nordihydroguaiaretic acid (NDGA, an inhibitor for plant ABA biosynthesis) and 10 mM diniconazole (DNCZ, an inhibitor for plant ABA catabolism), respectively. The similar incubation with distilled water was implemented as control. Twelve replications were maintained for each treatment. All detached leaves were incubated at 25 °C under complete darkness, the leaf segments were subsequently sampled at 0 day, 3 days, and 6 days after the initial incubation. The visual symptoms of leaf senescence for different treatments were recorded

with a digital camera, and the sampled leaf segments were kept at − 80 °C until further analysis.

Measurement of chlorophyll, sucrose, soluble sugar, hydrogen peroxide (H_2O_2), superoxideradical ($O_2^{\bullet-}$), and malondialdehyde (MDA) contents in rice leaves

The chlorophyll content in rice leaves was determined spectrophotometrically as described by Lichtenthaler (1987), using a Shimadzu UV–Vis 2450/2550 spectrophotometer. Sucrose contents and soluble sugar were assayed according to the method described previously by Yang et al. (2003). Superoxide radical ($O_2^{\bullet-}$) generation rate, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) contents were measured by following the procedure as described by Jiang and Zhang (2001). For all these parameters described above, the flag leaves and/or its detached leaf segments were used for each measurement, with 3–4 biological replicates.

Extraction and determination of ABA in rice leaves

The extraction and purification of ABA was performed according to the method of Kojima et al. (2009) with some modifications. One-gram aliquot of fresh leaf samples was normalized in liquid nitrogen and added to 5 mL of ice-chilled extraction buffer (methanol:formic acid:water = 15:1:4). After 24 h extraction at − 20 °C, the homogenate was centrifuged at 10,000×*g* for 15 min at 4 °C. The supernatants from each sample were transferred to a 96-well collection plate and the remaining residues were extracted again. Subsequently, the collection plate was placed in the automatic extraction of solid phase extraction system (SPE215; Gilson, Middleton, WI, USA). After extraction, the residual extraction was activated with 1 mol L^{-1} formic acid before through column. The ABA was washed out using methanol, the eluent was evaporated and further purified. Afterward, ABA content was measured by UPLC-ESI-qMS/MS method. The chromatographic conditions and MS parameters in UPLC-ESI-qMS/MS system was designed as described previously by Kojima et al. (2009). Three replications were detected for each sampling.

RNA isolation, cDNA preparation, and Quantitative real-time PCR

The procedures of RNA extraction and cDNA preparation for leaf tissues were performed as described by Wang et al. (2016b). Trizol reagent kit (Invitrogen, Carlsbad, CA, USA) was used for the extraction of total RNA, the First Stand cDNA Synthesis kit (Toyobo, Osaka, Japan) was used for cDNA synthesis by following the manufacturer instructions in manual. Quantitative real-time PCR was performed by using the SYBR Green real-time PCR Master Mix Reagent Kit (Toyobo, Osaka, Japan). The reactions were conducted in Bio-Rad CFX96 real-time system (Bio-Rad, CA, USA) by following the manufacturer's protocol. The amplification reagents contained 10 µL SYBR, 1 µL cDNA, 1.6 µL 10 mM primer pairs and 7.4 µL RNase free H_2O. All gene-specific primer pairs were designed by using online software GenScript, and the optimal primer of annealing temperature for each gene was listed in supplementary Table. The expression of *Actin* was used as internal control. The amplification of various genes was normalized by *ACTIN-1* expression and their relative expression levels were calculated by $2^{(-\Delta\Delta CT)}$ method (Rao et al., 2013). Each experiment was performed with three replicates.

Statistical analysis

All determinations were performed in at least three independent experiments. Statistical differences were analyzed by analysis of variance (ANOVA) using the SPSS statistical software package (Chicago, Illinois, USA). The mean was compared by the least significant difference (LSD) test at probability level of $p < 0.05$.

Results

Differences in the temporal patterns of sugar, ABA, and ROS concentrations during leaf senescence between the psf mutant and its wild type

The marked difference in the initiation and progression of leaf senescence was observed between the *psf* mutant and its wild type, with the significantly lower chlorophyll content and substantially higher MDA content for the *psf* mutant relative to its wild type at the middle and late stage of grain filling (Fig. S1). Interestingly, the *psf* mutant differed evidently from its wild type in the soluble sugar, sucrose, hexose (fructose + glucose) contents and also their temporal patterns during leaf senescence. The soluble sugar and sucrose contents in the flag leaves of *psf* mutant was significantly lower than those of its wild type at the middle and late stage of grain filling (Fig. 1a–c). Furthermore, the genotype-dependent alteration in ABA content in rice leaves was basically consistent with the senescence-associated change in O_2^- production rate, in terms of their temporal patterns during leaf senescence (Fig. 1f and Fig. S1). Considering the possible interaction between leaf senescence and sugar starvation, we inferred that the significantly lowered sugar level in the flag leaves of *psf* mutant was, at least partly, responsible for its induction and acceleration of leaf senescence, in addition to the marked increase in ABA concentration and ROS generation during leaf senescence.

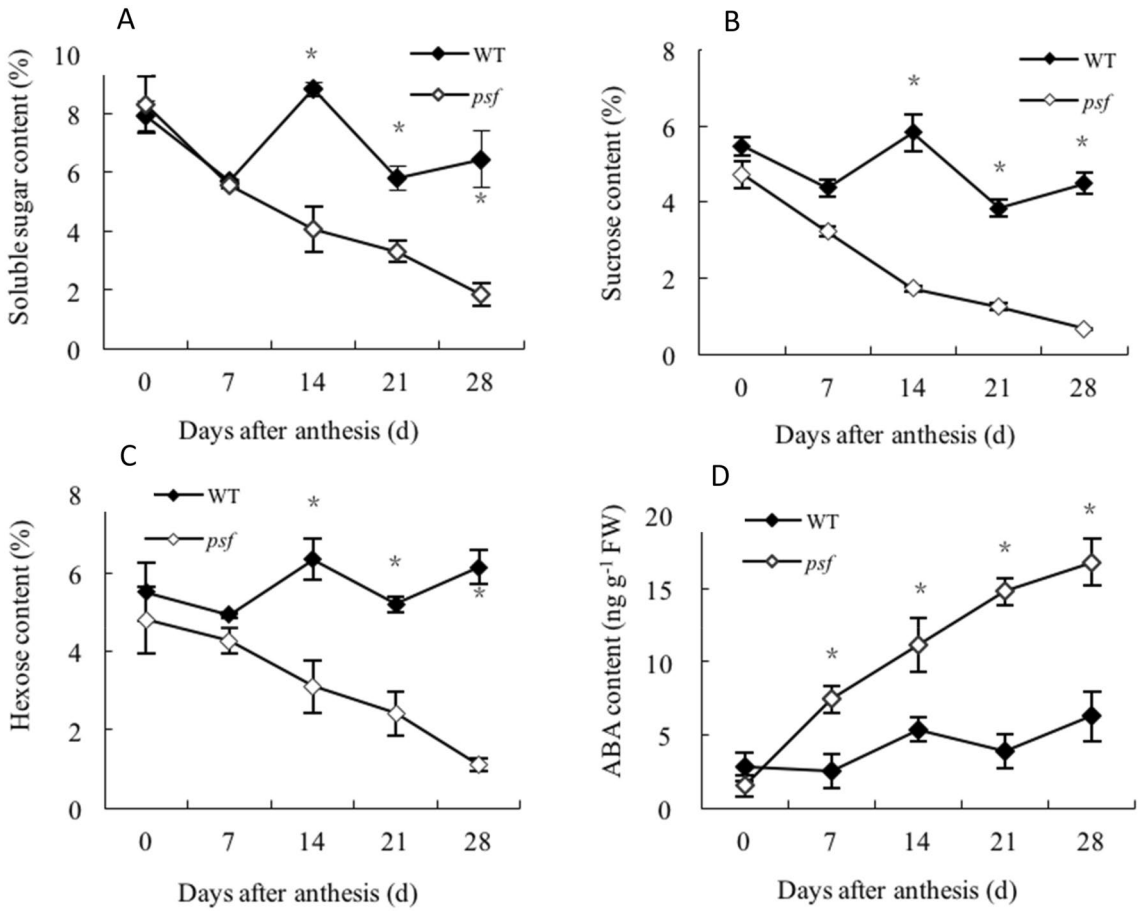


Fig. 1 Comparison of sugar and ABA contents between the *psf* mutant and its wild type at pre anthesis left: wild and right: *psf* mutant, temporal pattern of soluble sugar content (**a**), sucrose content (**b**), hexose content (**c**) and ABA content (**d**) in flag leaves of the *psf* mutant and its wild type after anthesis. Values are means with the standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between the *psf* mutant and its wild type taken at the same time interval

Linking sugar starvation-induced leaf senescence with the varying ABA and ROS concentrations in senescing leaves

To clarify the effect of sugar starvation on leaf senescence and its relation to ROS generation and ABA concentration in senescing leaves, the detached leaf segments of *psf* mutant and its wild type at 14 DAA were imposed to different incubation period of darkness (Fig. 2). The detached leaf segments of *psf* mutant displayed the chlorosis and senescence symptom after 3 days' darkness, with the aggravated senescence symptom and much lower chlorophyll content after 6 days' dark treatment, while for the detached leaf segments of wild type, the visual senescence symptom occurred after 6 days' darkness (Fig. 2a, b). Obviously, the concentration of endogenous sugars (soluble total sugar, sucrose and hexose contents) in detached leaf segments dropped rapidly under dark treatment, and the detached leaf segments of *psf* mutant had strikingly lower concentration of endogenous sugars than its wild type after the same duration of darkness (Fig. 2f–h). These results indicated clearly that the initiation and subsequent progression of senescence in rice leaves were visibly induced by sugar starvation under darkness, although the extent of leaf senescence induced by sugar starvation under darkness was largely variable, depending on different dark duration and rice genotypes. Interestingly, sugar starvation under darkness significantly enhanced the endogenous ABA concentrations in the detached leaf segments of the two rice genotypes (Fig. 2i), simultaneously with the markedly increasing levels of H_2O_2, O_2^- and MDA under sugar starvation (Fig. 2c–e). This phenomenon implied that the marked increases in ABA concentration and ROS generation in the leaf segments might contribute to the sugar starvation-induced leaf senescence under darkness.

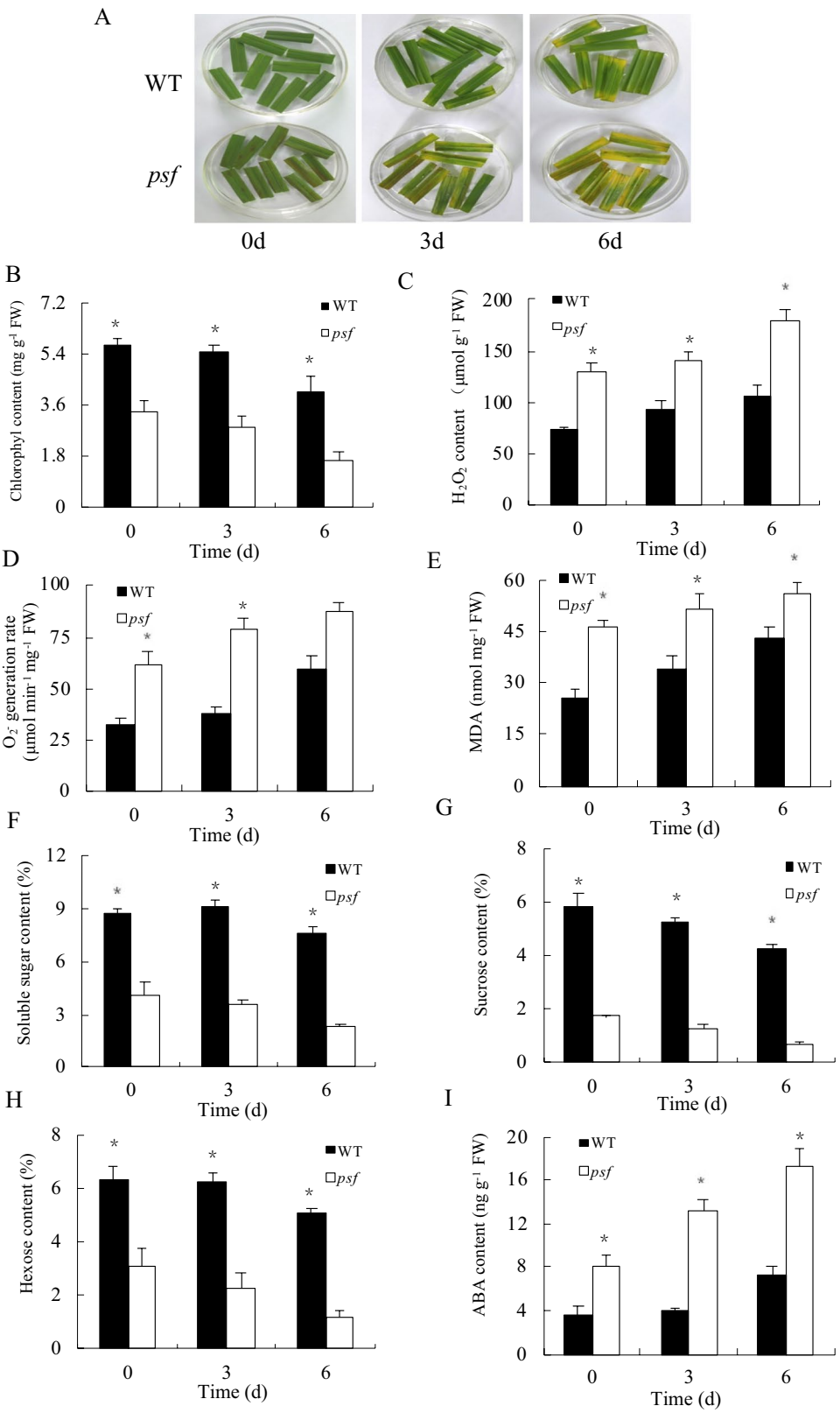


Fig. 2 Senescent symptoms and total chlorophyll content changes of detached leaf segments induced by dark treatment (sugar starvation treatment) after 0, 3 and 6-days' incubation, respectively. Visual color change of detached leaf segments (**a**). The chlorophyll contents in detached leaf segments after 0, 3 and 6-days' incubation (**b**). Effect of dark treatment (sugar starvation treatment) on H_2O_2 content (**c**), O_2^- generation rate (**d**), MDA content (**e**), soluble sugar content (**f**), sucrose content (**g**), hexose content (**h**) and ABA content (**i**) after 0, 3- and 6-days' incubation, respectively. Vertical bars represent standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between the *psf* mutant and its wild type taken at the same time interval

We further investigated the effect of exogenous sucrose supply on leaf senescence and its relation to H_2O_2 and ABA concentrations by using the detached leaf segments of *psf* mutant at 0 DAA, with a series of gradient sucrose concentrations (0 mM, 40 mM, 80 mM, 160 mM, 320 mM, 480 mM, 600 mM) (Fig. 3). Exogenous sucrose incubation inhibited the fading of leaf green (Fig. 3A) and significantly enhanced the chlorophyll content in detached leaf segments (Fig. 3B), while the detached leaf segments faded quickly along with decline in chlorophyll content when the similar concentrations of mannitol were supplied to sugar starvation-induced leaf senescence (Fig. 3a, b). Notably, the H_2O_2 content and O_2^- generation in detached leaves dropped sharply after 6 days' incubation of exogenous sucrose (Fig. 3d, e), and the ABA level and MDA content in the detached leaf segments being treated by > 80 mM concentration of exogenous sucrose were significantly lower than those incubated in mannitol solution (Fig. 3c, f). These results suggested that the contribution of exogenous sucrose incubation to the delayed leaf senescence in detached segments was closely associated with the inhibitory effect of exogenous sucrose supply on the ROS level and ABA concentration in detached segments.

Transcriptional profile and the temporal patterns of key genes involving in ABA biosynthesis and its degradation during leaf senescence

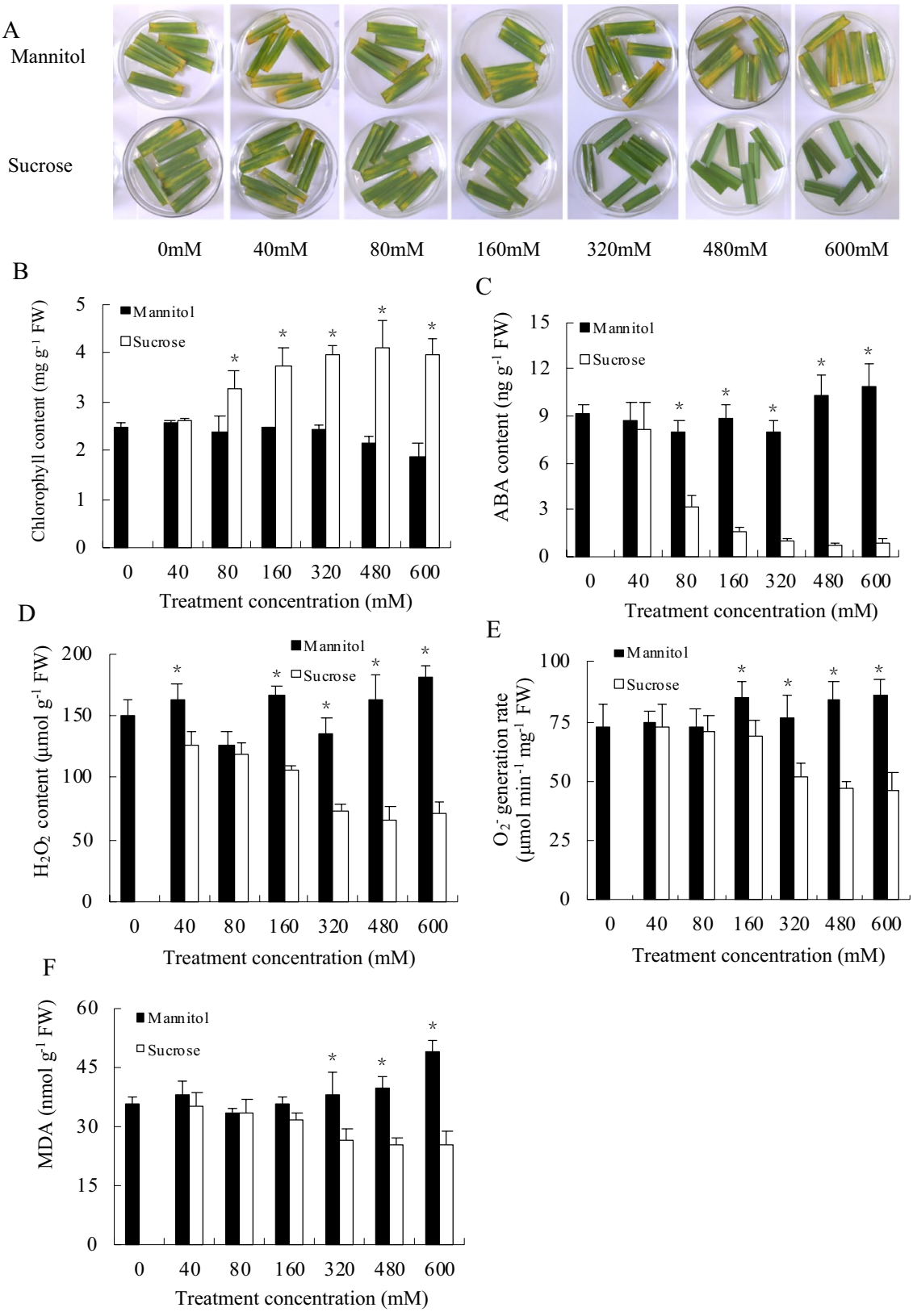
The genotype-dependent alterations in the transcriptional expression of several key genes involving in ABA biosynthesis and its degradation (*OsNCEDs* and *OsABA8oxs*) and also their temporal patterns during leaf senescence were comprehensively investigated by quantitative real-time reverse transcription PCR (Fig. 4). For five *OsNCED* genes, *OsNCED1* was much more abundant in rice leaves than *OsNCED4*, *OsNCED5* (Fig. 4a), while the transcripts of *OsNCED2* and *OsNCED3* in rice leaves showed extremely low transcript levels (Fig. 4h). Three *OsABA8ox* genes also showed obvious differences in their relative transcript amounts in rice leaves, with the highest level for *OsABA8ox3* among three *OsABA8ox* isoforms (Fig. 4a). In comparison of the wild type, the *psf* mutant exhibited significantly lower transcript levels of *OsNCED1* and *OsABA8ox3* over the entire sampling stage, with the gradually descending trend along with leaf senescence (Fig. 4b, g). This result indicated that *OsNCED1* and *OsABA8ox3* were among the important isoforms associated closely with the ABA concentration in rice leaves, and the down-regulation of *OsNCED1* and *OsABA8ox3* transcripts was strongly responsible for the genotype-dependent alterations in the endogenous ABA concentration and its temporal patterns during leaf senescence for the *psf* mutant and its wild type. Considering the senescence-associated change in ABA concentration, we inferred that the strikingly increased ABA concentration in the senescent leaves of *psf* mutant was more attributable to the suppression of ABA degradation, rather than the enhancement of ABA biosynthesis during leaf senescence.

To elucidate the relationship of ABA biosynthesis and its catabolism with sugar starvation-induced leaf senescence, we further examined the transcripts of several genes involving in ABA biosynthesis and its catabolism by using the detached leaf segments under darkness (Fig. 5). Sugar starvation severely suppressed the transcript *OsABA8ox3* in the detached leaf segments of both the rice genotypes, and the *psf* mutant displayed significantly lower amount of *OsNCED1* and *OsABA8ox3* transcripts than its wild type after the same duration of sugar starvation (Fig. 5a, f). Interestingly, sugar starvation significantly enhanced the *OsABA8ox1* transcript in the detached leaf segments, with the remarkably higher *OsABA8ox1* transcript for the *psf* mutant relative to its wild type after 3-days' sugar starvation under darkness (Fig. 5d). Such diversity possibly played a complementary role in the regulation of ABA concentration in the senescent leaves under sugar starvation.

The transcripts of several genes involving in ABA biosynthesis and its catabolism in response to the varying sugar concentration was further investigated by the detached leaf segments floated in different exogenous sucrose concentration (Fig. 6). The transcripts of *OsNCED1* and *OsABA8ox3* in detached leaf segments were significantly up-regulated by the incubation of higher sucrose concentrations (>80 mM). However, the rising extent was greatly variable between the two genes. The *OsABA8ox3* transcript increased by 2-fold, while *OsNCED1* transcript showed only 0.8-fold increase under the same concentration of sucrose treatment (80 mM). These results implied that the enhancement of ABA catabolism as a result of strikingly increasing *OsABA8ox3* transcript might contribute greatly to the lowered ABA concentration in detached leaf segments under exogenous sucrose treatment, which in turn led to the inhibition of leaf senescence by exogenous sugar supply.

Participation of ABA metabolism in dark-induced leaf senescence

To detect whether endogenous ABA was the inducer of sugar starvation-induced leaf senescence, the detached flag leaves were treated by NDGA (an inhibitor of ABA biosynthesis) and DNCZ (an inhibitor of ABA degradation, respectively (Fig. 7). The result showed that NDGA significantly decreased the endogenous ABA concentration in the detached flag leaves, while the opposite trend was true for DNCZ (Fig. 7e). Obviously, NDGA incubation effectively retarded the visual senescence symptom in detached flag leaves (Fig. 7a), and the contents of chlorophyll, soluble sugar, and sucrose in the detached flag leaves being treated

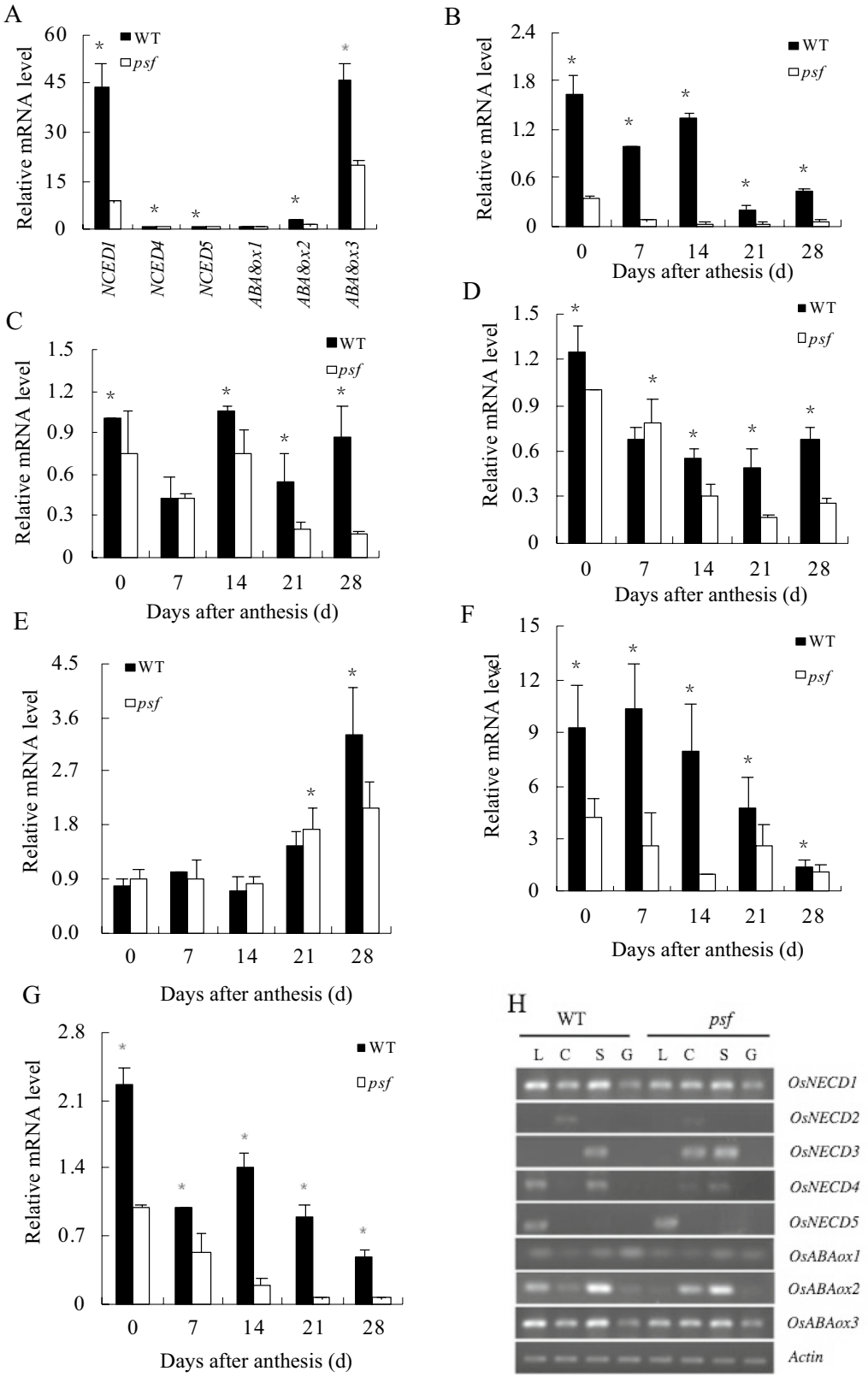

A
Mannitol
Sucrose
0mM
40mM
80mM
160mM
320mM
480mM
600mM
B
Chlorophyll content (mg g-1 FW)
Mannitol
Sucrose
Treatment concentration (mM)
C
ABA content (ng g-1 FW)
Mannitol
Sucrose
Treatment concentration (mM)
D
H2O2 content (μmol g-1 FW)
Mannitol
Sucrose
Treatment concentration (mM)
E
O2- generation rate (μmol min-1 mg-1 FW)
Mannitol
Sucrose
Treatment concentration (mM)
F
MDA (nmol g-1 FW)
Mannitol
Sucrose
Treatment concentration (mM)

◂**Fig. 3** Senescent symptoms, total chlorophyll content changes and its relation to the ABA content of detached leaf segments induced by a gradient concentration of exogenous sucrose and mannitol (0 mM, 40 mM, 80 mM, 160 mM 320 mM, 480 mM and 600 mM) after 6 days' incubation. Visual color change of detached leaf segments (**a**), total chlorophyll contents in detached leaf segments (**b**), ABA content in detached leaf (**c**), H_2O_2 content (**d**), O_2^- generation rate (**e**) and MDA content (**f**) at 6 days' incubation. Vertical bars represent standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between mannitol and sucrose of same treatment concentration

by NDGA were significantly higher than those in control samples (CK) (Fig. 7b–d), concomitantly with the markedly lower values of H_2O_2 and MDA after the incubating durations of 3 days' or 6 days' NDGA incubation (Fig. 7f, g). Just opposite to NDGA, DNCZ significantly decreased the contents of chlorophyll, soluble sugar, and sucrose content, but it significantly increased the H_2O_2 and MDA concentrations in detached leaves, thereby the accelerated senescence symptom being observed for DNCZ incubation (Fig. 7). In addition, DNCZ inhibited the *OsNCEDs* transcripts, with only slight change in *OsABA8ox3* for NDGA treatment under darkness, while DNCZ incubation severely repressed the *OsABA8ox3* transcript in detached leaves under darkness (Fig. 8). These results further confirmed that ABA is one of mediator of sugar starvation induced-leaf senescence, in which the triggering effect of sugar starvation on leaf senescence was closely associated with the disturbance of ABA metabolism and varying ABA concentration and ROS generation in senescent leaves.

Discussion

Sucrose is the key carbon source for growth, development and stressful defense in higher plants, it also acts as the main end-product of photosynthesis used for the translocation from the source to sinks through the sieve element/companion cell complex of the phloem (Fan et al. 2010; Wingler et al. 2005). At the cellular level, sucrose metabolism is tightly coupled with sugar signaling by exerting the generation of sugar signaling molecules such as sucrose itself, glucose, fructose, and trehalose-6-phosphate (T6P) (Wingler et al. 2005; Wingler and Roitsch 2008). Previous studies have disclosed that sugar signaling modulates plant development and stress response either directly or through interactions with other signaling pathways, including hormone- and redox-mediated processes (Chen et al. 2015). Over the past few decades, several sugar-signaling pathways have been found to be involved in the regulation of leaf senescence (Ruan et al. 2009). However, it is still debated whether or not the endogenous ABA participates in the regulation of sugar starvation-induced leaf senescence. For the association of ABA with sugar level in plant growth and development, Morita-Yamamuro et al. (2004) revealed that sugar starvation promoted endogenous ABA level and ABA sensitivity in *Arabidopsis*. On the other hand, ABA is an inducer of ROS generation leading to stomatal closure under water deficiency, and the occurrence of drought-induced stomatal closure was poorly associated with the changes of sugar concentration in leaf tissues (Brouwer et al. 2012). A study on sunflower indicated that the decrease in leaf sugar concentration by shading treatment (36% of full light intensity) was not necessarily accompanied by the increase in ABA concentration in leaves (Ono et al. 2001). In *Arabidopsis*, sugar-insensitive mutants sis4 and sis5 are defective in ABA synthesis, with the retarded leaf senescence phenotype and significantly lower ABA concentration in sugar-insensitive mutant leaves relative to its wild type (Laby et al. 2000; Song et al. 2016), and the deficit mutations of ABA synthesis (aba mutant) or signaling (abi mutant) suppressed ABA accumulation in leaves and their seedling development was insensitive to externally supplied sucrose. (Kukavica and Jovanovic, 2004; Liebsch and Keech 2016). In this paper, the *psf* mutant showed strikingly lower concentrations of soluble sugar, sucrose and hexose in senescent leaves than its wild type, while the opposite changes was observed for genotype-dependent alteration in the temporal patterns of ABA and ROS concentrations during leaf senescence (Fig. 1). Darkness treatment significantly enhanced the endogenous ABA concentrations in detached leaf segments, concurrently with the significant lowered sugar concentration and markedly increased ROS accumulation at 3–6 days' dark treatment (Fig. 2). Notably, the induction of sugar starvation to leaf senescence under darkness was effectively alleviated by NDGA (an inhibitor of ABA biosynthesis), while the aggravated senescent symptom of sugar starvation-induced leaf senescence under darkness was observed for DNCZ (an inhibitor of ABA degradation) incubation (Fig. 7). Our present investigation on the *psf* mutant and its wild type indicated the presence of a link between ABA and sugar in regulating leaf senescence, and these results of exogenous incubation experiment in detached leaves can provide the reliable evidence that the induction of sugar starvation to leaf senescence is effectively disturbed by the modifying change in ABA-induced ROS generation in rice leaves. Our results give a strong support for the hypothesis that ABA may function as a bridge between sugar starvation and leaf senescence (Pourtau et al. 2004; Song et al. 2016). Nevertheless, leaf senescence was finely regulated by a complex signaling networks (Brouwer et al. 2012; Han et al. 2018), and the interaction between sugar signaling and plant hormones for the regulation of plant growth and leaf senescence also associated with root nitrogen assimilation, leaf nitrogen status and photosynthetic efficiency, grain filling capacity, and translocation/remobilization of nutrition substances from


A
Relative mRNA level
WT
psf
NCED1
NCED4
NCED5
ABA8ox1
ABA8ox2
ABA8ox3
B
Relative mRNA level
Days after athesis (d)
C
Relative mRNA level
Days after anthesis (d)
D
Relative mRNA level
Days after anthesis (d)
E
Relative mRNA level
Days after anthesis (d)
F
Relative mRNA level
Days after anthesis (d)
G
Relative mRNA level
Days after anthesis (d)
H
WT
psf
L C S G L C S G
OsNECD1
OsNECD2
OsNECD3
OsNECD4
OsNECD5
OsABAox1
OsABAox2
OsABAox3
Actin

◂**Fig. 4** Genotypic difference in the mRNA transcript levels of several key genes involved in ABA synthesis and catabolism and their temporal patterns in the flag leaves of the *psf* mutant and its wild type after anthesis. Comparison of *OsNCEDs* and *OsABA8oxs* isoform genes expressions at 0 day after anthesis (**a**). Relative mRNA level of *OsNCED1* (**b**), *OsNCED4* (**c**), *OsNCED5* (**d**), *OsABA8ox1* (**e**), *OsABA8ox2* (**f**) and *OsABA8ox3* (**g**), spatial pattern of *OsNCEDs* and *OsABA8oxs* isoform genes expressions in leaves (L), culm (C), sheaths ((S) and grains (G) in Fig. 4-H. Vertical bars represent standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between the *psf* mutant and its wild type taken at the same time interval

source to sink for the situation of the whole-plant (Wingler et al. 2009; Asad et al. 2019).The orderly changes in physiology and biochemistry during leaf senescence were accompanied by different expression of thousands of senescence-associated genes (*SAG*) (Bolouri-Moghaddam et al. 2010; Lv et al. 2020). The partial source removal might induce a series of physiological responses and thus caused a burst of ROS and membrane oxidation, which in turn, resulted in premature senility of wheat plants (Srivalli and Khanna-Chopra 2009). Therefore, further study should be required to investigate the coordination of sugar starvation with ABA-induced ROS generation in regulating leaf senescence under the backgrounding of source-sink communication by regulatory network analysis.

General agreements indicate that environment adversity affected ABA concentration ABA and its metabolic response by breaking/disturbing the homeostasis between ABA biosynthesis and degradation in plant tissues (Ikegami et al. 2009; Nambara and Marion-Poll 2005; Qiu et al. 2017). The studies on tobacco and tomato revealed that the increased ABA accumulation in leaves under low light adversity was dominantly caused by the impairment of ABA degradation, while ABA biosynthesis was unaffected by weaker light-intensity (Nambara and Marion-Poll 2005; Qiu et al. 2017). In rice, chilling stress induced the up-regulations of *OsABA8ox2* and *OsABA8ox3*, but it had little impact on the expressions of OsABA8ox1 (Mega et al. 2015). Nitrogen deficiency caused a marked increase in the endogenous ABA concentration of rice leaves both by activating the expression of these genes related ABA biosynthetic genes and by suppressing the expression of genes related to ABA degradation (Zakari et al. 2020). Results of our present study on detached leaf segments indicated that the inhibitory effect of exogenous sucrose on the ABA concentration and ROS level in detached segments was clearly detectable in a dose dependent manner, and exogenous application of sucrose at higher concentration (> 160 mM) evidently retarded leaf senescence symptom, concomitantly with the markedly repressed ABA concentration and MDA accumulation in detached leaves (Fig. 3). This result was well consistent with several previous reports on *Arabidopsis*, wheat and barley, which concluded that exogenous sucrose supply decreased ROS generation and delayed leaf senescence (Fujiki et al. 2001; Hoeberichts et al. 2007; Xiao et al. 2010). Interestingly, our present investigation further revealed that the *psf* mutant differed evidently from its wild type in the expression of various genes involving in ABA metabolism and their temporal pattern during leaf senescence, in which *OsNCED1* and *OsABA8ox3* were among the important isoforms associated closely with the ABA concentration in rice leaves, and the down-regulation of *OsNCED1* and *OsABA8ox3* transcripts was strongly responsible for the genotype-dependent difference in endogenous ABA concentration between *psf* mutant and its wild type (Fig. 4). Furthermore, sugar starvation under darkness severely suppressed the transcriptional expression of both *OsNCED1* and *OsABA8ox3* in detached leaf segments, in addition to sugar starvation induced enhancements in endogenous ABA concentration and ROS generation (Fig. 5), while the opposite change was detected for the effect of exogenous sucrose incubation on the expression of *OsNCED1* and *OsABA8ox3* as well as endogenous ABA concentration and ROS generation in detached leaf segments (Fig. 6). These results, in conjunction with previous reports on ABA metabolism pathway and catalyzing function of NCED and ABA8 enzymes in the maintenance and/or disturbance of ABA homeostasis (Nambara and Marion-Poll 2005; Saika et al. 2007; Mega et al. 2015), suggested that the striking elevation in endogenous ABA concentration in the senescent leaves of *psf* mutant was more attributable to the suppression of ABA degradation, rather than the activation of ABA biosynthesis during leaf senescence. Correspondingly, the increase in ABA concentration under darkness-induced and the decline in ABA concentration under externally supplied sucrose incubation was dominantly controlled by disturbing ABA catabolic metabolism, while the effect of sugar starvation on ABA biosynthetic metabolism plays minor role in endogenous ABA concentration in senescent leaves. Hence, sugar starvation inducible increase in ABA concentration in senescing leaves was caused predominantly by the suppression of ABA catabolism, rather than the activation of ABA biosynthesis (Fig. 9). In previous studies, it was reported that high temperature above optimal growth notably enhanced the ABA concentration in the imbibed seeds and resulted in the thermo-inhibition of *Arabidopsis* seed germination by activating ABA biosynthesis (Toh et al. 2008). A study on rice revealed that external sugar incubation evidently suppressed *OsABA8ox3* expression and impaired ABA catabolism in the imbibed seeds, this occurrence in turn caused the rising elevation in endogenous ABA concentration and seed germination retardation, while the expression of five *OsNCED*s in the imbibed seeds was insensitive to exogenously supplied glucose, suggesting that sugar-induced delay of seed germination in rice is mediated by the suppression of ABA catabolism rather than an enhancement of ABA

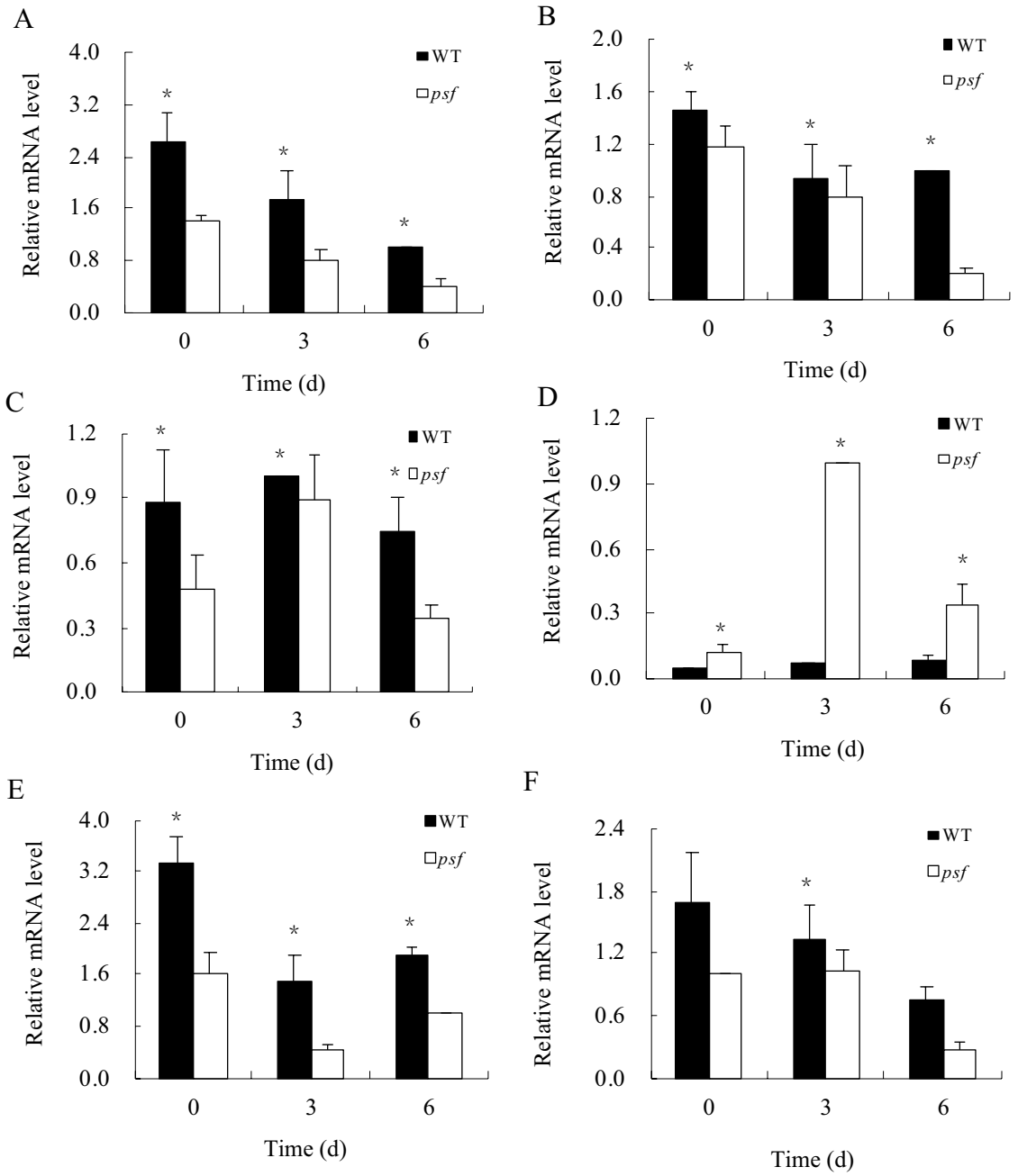


Fig. 5 Effects of dark treatment (sugar starvation treatment) on the transcript levels of key genes involved in ABA synthesis and catabolism in leaf segments of the *psf* mutant and its wild type after 0, 3 and 6 days' incubation. Relative mRNA level of *OsNCED1* (**a**), *OsNCED4* (**b**), *OsNCED5* (**c**), *OsABA8ox1* (**d**), OsABA8ox2 (**e**) and *OsABA8ox3* (F). Vertical bars represent standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between the *psf* mutant and its wild type taken at the same time interval

biosynthesis (Zhu et al. 2009). Our present results actually supported the findings of Zhu et al. (2009), and further comfirmed that the importance of ABA catabolic metabolism in regulating the endogenous ABA concentration in senescent leaves. However, our present result indicated that external sugar incubation caused the up-regulation of *OsNCED*s in senescent leaves, while sugar starvation-induced increase in endogenous ABA concentration was negatively correlated with the down-regulation of *OsNCED*s (Fig. 5 and Fig. 6). The reason for this phenomenon was possibly caused by

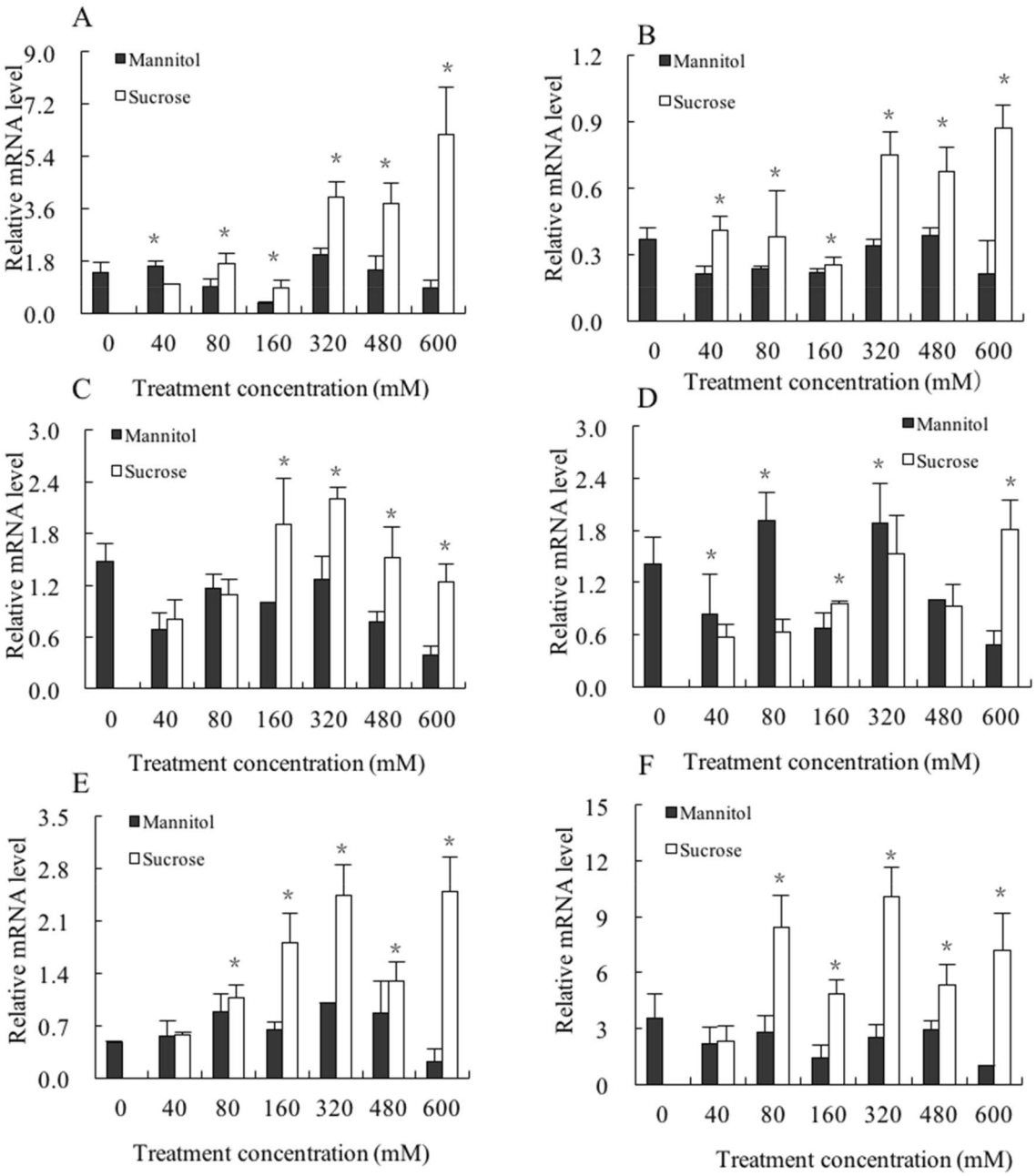


Fig. 6 Effect of exogenous gradient concentrations of sucrose treatment on the transcript levels of key genes involved in ABA synthesis and catabolism in the *psf* mutant and its wild type leaf segments. *OsNCED1* (**a**), *OsNCED4* (**b**), *OsNCED5* (**c**), *OsABA8ox1* (**d**), *OsABA8ox2* (**e**) and *OsABA8ox3* (**f**). Vertical bars represent standard errors (n = 3). The asterisks represent significant differences according to LSD ($P < 0.05$) between same treatment concentration of sucrose and mannitol

the different response to exogenous sugar incubation and its gradient concentration between the germinating seeds and senescent leaf tissues. Further investigation should be necessary to clarify the relationship of sugar starvation induced leaf senescence with *OsABA8ox3* expression and ROS generation in different cell compartments.

Conclusion

The onset and subsequent progression of leaf senescence was closely associated with the significant increase in ABA accumulation and rapid decline in sugar concentration in leaf tissues. ABA participants in the induction of sugar

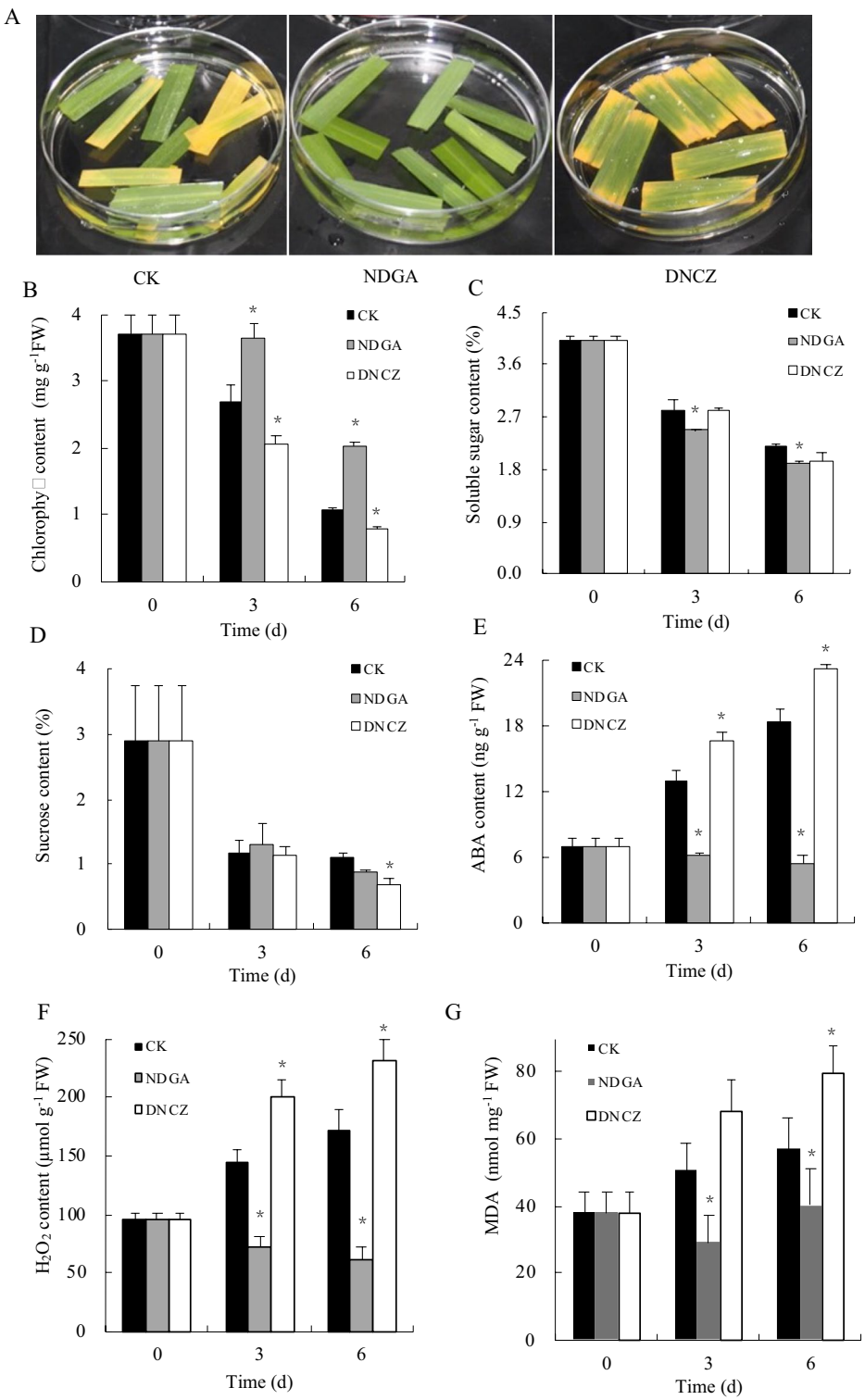


Fig. 7 Senescent symptoms, total chlorophyll content, soluble sugar content, sucrose content changes and its relation to the ABA content of detached leaf segments of *psf* mutant induced by sugar starvation, exogenous NDGA and DNCZ after 0, 3 and 6 days' incubation. Visual color change of detached leaf segments after 6 days' incubation (**a**), chlorophyll contents (**b**), soluble sugar contents (**c**), sucrose contents (**d**), ABA contents (**e**), H_2O_2 content (**f**) and MDA content (**g**). Vertical bars represent standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between different treatments (CK/NDGA and CK/DNCZ) taken at the same time interval

starvation to leaf senescence by activating ROS generation in leaf tissues, while exogenous sugar incubation severely suppressed the ABA concentration and evidently delayed leaf senescence. The induction of sugar starvation to leaf senescence could be effectively alleviated by the inhibitor of ABA biosynthesis (NDGA), while the opposite effect was shown for the inhibition of ABA catabolism by DNCZ. Hence, ABA may acts as a bridge between rapidly rising

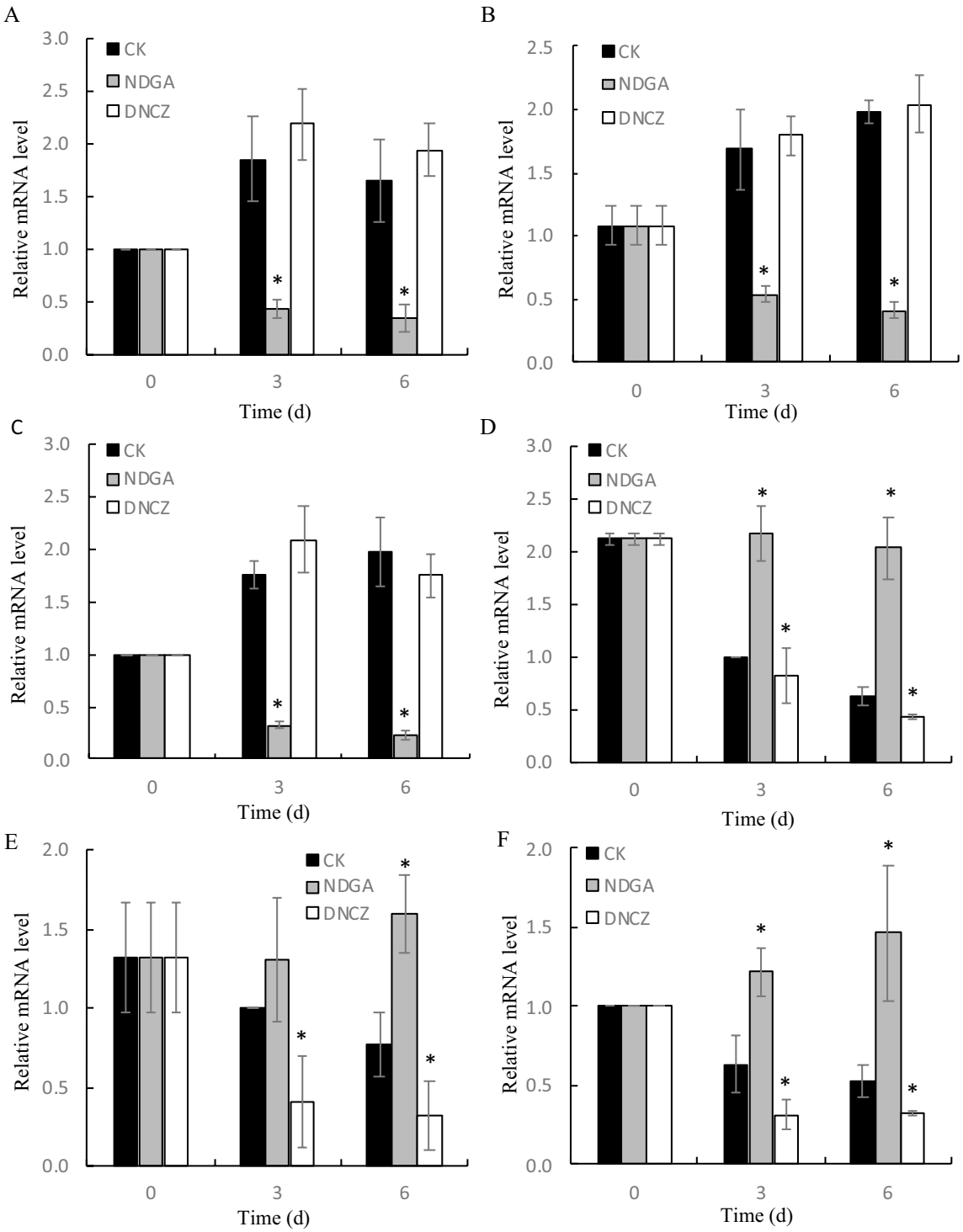


Fig. 8 Effect of sugar starvation with exogenous NDGA, DNCZ treatment on the transcript levels of key genes involved in ABA synthesis and catabolism genes after 0, 3 and 6 days' incubation. *OsNCED1* (**a**), *OsNCED4* (**b**), *OsNCED5* (**c**), *OsABA8ox1* (**d**), *OsABA8ox2* (**e**) and *OsABA8ox3* (**f**). Vertical bars represent standard errors (n = 3). The asterisks represent significant differences ($P < 0.05$) according to LSD between the different treatments (CK/NDGA and CK/DNCZ) taken at the same time interval

ROS generation and sharply varying sugar concentration for sugar starvation-induced leaf senescence. Among various

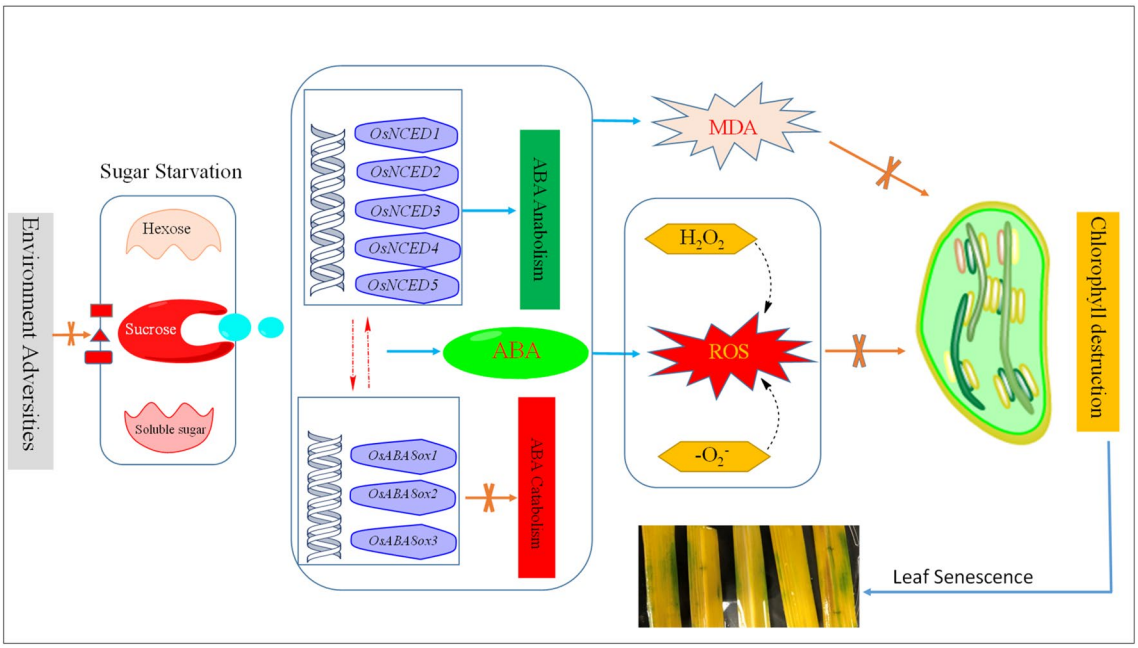


Fig. 9 The mechanism of sugar starvation induced leaf senescence. Sugar starvation increase ABA accumulation by inducing ABA anabolism and suppressing ABA catabolism. This increased ABA along with increase ROS and MDA contents decrease chlorophyll contents and induce chlorophyll destruction. →indicate activation and ⊣indicate suppression

genes involving in ABA biosynthesis and degradation, *OsNCED1* and *OsABA8ox3* were the important isoforms associated closely with the ABA concentration in rice leaves. Sugar starvation inducible increase in ABA concentration in senescing leaves was caused predominantly by the suppression of ABA catabolism, rather than the activation of ABA biosynthesis.

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Author contributions Conceived and designed the experiments: MAUA, FW and FC. Performed the experiments: MAUA FW, ZH, XG and LZ. Analyzed the data: MAU, FW, YY and ZH. Wrote the paper: MAU, FC, PG and WF. All of the authors reviewed the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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