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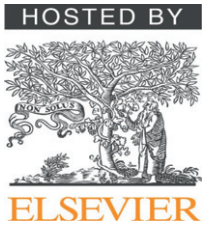

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Physiological and molecular studies of staygreen caused by pod removal and seed injury in soybean

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ABSTRACT

Leaves provide substances and signals for pod and seed development in soybean. However, the regulatory feedbacks of pod and seed to leaf development remain unclear. We investigated the effects of pod and seed on leaf senescence by conducting pod removal and seed injury experiments. Pod removal and seed injury delayed leaf senescence and caused the staygreen phenotype of leaves. There were dosage effects of pod number on the extent of staygreen in depodded plants. The concentrations of chlorophyll (SPAD value, an index of relative chlorophyll content), soluble protein, and soluble sugar in the leaves of depodded plants were higher than those of intact plants. During seed development, the content of IAA decreased, while that of ABA increased. This trend was more pronounced in intact than in depodded and seed-injured plants. The GA_3/ABA ratio decreased gradually in all treatments. The content of GA_3 was relatively stable and was higher in intact than in depodded plants. The expression levels of four senescence-related genes, *GmSARK*, *GmSGR1*, *GmCYN1*, and *GmNAC*, declined in depodded or seed-injured treatments and were positively correlated with the number of leaves retained on plants. *GmFT2a*, the major flowering-promoting gene, was expressed at a higher level while *E1*, a key flowering inhibitory gene, was expressed at a lower level in depodded than in intact plants. We propose that the pod or seed can regulate leaf development. When the seed is aborted owing to disease infection or pest attack, the leaves stay green because of the absence of the seed signals for senescence.

1. Introduction

The leaf not only plays a major role in photosynthesis but also is an organ perceiving environmental signals during plant development. For soybean, dry matter derived from leaf photosynthesis constitutes over 90% of overall dry matter accumulation and is considered a determinant of yield [1]. The role of the leaf in signal reception comprises the measurement

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of day-length changes and production of florigen that is transferred to the shoot apical meristem, resulting in adaptive changes in plant growth and development [2]. In soybean, a typical short-day (SD) plant, SD promotes and long day (LD) inhibits flowering and maturation [3,4]. For some photoperiod-sensitive varieties of soybean, continuous SD is required for the maintenance of post-flowering reproductive status; plants can revert to vegetative from reproductive growth if moved from SD to LD. New branches and leaves in the reverted plants stay green without SD conditions [5]. The floral stimuli are transmissible in soybean, and a late-maturing scion could be induced to flower when grafted onto an early-maturing stock with enough leaves [6].

The leaf produces photosynthates and exports them to seeds during the seed-filling period of crops. However, in the last stage of leaf development and seed filling, the function of leaves weakens, accompanied by the degradation of chlorophyll, protein, and nucleic acids and the remobilization and transportation of nutrients to sink organs [7]. Leaf senescence is controlled by an intricate genetic network that is programmed and regulated by growth stage and internal and external stimuli [7]. Several genes in metabolic and signaling pathways are involved in the senescence process [8–12]. Among them, *GmSARK* plays specific roles in senescence-inducing hormonal pathways [8,9], *SGR1* and *CYN1* are crucial in chlorophyll degradation, and *NAC* is a transcription factor for ABA synthesis [10–12].

Abnormal senescence, including premature death and staygreen, is caused by both genetic variation and environmental factors [13–15]. Staygreen is an abnormal crop developmental phenotype involving delayed leaf senescence [13]. Disruption of chloroplast degradation and related metabolic pathways leads to staygreen leaves [13,16,17]. In recent years, a soybean staygreen syndrome called “Zhengqing”, characterized by senescence-delayed leaves, aborted pods, and dead seeds has become a widespread problem in the Yellow-Huai-Hai river valley of China and has caused large soybean yield losses [18]. Compared with the staygreen phenomena in other crops, “Zhengqing” in soybean is a special type of staygreen caused by disease or insect attack.

Interactions between multiple organs are also involved in leaf senescence. Wittenbach [19] proposed that pod removal might exert an important influence on leaf senescence progress in soybean. Some physiological parameters in soybean were influenced by pod removal [20,21], although molecular changes in leaves caused by seed regulation have remained rarely reported. In the present study, we followed the leaf development process and measured the physiological parameters and expression of senescence-related and flowering-timing genes in response to pod removal and seed injury treatments. Our aim was to evaluate the effects of pod/seed status on leaf development, to characterize the relationship between source and sink in soybean, and to identify the cause of “Zhengqing” outbreaks.

2. Materials and methods

2.1. Plant materials

Zhonghuang 30, a mid-maturing (maturity group III) variety of soybean [*Glycine max* (L.) Merr.], was used in a two-year pot experiment conducted in 2014 and 2015 at the Institute of Crop Science, the Chinese Academy of Agricultural Sciences, Beijing, China (39°54′ N, 116°46′ E). Seeds were sown on June 28, 2014 and July 1, 2015 in plastic pots of 26 cm height × 30 cm diameter at the top and 22 cm at the bottom. Each pot contained 4 kg of soil (turf:loam:vermiculite 4:2:1, v/v/v). Seeds were thinned to five healthy plants in each pot at V2 (the second-node stage) [22]. Plants were placed outdoors and were irrigated as needed to avoid water stress. Other environmental conditions were controlled at the optimum level to minimize environmental effects on the results.

2.2. Experiment design

The experiment was arranged in a randomized complete block design with three replications. At the R4 (full pod) [22] stage, the pots were randomly divided into five groups for five treatments. In treatment 1, 0 pods were retained (0-pod) in each plant (all pods were removed) after R4; in treatments 2 and 3, 10 (10-pod) and 20 pods (20-pod), respectively, were retained in each plant after R4. Pod removal was performed by excision of the pods at the carpopodium with scissors, with the remaining pods evenly distributed on 10 nodes of the main stem. In treatment 4, all (approximately 30) pods were retained, but the seeds were destroyed by puncturing with a syringe in the pod cavity. Intact (fully podded) plants (treatment 5) were used as controls. After R4, plants were checked and continuously depodded (treatments 1–3) or new pods were punctured (treatment 4) every other day to meet the designed pod numbers or conditions.

2.3. Measurement of physiological parameters

Trifoliate leaves at the seventh node (from bottom) on the main stem were sampled at intervals of 5 days for analysis of physiological parameters and of expression of senescence-related genes (Table 1). The leaves on the same node of the 0-pod and intact plants were sampled daily in the first week after R4 for expression analysis of the flowering genes *GmFT2a* [23,24] and *E1* [25]. Samples were taken from each treatment and replication, frozen in liquid nitrogen, and stored at −80 °C until processing. Each sample was extracted separately and measured three times.

2.3.1. Chlorophyll concentration

SPAD value, an index of relative chlorophyll content, was measured with a SPAD-502 chlorophyll meter (Konica Minolta Inc., Tokyo, Japan), as described by Li et al. [26].

2.3.2. Soluble sugar and protein

The soluble protein content of leaves was measured using Coomassie Brilliant Blue G250 [27] and soluble sugar content by anthrone colorimetry [27].

2.3.3. Plant hormone content

IAA, GA_3, and ABA contents were measured by Huakong Center, College of Agronomy and Biotechnology, China Agricultural University, using enzyme-linked immunosorbent assay (ELISA) methods [28].