RESEARCH PAPER

Shading of the mother plant during seed development promotes subsequent seed germination in soybean

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Abstract

The effect of shading during seed development on subsequent germination remains largely unknown. In this study, two soybean (Glycine max) seed production systems, monocropping (MC) and maize–soybean intercropping (IC), were employed to examine the effects of shading of the mother plant on subsequent seed germination. Compared to the MC soybean seeds, which received light, the developing IC seeds were exposed to shade resulting from the taller neighboring maize plants. The IC seeds germinated faster than the MC seeds, although there was no significant difference in the thickness of the seed coat. The concentration of soluble proanthocyanidin in the IC seed coat was significantly lower than that in the MC seed coat. Changes in the concentrations of several types of fatty acids in IC seeds were also observed, the nature of which were consistent with the effect on germination. The expression levels of genes involved in abscisic acid (ABA) biosynthesis were down-regulated in IC seeds, while the transcription levels of the genes related to gibberellin (GA) biosynthesis were up-regulated. This was consistently reflected in decreased ABA concentrations and increased active GA₄ concentrations in IC seeds, resulting in an increased GA₄/ABA ratio. Our results thus indicated that shading of the mother plant during seed development in soybean promoted subsequent germination by mediating the biosynthesis of proanthocyanidins, fatty acids, and phytohormones.

Keywords: Glycine max, parental environment, phytohormone, proanthocyanidins, seed germination, shade, soybean.

Introduction

Seed germination is one of the most important stages of the life-cycle of an angiosperm. In agricultural production systems, the timely and uniform processes of germination and seedling emergence are key determinants of crop yield (Ashraf and Foolad, 2005; Chauhan and Opeña, 2012). Consequently, it is essential to understand the molecular mechanisms that regulate seed germination, and this field of research has attracted the attention of investigators from various plant disciplines,
including ecologists, geneticists, physiologists, molecular biologists, and plant breeders.

Both endogenous signals and exogenous environmental cues precisely regulate seed germination, and the underlying molecular mechanisms have been studied both extensively and intensively (Bentsink and Koornneef, 2008; Graeber et al., 2012; Shu et al., 2013, 2016b). The phytohormones abscisic acid (ABA) and gibberellins (GAs) have been found to be the most important factors, and the stimulatory effect of GAs and the inhibitory effect of ABA on seed germination are well documented (Finkelstein et al., 2008; Shu et al., 2013, 2016b; Vaistij et al., 2013). Enhanced GA signaling or elevated GA concentrations can accelerate seed germination, resulting from the effect of increased secretion of hydrolytic enzymes on a weakened testa structure (Seo et al., 2006; Holdsworth et al., 2008). In contrast, ABA delays germination, so that seeds of various mutants exhibiting altered ABA biosynthesis or signaling, such as abi3, abi4, and abi5, show changes in germinability compared to the wild-type (Piskuriewicz et al., 2008; Kanai et al., 2010; Frey et al., 2012; Shu et al., 2013). ABA acts through the PYR/PYL/R/CAR–PP2C–SnRKs signaling pathway (Cutler et al., 2010), and key factors in this pathway regulate germination (Née et al., 2017; Nishimura et al., 2018).

Seed germination is defined by the emergence of the radicle (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006), which is driven by the energy stored in the seed itself (Eastmond, 2004, 2006; Chen et al., 2016). During the imbibition of oil-containing seeds, the hydrolysis of triacylglycerol releases glycerol and fatty acids, and the conversion of the latter to sugars then fuels germination (Eastmond, 2004, 2006; Quettier and Eastmond, 2009; Theodoulou and Eastmond, 2012). The association between the seed fatty acid concentration and germination ability has been examined. In soybean (Glycine max), a negative correlation between the oleic acid concentration and germination vigor has been reported (Bachleda et al., 2017), while in sweet pepper (Capsicum annuum), the linoleic acid concentration is positively correlated with germination vigor, with higher concentrations of palmitoleic acid leading to poorer seed germination (Kaymak, 2014). Changes in the concentrations of several fatty acids and sugars during soybean seed germination have also been documented (Zhou et al., 2019).

In addition to the roles of phytohormones and fatty acids described above, polyphenolic compounds such as anthocyanins and pro-anthocyanidins (PAs) are also involved in the control of seed germination (Jia et al., 2012b; Snykal et al., 2014; MacGregor et al., 2015; Shah et al., 2018; Zhao et al., 2019). These compounds play an indirect restrictive role during seed imbibition by hampering radicle protrusion, and are therefore important determinants of seed coat-regulated germination (Debeaujon et al., 2000). Arabidopsis TRANSPARENT TESTA 12 (TT12) encodes a protein with similarity to prokaryotic and eukaryotic multi-drug secondary transporters, and tt12 mutant seeds show reduced dormancy and significantly lower PA concentrations than the wild-type (Debeaujon et al., 2001). The tt12 mutation systematically leads to the complete disappearance of PAs from the seed coat, confirming the negative relationship between PA concentration and germination. In Chinese tallow tree, Sapindus sebiferum, high concentrations of PAs in the seed coat inhibits the germination processes, during which PAs interact with the metabolic pathways of ABA, GA, and reactive oxygen species (Shah et al., 2018, Preprint).

In Arabidopsis, PAs cause a delay in seed germination, and the underlying mechanism has been identified as the maintenance by PAs of high concentrations of ABA (Jia et al., 2012a). Further insights have come from studies of sheepgrass (Leymus chiniensis), in which the LcbHLH92 transcription factor acts as a negative regulator of anthocyanin and PA biosynthesis, and the overexpression of LcbHLH92 in Arabidopsis results in lower concentrations of PAs and higher germination ability (Zhao et al., 2019).

Most studies on seed germination have focused on the roles of endogenous or environmental cues specifically during imbibition (Kanai et al., 2010; Martínez-Andújar et al., 2011; Shu et al., 2013, 2016b; Barrero et al., 2014; Nonogaki et al., 2014; Née et al., 2017), and there have been relatively few studies into the effects of exposure of the parent plant to environmental cues on the germination of subsequent seeds (Kvaalen and Johnsen, 2008; Postma and Ågren, 2015). Further examination of the effects of the parental environmental on subsequent seed germination is therefore required.

Light regulates numerous physiological processes throughout the plant life-cycle. Red light promotes seed germination through repression of the transcription of ABA biosynthesis genes, while far-red light delays germination by inducing the expression of ABA biosynthesis genes (Barrero et al., 2014). The shade environment that results from canopy mixing among neighboring plants, especially under close planting or maize–soybean relay-intercropping systems (Yang et al., 2014, 2015; Liu et al., 2017b), markedly affects plant architecture, seed quantity, and quality (Casal, 2013; Yang et al., 2014, 2018; Huang et al., 2018), and is the result of a decreased red:far-red ratio and lower photosynthetic photon flux density. Under shade conditions, plants show a shade-avoidance syndrome, including excessive elongation of the hypocotyl, stem, and petiole, and early flowering (Casal, 2013), which enables them to grow and compete effectively with their neighbors (Casal, 2013; Huang et al., 2018). However, the effects of a parental shade environment during the reproductive stage on the germination of subsequent seeds is largely unknown.

Some previous studies have focused on the parental effects of native ecosystems or species on subsequent plant growth stages, including seed germination (Galloway, 2001; Kvaalen and Johnsen, 2008; Awan et al., 2018). The effects of shading that result from cultivation systems are less well studied and are worthy of examination. Our present study reported that shade during the seed development stage in soybean promotes subsequent germination by regulating the endogenous levels of PAs, fatty acids, and the phytohormones ABA and GAs. We studied seeds produced in two soybean production systems, namely monocropping (MC) and maize–soybean intercropping (IC). The control group seeds (MC) received light, while the IC seeds suffered from shade stress during seed development from the taller neighboring maize plants. The results showed that IC seeds germinated faster than MC seeds. Subsequent biochemical analysis revealed that the concentrations of soluble PAs and
several fatty acids in the IC seeds were altered, and these were consistent with the changes observed in germination. qPCR assays showed that the transcription levels of ABA biosynthesis genes were down-regulated in IC seeds, while the transcription levels of GA biosynthesis genes were up-regulated. Decreases in ABA and increases in active GA_{4} concentrations were consistently detected in IC seeds, so that the GA_{4}/ABA ratio was increased. Our results improve our understanding of the effects of shading of the parent plant during seed development on the germination of the subsequent seeds.

Materials and methods

Seed production

Using a maize–soybean (Zea mays–Glycine max) relay intercropping system previously developed by our group (Yang et al., 2014, 2015; Liu et al., 2017a), we obtained intercropped soybean seeds (IC) and monocropped seeds (MC). The seeds used in this study were produced and studied at two different locations in 2016–2017, namely Heze in Shandong Province, China (35°15′S, 115°25′E) and Chengdu in Sichuan Province (30°33′N, 103°38′E). Climatic data for these locations during the soybean growing seasons in 2016 to 2017 are shown in Supplementary Table S1 at line. The MC seeds that were subject to light during development acted as the control, while the IC seeds suffered shade stress resulting from the canopy of the taller neighboring maize plants during development. At the Shandong site, the soybean cultivar Qihuang 34 (QH-34) and the maize cultivar Fundan 20 (JD-20) were used, whilst at Sichuan, the soybean cultivars Nandou 12 (ND-12) and C-103, and the maize cultivar Zhenghong 505 (ZH-505) were used.

The maize–soybean intercropping and soybean monocropping patterns were established according to our previous protocol (Yang et al., 2014, 2015; Liu et al., 2017a). In detail, in the relay intercropping system there were two rows of maize alternated with two rows of soybean, with a row spacing of 0.4 m and a distance between plants within a row of 0.6 m. For the soybean monocropping, the row spacing was 0.5 m. The area of each experimental plot was 36 m^{2} (6×6 m). At noon at the Shandong site, the red to far-red (R/FR) ratio at the top of the soybean canopy in the intercrop was ~0.4 and the photosynthetic photon flux density (PPFD) was ~740 μmol m^{-2} s^{-1} (49% of that of the monoculture soybean plants). For the Sichuan site, the R/FR ratio was ~0.65 and the PPFD was ~1050 μmol m^{-2} s^{-1} (62% of that of the monoculture soybean plants). The length of the photoperiods and the day/night ratios during the growing seasons for the two sites are given in Supplementary Table S2. All the experiments consisted of a randomized complete block design with three replicates. In addition, we also grew soybean plants of the ND-12 variety in a greenhouse under artificial shade produced by green filters, as described previously (Sasidharan et al., 2006). Seeds were ground in liquid nitrogen and then freeze-dried. Various types of fatty acids were extracted from the soybean seeds at different stages of development according to our previously published protocol (Liu et al., 2016; Zhou et al., 2019). The development stages were characterized according to Fehr and Caviness (1977) and Han et al. (2006). Seeds were ground in liquid nitrogen and then freeze-dried. Powdered samples of 30 mg were placed in 10–ml centrifuge tubes and 3 ml of n-hexane was added. The samples were extracted ultrasonically (at 40 kHz) for 15 min and kept at room temperature for 3 h. The solution was centrifuged for 15 min at 13 200 g at 4 °C. Then, 3 ml of 0.4 M methanolic potassium hydroxide solution was added to the supernatant, the tubes were subjected to vortex oscillation for 30 s, and then kept at room temperature for 1 h. The upper liquid layer was transferred to a 5-ml capacity bottle, made up to full volume by addition of n-hexane, and then injected into a GCMS-QP2010 system (Shimadzu) through a 0.45-μm organic-phase filter.

The fatty acid concentrations of the samples were quantified by comparing the retention times and area spectra with a fatty acid methyl ester (FAME) standard mixture containing 37 compounds (Nu-Chek-Prep Inc., USA). This standard mixture included oleic, linoleic, palmitic, stearic, and α-linolenic acid. Three biological replications were performed. To determine the relationships among the levels of fatty acids in the different samples, heat maps were created using the Adobe Illustrator software as described previously (Liu et al., 2016; Zhou et al., 2019).

Measurement of sugars

Developing soybean seeds at different stages were sampled, heated at 105 °C for 30 min, and then dried at 80 °C until constant weight was obtained. The dried samples were ground, and 100 mg samples of powder were placed in 10–ml centrifuge tubes to which 4 ml of 80% (v/v) ethanol was then added. The tubes were placed in a water bath at 80 °C for 40 min and then centrifuged at 4500 g for 10 min. This extraction procedure was repeated three times, and the combined supernatants were then diluted to 50 ml with ethanol (80%). The contents of sucrose, fructose, total soluble sugars, and reducing sugars were then quantified as described previously (Cai et al., 2016; Shi et al., 2016; Zhou et al, 2019).

Measurement of thickness of seed coat

The thickness of the soybean seed coat was measured using a M165C stereomicroscope (Leica). At least 10 seeds per sample were used, and the seed coats were peeled off and each one was measured five times at different angles.

Measurement of pro-anthocyanidins in the seed coat

Soluble and insoluble PAs were extracted from the seed coats according to the protocol described by Senda et al. (2017). The seed coats were peeled off, ground in liquid nitrogen, and freeze-dried. Samples of 50 mg of the powder were extracted in the dark at 4 °C for 1 h in 1 ml 70% (v/v) acetonitrile aqueous solution containing 0.1% (w/v) acetic acid. The samples were centrifuged at 11 000 g for 10 min, and the extraction procedure was repeated three times (2×1 h, and once overnight). The residue was then used to quantify the level of insoluble PAs, whilst the combined supernatants were made up to 4 ml with the acetonitrile aqueous solution. The supernatant was mixed with 3 ml of diethyl ether at −20 °C, and then the lower phase was transferred to another centrifuge tube for determination of soluble PAs. The contents of soluble and insoluble PAs were determined using the method described previously by Senda et al. (2017).

Gene expression analysis

Quantitative PCR (qPCR) was performed as described previously (Shu et al., 2013; Zhou et al., 2019). Total RNA was extracted from dry and
imbibed soybean seeds (0–9 h after sowing). Then, 2-μg samples were treated with DNase I and reverse-transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (200 units per reaction; Promega). qPCR was performed using a QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific) and Vazyme™ AceQ qPCR SYBR Green Master mix. The expression level of genes involved in ABA/GA biosynthesis and signaling cascades was analysed. Gene expression was quantified at the logarithmic phase using the expression of the housekeeping GmTubulin gene as an internal control. Expression analysis of each gene was repeated three times. Primers sequence are given in Supplementary Table S3.

Quantification of ABA and GA in seeds

Quantification of ABA and GA were performed according to the methods described in our previous studies (Chen et al., 2011; Shu et al., 2013, 2016a).

For ABA, dry and imbibed (6 h after sowing, 400 mg) soybean seeds were ground in liquid nitrogen and extracted for 24 h in methanol containing D6-ABA (OIChemIm Co. Ltd.) as an internal standard. Purification was performed using an Oasis Max solid-phase extract cartridge (Waters) and eluted with 5% formic acid in methanol. The elution was then dried and reconstituted, and injected into a LC–tandem MS system consisting of an Acquity ultra-performance LC (Acquity UPLC; Waters) and a triple-quadrupole tandem MS (Quatro Premier XE; Waters). Three biological replications were performed.

For GA, dry and imbibed (6 h after sowing, 400 mg) soybean seeds were ground in liquid nitrogen and extracted with 80% (v/v) methanol. GA d2 isotope standards were added to the samples before grinding. The crude extracts were purified by reversed-phase solid-phase extraction, ethyl-ether extraction, and derivatization. The resulting mixture was injected into a capillary electrophoresis-MS system (Agilent Technologies) for quantitative analysis. Three biological replications were performed.

Statistical analysis

The data were analysed using Student’s t-test. Time-to-event analysis of seed germination data was performed using the R function lifetab() in the KMsurv package, according to the method described by McNair et al. (2012).

Results

Ecological characterization of inter- and monocropping seeds

To examine the effects of the shade environment of the parental plant on the germination of subsequent seeds, soybean plants were grown under two different production systems, namely monocropping (MC, control treatment) and intercropping (IC) with maize. As already demonstrated by our previous studies (Yang et al., 2014, 2015; Liu et al., 2017a), the IC soybean plant canopy suffered from shade stress imposed by the taller neighboring maize plants during seed development, in contrast to the MC plants that received the light (Fig. 1A). The R/FR ratio and the PPFD received by the IC soybean plants were decreased significantly compared to the MC plants (Yang et al., 2014, 2015; Liu et al., 2017a). There were no obvious morphological differences between the IC and MC soybean seeds (Fig. 1B), which were comparable with regard to their 100-seed weight, length, width, and the ratio of length to width (Supplementary Fig. S1).

Intercropping seeds germinate faster than monocropping seeds

Seed germination assays conducted on the different soybean cultivars at the two experimental sites demonstrated that the IC seeds germinated faster than the MC seeds (Figs 2, 3, Supplementary Figs S2, S3). For the QH-34 cultivar, the seeds of which were produced in Shandong Province, the germinability of IC seeds was clearly higher than that of MC seeds (Fig. 2A). For example, at 18 h after sowing the percentage germination of IC soybean seeds was nearly 60% whilst that of MC seeds was only 30%. The faster germination of IC seeds was apparent throughout the germination processes. We performed similar assays with seeds from two other cultivars, ND-12 and C103, which come from different genetic backgrounds to QH-34, (B) Representative images of seeds harvested from MC and IC plants. The cultivars are indicated at the top. Scale bars are 5 mm. (This figure is available in colour at JXB online.)
The early visible stages of germination involve several processes, including rupture of the seed coat and protrusion of the radicle. We therefore examined germination under a microscope and found that rupture and radicle protrusion occurred earlier in IC seeds than in MC seeds (Fig. 3A). In contrast to MC seeds, at 15 h after sowing the IC seeds showed obvious rupture of the seed coat, and at 18 h the radicle of IC seeds was markedly longer than that of MC seeds.

We next examined the effects of maternal plant shade on post-germination seedling growth, using seeds of the ND-12 cultivar produced at the Sichuan Province site. The length of the radicle of germinated IC seeds was significantly longer than that of MC seeds (Fig. 3B), and there were also significant increases in the fresh and dry weights of the roots of the IC seeds compared to the MC seeds (Fig. 3C, D). Seeds of the QH-34 cultivar produced at the Shandong site were also examined and their germination characteristics showed trends similar to those of ND-12 (Supplementary Fig. S2).

In order to further confirm the seed germination phenotype of IC seeds caused by the maternal plant shade environment, we produced seeds from plants grown in a greenhouse in which shade was imposed artificially by the use of green filters. The results of the germination assays were consistent with those of seeds obtained under natural shading in the field (Fig. 2D), and the results of time-to-event analysis were also consistent (Fig. S3D). Taken together, these phenotypic analyses consistently demonstrated that maternal shading increased the germination of subsequent seeds.
Fig. 3. Development of soybean seeds from parent plants subjected to shading is faster than that of seeds from unshaded plants. (A) Representative images of seeds during the course of imbibition of the cultivar ND-12 from plants grown at the Sichuan experimental site with monocropping (MC) and with intercropping (IC). The protrusion of the radicle is indicated by arrows. The scale bar is 10 mm. (B) Length of the radicle, and (C) fresh and (D) dry weights of germinated seeds at 48 h after sowing. Data are means (±SE) of four replicates. Significant differences were determined using Student’s t-test: *P<0.05. (This figure is available in colour at JXB online.)

Fig. 4. Variation of contents of fatty acids during development of soybean seeds from parent plants grown under different shading environments. Data are from the cultivar ND-12 from plants grown at the Sichuan experimental site with monocropping (MC) and with intercropping (IC). The developmental stages are: R5, seed length ~3 mm; R6, green seed fills the pod cavity; R7, one normal pod reaches mature pod color; and R8, fully mature seed. A heatmap of fatty acid content is shown and the scale indicates the variation from low (L) to high (H) content. Fatty acids that are known to be associated with the control of seed germination are indicated (*). (This figure is available in colour at JXB online.)
were consistent across the three cultivars produced at the different locations and between natural and greenhouse conditions (Figs 2, 3, Supplementary Figs S2, S3), we selected one cultivar, ND-12, for further biochemical and gene transcription analysis.

**Variations in fatty acid and sugar concentrations between inter- and monocropping seeds**

Several previous studies have shown that during seed development in oilseed crops (such as soybean, rapeseed, and Arabidopsis), the sucrose produced by photosynthesis is converted to hexoses for the biosynthesis of oil (triacylglycerol, TAG), while fatty acids are important mediators that are necessary for subsequent germination and early seedling establishment (Eastmond, 2004, 2006; Quettier and Eastmond, 2009; Theodoulou and Eastmond, 2012; Xu and Shanklin, 2016). In order to determine the mechanisms underlying the faster germination phenotype of the IC seeds, we therefore examined the concentrations of several sugars and fatty acids between IC and MC seeds during their development in the ND-12 cultivar.

Examination of sugars suggested that there were no significant differences between the IC and MC seeds at most of the sampled time points, from stages R5 (seed length ~3 mm) to R8 (fully mature seed; Supplementary Fig. S4).

For fatty acids, GC-MS analysis revealed that, by the R6 stage of development (green seed fills the pod cavity), most of the concentrations had peaked in both the IC and MC of seeds (Fig. 4). From the R6 to the R8 stage, the concentrations decreased in both seed types but whilst it was gradual in the MC seeds, the decline was much steeper in IC seeds, especially at stage R7 (one normal pod reaches mature pod color). At stage R8, differences were observed between the IC and MC seeds in the concentrations of some fatty acids that are known to be involved in seed germination, namely oleic, linolenic, and linoleic acid, and this was consistent with the faster germination phenotype of the IC seeds. Thus, the concentrations of oleic and linolenic acid decreased in IC seeds compared to MC seeds, while the concentration of linoleic acid increased (Fig. 4). Overall, these results indicated that the shade environment of the parental plant influenced the concentrations of fatty acids during seed development, some of which are known to be associated with germination processes.

**Variations in pro-anthocyanidin concentrations in inter- and mono-cropping seed coats**

Previous studies have demonstrated that the thickness and composition of the seed coat play important roles during germination (Noodén et al., 1985; Smýkal et al., 2014; MacGregor et al., 2015; Shah et al., 2018, Preprint). We therefore examined the thickness of the seed coats of the IC and MC seeds of the ND-12 cultivar, but found that there was no significant difference between the two (Fig. 5A, B). We then examined the contents of (C) insoluble and (D) soluble PAs in the seed coats. Data are means (±SE) of four replicates. Significant differences were determined using Student’s *t*-test: *P*<0.05. (This figure is available in colour at JXB online.)
concentrations of pro-anthocyanidins (PAs) in the seed coats. Whilst the concentrations of insoluble PAs were not significantly different (Fig. 5C), the concentrations of soluble PAs in the IC seed coat were significantly lower than those in the MC seeds (Fig. 5D). According to previous studies, the Flowering Locus T (FT) protein inhibits the biosynthesis of PAs (Chen et al., 2014), and in soybean GmFT2a is a key regulator that mediates reproductive development (Liu et al., 2018). We therefore determined the expression level of GmFT2a, and found that its transcription in IC seeds was higher than that in MC seeds (Supplementary Fig. S6), which was consistent with the decrease in concentration of PAs (Fig. 5D). To further test the robustness of these findings, we also examined the QH-34 cultivar and found that the results were similar to those observed for ND-12 (Supplementary Fig. S5). Taken together, our results indicated that the shade environment of the parent plant had no effect on the thickness of the seed coat or its concentration of insoluble PAs, but shading did negatively affect the concentration of soluble PAs.

Parental shading increases GA biosynthesis but decreases ABA biosynthesis

Given that the phytohormones ABA and GAs play key roles in seed germination processes (Bewley, 1997; Shu et al., 2013, 2016b; Nishimura et al., 2018), our next step was to compare the transcription patterns of key genes involved in their biosynthesis/signaling pathways during germination sensu stricto. The results showed that the expression of the ABA biosynthesis gene GmABA2 was down-regulated in IC seeds during imbibition (Fig. 6A), while the transcription of the ABA inactivation gene GmCYP707A1 increased (Fig. 6B). The expression levels in IC seeds of GmRD29-A, GmABI4 and GmABI5, which encode positive regulators of the ABA signaling pathway, were significantly lower than those in MC seeds at most of the sampled time-points (Fig. 6C–E). qPCR assays showed that the expression levels of the GA biosynthesis genes GmGA3ox1, GmKAO, and GmGA3 were significantly higher during seed imbibition in IC seeds than in MC seeds (Fig. 6FH), while the expression of GmRGL1, a negative mediator in the GA signaling pathway, was down-regulated during imbibition in IC seeds compared to MC seeds (Fig. 6I). The results therefore indicated that parental shading negatively regulated ABA biosynthesis and positively mediated GA biosynthesis via transcriptional control of key genes, acting to promote germination in IC seeds significantly more than in MC seeds.

We next examined ABA and GA, during imbibition and found that the ABA concentration declined in both IC and MC seeds, but it was significantly lower in IC seeds (Fig. 7A). In contrast, the active GA, concentration increased in IC seeds during imbibition whilst remaining unchanged in MC seeds (Fig. 7). As a consequence, the GA,/ABA ratio in IC seeds was significantly higher than in IC seeds after 6 h imbibition (Fig. 7C). These results were consistent with the gene expression data (Fig. 6) and the seed germination phenotypes for the two seed types (Figs 2, 3, Supplementary Figs S2, S3).

**Discussion**

Our data for seed germination phenotypes, contents of fatty acids and soluble PAs, gene expression, and concentrations of ABA and GAs indicated that parental shading during seed development in soybean promoted the subsequent germination of seeds. We propose that the molecular and physiological mechanisms underlying this positive effect of shade are as follows. Parental shading results in a reduction of biosynthesis of soluble PAs and changes the concentrations of several fatty acids that are associated with seed germination control, namely
α-linolenic, oleic, and linoleic acid. In addition, increased biosynthesis of active GA4 and decreased biosynthesis of ABA during imbibition of seeds that develop under shade are also responsible for the faster germination. Our study therefore contributes to a better understanding of the effects of environmental cues during the seed development stage on the subsequent processes of germination.

Parental environmental cues regulate subsequent development stages in offspring

One of the most interesting topics within developmental biology is whether and how parental environment cues have transgenerational influences on development processes, epigenetic modifications, and molecular adaptations of the offspring. There have been numerous studies on this topic in the animal kingdom; for example, adverse or unfavorable prenatal environments can cause metabolic diseases in the offspring (Radford et al., 2014; Vogt et al., 2014; Bauer et al., 2016; Haire-Joshu and Tabak, 2016; Sweeney and MacBeth, 2016). There have also been some pioneering studies that have examined the effects of parental environmental signals on subsequent growth and development in plants.

In Norway spruce (Picea abies), the timing of bud set is regulated by the memory of temperature during zygotic and somatic embryogenesis (Kvaalen and Johnsen, 2008), with plants from warmer seed production areas being taller and setting buds later compared with plants from colder parental environments. Parental reproductive temperatures also regulate root growth in the offspring of Arabidopsis, as determined by Blödner et al. (2007) who found that seed progeny from a warm parental environment exhibit faster germination, faster root elongation, greater leaf biomass, and increased seed production at various temperatures compared with seed produced in a cold parental environment.

With regard to the control of seed germination, Chen et al. (2014) showed that the temperature environment of the parent in Arabidopsis is transduced into a signal through Flowering Locus T (FT) via the silique phloem, with FT mediating the degree of seed dormancy through inhibition of PA synthesis in the seeds, resulting in a change in the concentration of tannins in the seed coat. Another study in Brassica oleracea has shown that exposure to an environmental stress during the reproductive stage has a large impact on seed performance, including germination speed and resistance to controlled deterioration (Awan et al., 2018). Pioneering investigations such as these have suggested that parental environment cues can play key roles in the subsequent growth and development in the progeny.

Positive effects of parental shading on subsequent seed germination

Most studies of germination have focused on the roles of endogenous or environmental cues specifically during seed imbibition (Kanai et al., 2010; Martínez-Andújar et al., 2011; Shu et al., 2013, 2016b; Barrero et al., 2014; Nonogaki et al., 2014; Née et al., 2017). Although some reports have been published on the effects of parental environmental cues on the subsequent germination of the seeds generated (Kvaalen and Johnsen, 2008; He et al., 2014; Postma and Ågren, 2015; Awan et al., 2018), the detailed regulatory mechanisms remain largely unknown, especially the roles of exposure to diverse abiotic stress cues during the development stage on subsequent seed germination. This is in contrast to the more detailed knowledge that is available in the animal kingdom.

There is limited information available on the relationship between shading of the parent plant and the germination characteristics of the seed produced. Red light is known to promote germination while far-red light inhibits the red light-induced processes in some species (Bewley, 1997). Some studies have detected an inhibitory effect of shade on germination (Frankland and Taylorson, 1983; Mathews, 2006; Lee and Lopez-Molina, 2012), but they focused only on the
Shading in soybean promotes germination of subsequent seeds

Effects of shade during seed imbibition, rather than examining the effects of shade stress of the parent on subsequent seed germination. Early studies indicated that Arabidopsis seeds that had developed under a low ratio of FR to R light germinated faster than seeds grown under a high ratio (Mccullough and Shropshire, 1970; Hayes and Klein, 1974). It has been suggested that subsequent seed germination processes are regulated by the spectral quality to which the parent plant was exposed (Chahtane et al., 2017); however, the molecular mechanisms underlying this have remained elusive. In our present study we used seeds from three different soybean cultivars produced under different environmental conditions (at two different locations), as well as seeds produced in greenhouses, and found that the parental shade signal promoted subsequent germination processes (Figs 2, 3, Supplementary Figs S2, S3). Physiological and biochemical analyses showed that parental shading attenuated the biosynthesis of soluble PAs (Fig. 5) and resulted in changes in the concentrations of several fatty acids (Fig. 4), results that were consistent with the patterns of germination. The parental shade signal also down-regulated ABA biosynthesis while up-regulating GA biosynthesis in seeds produced by shaded parent plants, via effects on the transcription of specific genes (Figs 6, 7). Overall, our study demonstrated that shading of the parent during the reproductive stage increased the speed of soybean seed germination via mediation of the biosynthesis of soluble PAs, key fatty acids, and the phytohormones ABA and GA (Fig. 8).

Phytochromes sense R and FR light (Klose et al., 2015; Pham et al., 2018), and phyA and phyB are also involved in the regulation of seed germination through auxin and ABA-mediated pathways in Arabidopsis (Lee et al., 2012; Ibarra et al., 2013). Compared to the extensive studies of the molecular functions of phyA and phyB in model plants, there have been few studies of GmphyA and GmphyB in soybean. Some studies have focused on the genetic redundancy of soybean phytochromes (Liu et al., 2008; Wu et al., 2013), and have also shown that GmPHYAs are involved in post-flowering photo-period responses (Xu et al., 2013). Given the importance of photoreceptors in plant shade responses (Fraser et al., 2016), we speculate that in soybean, GmphyA and GmphyB might sense the change of ratio of R:FR under shaded environments during seed development and then mediate the subsequent seed germination processes through as yet unknown cascades. This is clearly worthy of future study.

In the field of animal research, it is known that undernourishment during the prenatal stage alters DNA methylation in the germline of the offspring, compromising the development of male offspring (Radford et al., 2014). Our current work provides a good case study for investigating cross-generational effects in plants induced by environmental cues. In ongoing research, we are examining the underlying genetic mechanisms using epigenetic approaches, including genomic DNA methylation and other molecular effects, and we hope that the precise mechanisms underlying the positive effect of parental shade signals on subsequent seed germination will be uncovered in the near future.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Analysis of agronomic traits of MC and IC seeds of the different cultivars.
Fig. S2. Length of radicle, and fresh and dry weights of MC and IC seeds during imbibition for the cultivar QH-34.
Fig. S3. Germination phenotypes of IC and MC seeds of the different cultivars as determined by life-table estimates.
Fig. S4. Effects of parental shading on sugars level during different stages of development of IC and MC seeds in the ND-12 cultivar.
Fig. S5. Seed coat thickness and content of PAs in IC and MC seeds in the cultivar QH-34.
Fig. S6. Expression of GmFT2a in MC and IC seeds in the ND-12 cultivar.
Table S1. Air temperature, rainfall, and duration of sunshine during the 2016–2017 soybean growing seasons at the two experimental locations.

Table S2. Data for daylength during the 2016–2017 soybean growing seasons at the two experimental locations.

Table S3. Sequences of primers used in this study.

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References

Ashraf M, Foolad MR. 2005. Pre-sowing seed treatment—a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Advances in Agronomy 88, 223–271.

Awan S, Footitt S, Finch-Savage WE. 2018. Interaction of maternal environment and allelic differences in seed vigour genes determines seed performance in Brassica oleracea. The Plant Journal 94, 1098–1108.

Bachleda N, Grey T, Li Z. 2017. Effects of high oleic acid soybean on seed yield, protein and oil contents, and seed germination revealed by near-isogenic lines. Plant Breeding 136, 539–547.

Barrero JM, Downie AB, Xu Q, Gubler F. 2014. A role for barley CRYPTOCHROME1 in light regulation of grain dormancy and germination. The Plant Cell 26, 1094–1104.

Bauer T, Trump S, Ishaque N, et al. 2016. Environment-induced epigenetic reprogramming in genomic regulatory elements in smoking mothers and their children. Molecular Systems Biology 12, 861.

Bentsink L, Koornneef M. 2008. Seed dormancy and germination. The Arabidopsis Book 6, e0119.

Bewley JD. 1997. Seed germination and dormancy. The Plant Cell 9, 1055–1066.

Blödner C, Goebel C, Feussner I, Gatz C, Polle A. 2007. Warm and cold parental reproductive environments affect seed properties, fitness, and cold responsiveness in Arabidopsis thaliana progeny. Plant, Cell & Environment 30, 165–175.

Cai Y, Shao L, Li X, Liu G, Chen S. 2016. Gibberellin stimulates regrowth after defoliation of sheepgrass (Leymus chinensis) by regulating expression of fructan-related genes. Journal of Plant Research 129, 935–944.

Casal JJ. 2013. Photoreceptor signaling networks in plant responses to shade. Annual Review of Plant Biology 64, 403–427.

Chaitane H, Kim W, Lopez-Molina L. 2017. Primary seed dormancy: a temporally multilayered riddle waiting to be unlocked. Journal of Experimental Botany 68, 857–869.

Chauhan BS, Opeña J. 2012. Effect of tillage systems and herbicides on weed emergence, weed growth, and grain yield in dry-seeded rice systems. Field Crops Research 137, 56–69.

Chen HH, Chu P, Zhou YL, Ding Y, Li Y, Liu J, Jiang LW, Huang SZ. 2016. Ecotoxic expression of NiPRT1, a Nelumbo nucifera 1-cysteine peroxidoxin/antioxidant, enhances seed longevity and stress tolerance in Arabidopsis. The Plant Journal 88, 608–619.

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA, Penfield S. 2014. Maternal temperature history activates flowering Locus T in fruits to control progyny dormancy according to time of year. Proceedings of the National Academy of Sciences, USA 111, 18787–18792.

Chen ML, Huang YQ, Liu JQ, Yuan BF, Feng YQ. 2011. Highly sensitive profiling assay of acid plant hormones using a novel mass probe by capillary electrophoresis-time of flight-mass spectrometry. Journal of Chromatography B 879, 938–944.

Cutter SR, Rodriguez PL, Finkelstein RR, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. Annual Review of Plant Biology 61, 651–679.

Debeaujon I, Léon-Kloosterziel KM, Koornneef M. 2000. Influence of the tests on seed dormancy, germination, and longevity in Arabidopsis. Plant Physiology 122, 403–414.

Debeaujon I, Peeters AJ, Léon-Kloosterziel KM, Koornneef M. 2001. The TRANSPARENT TESTA12 gene of Arabidopsis encodes a multidrug secondary transporter-like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium. The Plant Cell 13, 853–871.

Eastmond PJ. 2004. Glycerol-insensitive Arabidopsis mutants: gift seedlings lack glycerol kinase, accumulate glycerol and are more resistant to abiotic stress. The Plant Journal 37, 617–625.

Eastmond PJ. 2006. SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. The Plant Cell 18, 665–675.

Fehr WR, Caviness CE. 1977. Stages of soybean development. Special Report 87. Ames, IA, USA: Iowa State University.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. New Phytologist 171, 501–523.

Finkelstein RR, Reeves W, Arizumi T, Steber C. 2008. Molecular aspects of seed dormancy. Annual Review of Plant Biology 59, 387–415.

Frankland B, Taylorson R. 1983. Light control of seed germination. In: Shropshire W, Mohr H, eds. Photomorphogenesis. Heidelberg: Springer. 428–456.

Fraser DP, Hayes S, Franklin KA. 2016. Photoreceptor crosstalk in shade avoidance. Current Opinion in Plant Biology 33, 1–7.

Frey A, Effroy D, Lefebvre V, Sea M, Perreau F, Berger A, Sechet J, To A, North HM, Marion-Poll A. 2012. Epoxycarotenoid cleavage by NCED5 confers increased cumacteral ABA accumulation and affects seed dormancy and drought tolerance with other NCED family members. The Plant Journal 70, 501–512.

Galloway LF. 2001. Parental environmental effects on life history in the herbaceous plant Campanula americana (Campanulaceae). Ecology 82, 2781–2789.

Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ. 2012. Molecular mechanisms of seed dormancy. Plant, Cell & Environment 35, 1769–1786.

Haire-Joshu D, Tabak R. 2016. Preventing obesity across generations: evidence for early life intervention. Annual Review of Public Health 37, 253–271.

Han TF, Xu C, Tong Z, Mentreddy RS, Tan KH, Gai JY. 2006. Postflowering photoperiod regulates vegetative growth and reproductive development of soybean. Environmental and Experimental Botany 55, 120–129.

Hayes RG, Klein WH. 1974. Spectral quality influence of light during development of Arabidopsis thaliana plants in regulating seed germination. Plant & Cell Physiology 15, 643–653.

He H, de Souza Vidigal D, Snoek LB, Schnabel S, Nijveen H, Holdsworth MJ, Léon-Kloosterziel KM, Koornneef M. 2014. Interaction between parental environment and genotype affects plant and seed performance in Arabidopsis. Journal of Experimental Botany 65, 6603–6615.

Holdsworth MJ, Bentsink L, Soppe WJ. 2008. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytologist 179, 33–54.

Huang X, Zhang Q, Jiang Y, Yang C, Wang Q, Li L. 2018. Shade-induced nuclear localization of PIF7 is regulated by phosphorylation and 14-3-3 proteins in Arabidopsis. eLife 7, e31636.
Ibarra SE, Auge G, Sánchez RA, Botto JF. 2013. Transcriptional programs related to phytochrome A function in Arabidopsis seed germination. Molecular Plant 6, 1261–1273.

Jia L, Wu Q, Ye N, Liu R, Shi L, Xu W, Zhi H, Rahman AN, Xia Y, Zhang J. 2012a. Proanthocyanidins inhibit seed germination by maintaining a high level of abscisic acid in Arabidopsis thaliana seeds. Journal of Integrative Plant Biology 54, 663–673.

Jia LG, Sheng ZW, Xu WF, Li YX, Liu YG, Xia YJ, Zhang JH. 2012b. Modulation of anti-oxidation ability by proanthocyanidins during germination of Arabidopsis thaliana seeds. Molecular Plant 5, 472–481.

Kanai M, Nishimura M, Hayashi Y. 2010. A peroxisomal ABC transporter promotes seed germination by inducing pectin degradation under the control of ABI5. The Plant Journal 62, 936–947.

Kaymak H. 2014. Potential effect of seed fatty acid profile of pepper (Capsicum annuum L.) cultivars on germination at various temperatures. Zemdirbyste-Agriculture 101, 321–326.

Klose C, Viczián A, Kircher S, Schäfer E, Nagy F. 2015. Molecular mechanisms for mediating light-dependent nucleo/cytoplasmic partitioning of phytochrome photoreceptors. New Phytologist 206, 965–971.

Kvalen H, Johnsen O. 2008. Timing of bud set in Ricea abies is regulated by a memory of temperature during zygoti and somatic embryogenesis. New Phytologist 177, 49–59.

Lee KP, Lopez-Molina L. 2012. Control of seed germination in the shade. Cell Cycle 11, 4498–4490.

Lee KP, Piskurewicz U, Turečková V, Carat S, Chappuis R, Strnad M, Fankhauser C, Lopez-Molina L. 2012. Spatially and genetically distinct control of seed germination by phytochromes A and B. Genes & Development 26, 1984–1996.

Liu B, Kanazawa A, Matsumura H, Takahashi R, Harada K, Abe J. 2008. Genetic redundancy in soybean photoresponses associated with duplication of the phytochrome A gene. Genetics 180, 995–1007.

Liu J, Yang CO, Zhang Q, Lou Y, Wu HJ, Deng JC, Yang F, Yang WY. 2016. Partial improvements in the flavor quality of soybean seeds using intercropping systems with appropriate shading. Food Chemistry 207, 107–114.

Liu W, Jiang B, Ma L, et al. 2018. Functional diversification of Flowering Locus T homologs in soybean: GmFT1a and GmFT2a/5a have opposite roles in controlling flowering and maturation. New Phytologist 217, 1335–1345.

Liu X, Rahman T, Song C, Su BY, Yang F, Yong TW, WuYS, Zhang CY, Yang WY. 2017a. Changes in light environment, morphology, growth and yield of soybean in maize–soybean intercropping systems. Field Crops Research 200, 38–46.

Liu X, Rahman T, Yang F, Song C, Yong T, Liu J, Zhang C, Yang W. 2017b. PAR interception and utilization in different maize and soybean intercropping patterns. PLoS ONE 12, e0169218.

MacGregor DR, Kendall SL, Florence H, Fedi F, Moore K, Paszkiewicz K, Smirniotopoulos N, Penfield S. 2015. Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. New Phytologist 205, 642–652.

Martinez-Andújar C, Ordiz MI, Huang Z, Nonogaki M, Beachy RN, Nonogaki H. 2011. Induction of 9-cis-epoxycarotenoid dioxygenase in Arabidopsis thaliana seeds enhances seed dormancy. Proceedings of the National Academy of Sciences, USA 108, 17225–17229.

Matthews S. 2006. Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. Molecular Ecology 15, 3483–3503.

McCullough JM, Shropshire W. 1970. Physiological predetermination of germination responses in Arabidopsis thaliana (L) HEYNH. Plant & Cell Physiology 11, 139–148.

McNair JN, Sunkara A, Frobish D. 2012. How to analyse seed germination data using statistical time-to-event analysis: non-parametric and semi-parametric methods. Seed Science Research 22, 77–95.

Née G, Kramer K, Nakabayashi K, Yuan B, Xiang Y, Miatton E, Finkemeier I, Soppe WJJ. 2017. DELAY OF GERMINATION1 requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. Nature Communications 8, 72.

Nishimura N, Tsuchiya W, Moresco JJ, et al. 2018. Control of seed dormancy and germination by DOG1-AHG1 PP2C phosphatase complex via binding to heme. Nature Communications 9, 2132.

Nonogaki M, Sall K, Nambara E, Nonogaki H. 2014. Amplification of ABA biosynthesis and signaling through a positive feedback mechanism in seeds. The Plant Journal 78, 527–539.

Nooteboom LD, Blakken KA, Fryzlewicz JM. 1985. Control of seed coat thickness and permeability in soybean: a possible adaptation to stress. Plant Physiology 79, 543–545.

Pham VN, Kathare PK, Huu E. 2018. Phytochromes and phytochrome interacting factors. Plant Physiology 176, 1025–1038.

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. 2008. The gibberellin acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. The Plant Cell 20, 2729–2745.

Postma FM, Ågren J. 2015. Maternal environment affects the genetic basis of seed dormancy in Arabidopsis thaliana. Molecular Ecology 24, 785–797.

Quettel AL, Eastmond PJ. 2009. Storage oil hydrolysis during early seedling growth. Plant Physiology and Biochemistry 47, 485–490.

Radford EJ, Ito M, Shi et al. 2014. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. Science 345, 1259093.

Sasidharan R, Chinnappa CC, Voesenek LA, Pierik R. 2008. The regulation of cell wall extensibility during shade avoidance: a study using two contrasting ecotypes of Stellaria longipes. Plant Physiology 148, 1557–1569.

Senda M, Yamaguchi N, Hiraoka M, Kawada S, Ilyoshi R, Yamashita K, Sonoki T, Maeda H, Kawasaki M. 2017. Accumulation of proanthocyanidins and/or lignin deposition in buff-pigmented soybean seed coats may lead to frequent defective cracking. Planta 245, 659–670.

Seo M, Hanada A, Kuwahara A, et al. 2006. Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. The Plant Journal 45, 354–366.

Shah FA, Ni J, Chen J, Wang G, Liu W, Chen X, Tang C, Fu S, Wu L. 2018. Proanthocyanidins in seed coat tegmen and endospermic cap inhibit seed germination in Sapium sebiferum. PeerJ 6, e4690.

Shi H, Wang B, Yang P, Li Y, Miao F. 2016. Differences in sugar accumulation and mobilization between sequential and non-sequential senescence wheat cultivars under natural and drought conditions. PLoS ONE 11, e0166155.

Shu K, Chen Q, Wu Y, et al. 2016a. ABI4 mediates antagonistic effects of abscisic acid and gibberellins at transcript and protein levels. The Plant Journal 85, 348–361.

Shu K, Liu XD, Xie Q, He ZH. 2016b. Two faces of one seed: hormonal regulation of dormancy and germination. Molecular Plant 9, 34–45.

Shu K, Qi Y, Chen F, et al. 2017. Salt stress represses soybean seed germination by negatively regulating GA biosynthesis while positively mediating ABA biosynthesis. Frontiers in Plant Science 8, 1372.

Shu K, Zhang H, Wang S, Chen M, Wu Y, Tang S, Liu C, Feng Y, Cao X, Xie Q. 2013. ABI4 regulates primary seed dormancy by regulating the biosynthesis of abscisic acid and gibberellins in Arabidopsis. The Plant Journal 78, 785–797.

Shu K, Zhou W, Yang W. 2018. APETALA 2-domain-containing transcription factors: focusing on abscisic acid and gibberellins antagonism. New Phytologist 217, 977–983.

Smýkal P, Vernoud V, Blair MW, Soukup A, Thompson RD. 2014. The role of the testa during development and in establishment of dormancy of the legume seed. Frontiers in Plant Science 5, 351.

Sweeney S, MacBeth A. 2016. The effects of paternal depression on child and adolescent outcomes: a systematic review. Journal of Affective Disorders 205, 44–59.

TheoDouli FL, Eastmond PJ. 2012. Seed storage oil catabolism: a story of give and take. Current Opinion in Plant Biology 15, 322–328.

Vaistij FE, Gan Y, Penfield S, Gilday AD, Dave A, He Z, Josse EM, Choi G, Halliday KJ, Graham IA. 2013. Differential control of seed primary dormancy in Arabidopsis ecotypes by the transcription factor SPATULA. Proceedings of the National Academy of Sciences, USA 110, 10866–10871.

Vogt MC, Paeger L, Hess S, et al. 2014. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. Cell 156, 495–509.
Wu FQ, Fan CM, Zhang XM, Fu YF. 2013. The phytochrome gene family in soybean and a dominant negative effect of a soybean PHYA transgene on endogenous Arabidopsis PHYA. Plant Cell Reports 32, 1879–1890.

Xu C, Shanklin J. 2016. Triacylglycerol metabolism, function, and accumulation in plant vegetative tissues. Annual Review of Plant Biology 67, 179–206.

Xu M, Xu Z, Liu B, et al. 2013. Genetic variation in four maturity genes affects photoperiod insensitivity and PHYA-regulated post-flowering responses of soybean. BMC Plant Biology 13, 91.

Yang C, Xie F, Jiang Y, Li Z, Huang X, Li L. 2018. Phytochrome A negatively regulates the shade avoidance response by increasing auxin/indole acidic acid protein stability. Developmental Cell 44, 29–41.e4.

Yang F, Huang S, Gao R, Liu W, Yong T, Wang X, Wu X, Yang W. 2014. Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio. Field Crops Research 155, 245–253.

Yang F, Wang XC, Liao DP, et al. 2015. Yield response to different planting geometries in maize–soybean relay strip intercropping systems. Agronomy Journal 107, 296–304.

Zhao P, Li X, Jia J, Yuan G, Chen S, Qi D, Cheng L, Liu G. 2019. LcbHLH92 from sheepgrass acts as a negative regulator of anthocyanin/proanthocyanin accumulation and influences seed dormancy. Journal of Experimental Botany 70, 269–284.

Zhou W, Chen F, Zhao S, et al. 2019. DA-6 promotes germination and seedling establishment from aged soybean seeds by mediating fatty acid metabolism and glycometabolism. Journal of Experimental Botany 70, 101–114.