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Effect of exogenous gibberellin on reserve accumulation during the seed filling stage of oilseed rape

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ABSTRACT. Exogenous gibberellins (GAs) are widely applied to increase crop yields, with knowledge about the physiological functioning and biochemistry mechanisms of these phytohormones improving; however, information remains limited about the effect of GAs on seed filling. In this study, the siliques (containing the seeds) of oilseed rape (*Brassica napus* L.) were treated with GA_3 at 3 stages of seed filling. We confirmed that GA_3 regulates the deposition of storage reserves in developing seeds. The percentage of crude fat in the seeds increased during the early stage, but remained stable during the middle and late stages. In comparison, the percentage of total protein decreased during the early and middle stages, but significantly increased during the late stage. In addition, Q-PCR was employed to analyze the expression level of related genes in

response to GA_3. It was found that the expression of *WRI* and *ABI3* transcription factors corresponded to crude fat content and total protein content, respectively. The expression of storage reserve related genes *DGAT*, *MCAT*, *SUC2*, and *GPT* was consistent with crude fat content, whereas the expression of *Napin* corresponded to total protein content. The results of this study indicate that exogenous GA_3 has a different effect on storage reserve deposition in seed during different stages of seed filling, and the effect might be achieved via changing the expression of related genes.

Key words: Gibberellin; Reserve accumulation; Gene expression; Seed filling; Oilseed rape

INTRODUCTION

Gibberellins (GAs) are important phytohormones that are involved in regulating many aspects of plant development, such as seed germination, stalk elongation, leaf extension, flowering time, and fruit maturation (Kahn et al., 1957; Davies, 1995). Today, GAs are widely applied in agricultural processes to increase crop yield. GAs are frequently used to increase fruit and vegetable weight and yields, such as tomato (Naeem et al., 2001), in addition to increasing the 1000 grain weights of *Bashaier*, a drought tolerant maize genotype (Shaddad et al., 2011). The GA content varies in different plant organs, and is primarily abundant in the meristem, particularly the seed at maturity (Csukasi et al., 2011). There is evidence that the gibberellin/abscisic acid balance regulates the germination versus maturation pathways during the seed development of maize (White et al., 2000). Active GA in the endosperm is essential for the normal seed development of *Arabidopsis* (Singh et al., 2010); yet, its regulating mechanism remains poorly understood.

Oilseed rape (*Brassica napus* L.) is an important oil crop worldwide, and forms an important component of our diets, as well as in the bio-energy sector. Previous studies have indicated that GAs regulate oilseed rape shoot elongation, in addition to controlling shoot elongation responses to light conditions (Potter et al., 1999). GAs end seed dormancy by accelerating the early germination of rapeseed (Fu and Lu, 1991); yet, little is known about the effect of GAs on the seed development of rapeseed.

In the seed storage reserves of oilseed rape, lipids are the predominant component, followed by storage proteins and saccharides. The accumulation of reserves is a complicated process, involving carbon partitioning among oil, storage proteins, and carbohydrates. During seed filling, sucrose is transported from the leaves to the seeds, and then it is broken into monosaccharides for use as a source for the synthesis of different storage products. To date, there is a paucity of studies about the regulation of seed storage reserve deposition by GAs.

Here, we investigated the effect of GAs on seed development and storage production accumulation. Specifically, we treated the siliques (containing the seeds) of oilseed rape with GA_3 at 3 different stages of seed filling. At each stage, we measured the contents of the seed storage reserves, including crude fat, total protein, total sugar, and glucosinolates. The expression levels of genes related to storage reserve accumulation were analyzed.

MATERIAL AND METHODS

Plant material

The seeds of a conventional oilseed rape cultivar W6 (*Brassica napus* L.) were obtained from the Academy of Seed Industry of Hunan Yahua, China. W6 has a 200 day growth cycle in Southern China, with a 40 day period from flowering to seed harvest. The seeds were sown 0.5 m apart in the experimental field of Hunan University in Changsha on 11/10/2009. Changsha (28°12' N latitude, 112°59' E longitude) belongs to the subtropical zone, and has a humid monsoon climate. The experimental plot contained 4 treatments, with 3 replicates, and a random block design, with a total of 12 zones. Each zone covered an area of 4 x 5 m, with 0.5 m cell intervals, with 30 seedlings remaining after thinning. During flowering in April 2010, all of the flowers that blossomed on the same day from the main inflorescence were self-pollinated and marked.

GA_3 treatment

The siliques of oilseed rape at 15 DAF (days after flowering), 25 DAF, and 35 DAF (Figure 1), representing the early, middle, and later stages of seed filling, respectively, were selected for GA_3 treatment. One hundred micromolar GA_3 solution was used in this experiment, following the methods used in previous studies by our research group (Zhao et al., 2010; Zhou et al., 2011). The siliques were either treated with GA_3 solution or distilled water (control) using a small brush from 9:00 a.m. to 9:30 a.m. About 10 of the treated siliques were sampled 3 h later, immediately frozen in liquid nitrogen, and stored at -80°C for RNA extraction.

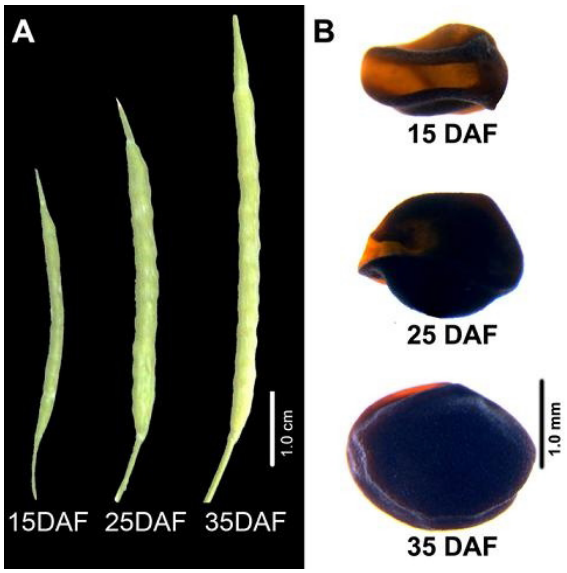

Figure 1. Fresh siliques and dry seeds used in this article. Fresh siliques and dry seeds: **A.** Three fresh siliques respectively represent the early, middle, and later stage of maturing siliques (15, 25 and 35 DAF). **B.** Dry seeds taken separately from three stages of maturing siliques.

Evaluation of seed related traits and measurement of storage reserves

The treated siliques were harvested at 40 DAF, oven-dried at 60°C until a constant weight was reached. Subsequently, the length and weight of the siliques, in addition to the 1000 seed weight, were measured. Ten individual plants from each zone were randomly selected for the evaluation of these seed related traits. All of the seeds from the 4 treatments were used in the measurement of storage reserves.

The crude fat content of seeds was determined using a Soxhlet extractor, as described by von Soxhlet (1879). Total protein was detected using the Micro-Kjeldahl method, as described by Stuart (1936), using fully automatic Kjeldahl apparatus (Shanghai HongJi Instrument Co Ltd, Shanghai, China). Total sugar was measured by Anthrone-sulfuric acid colorimetry (Grande et al., 1953). Glucosinolate content was determined by Fourier transform near-infrared reflectance (NIR) spectroscopy (Font et al., 2004). Three parallel experiments were carried out for the measurement of storage reserves.

Transcript level analysis

Total RNA was extracted from the siliques using an Easy Way RNA Plant Mini Kit (Ambiogen Co. Ltd., San Jose, USA), and were treated with RNase-free DNase I (Promega Biotech Co., Fitchburg, USA). First-strand cDNA was reverse transcribed from the Dnase-digested RNA samples using an M-MLV Reverse Transcript Kit and an Oligo(dT) 18 primer, following manufacturer protocols (Life Technologies Co., Carlsbad, USA). Q-PCR reactions were performed by the SYBR Green PCR Master Mix (ABI, Inc., London, England) in an Mx3000P QPCR System (Agilent Technologies, Inc., Santa Clara, USA). The housekeeping gene *Actin7* was amplified as an internal control. PCR were completed at 94°C denaturation for 10 min, followed by 40 cycles at 94°C denaturation for 30 s, 58°C annealing for 20 s, and 72°C elongation for 20 s. Triplicate sets of PCR samples were carried out. The DNA sequences of all of the primers used in this study were listed in **Table S1**.

To determine whether the changes of seed reserve compositions in response to GA_3 could be a result of altered genes expression, the expression profiles of 27 genes was analyzed. Among them, 5 genes are master seed transcription factors (*LEC1*, *LEC2*, *FUS3*, *ABI3*, and *WRI*), which control seed development and filling; 13 genes are oil biosynthesis genes including 3 *ACCase* genes (homomeric acetyl-CoA carboxylase [*ACC*], alpha-carboxyltransferase [*α-CT*] and beta-carboxyltransferase [*β-CT*]), 6 genes involved in fatty acid elongation (Malonyl CoA-acyl carrier protein transacylase [*MCAT*], beta-ketoacyl-ACP synthase I [*KASI*], beta-ketoacyl-ACP synthase II [*KASII*], beta-ketoacyl-ACP synthase III [*KASIII*], fatty acid elongase 1 [*FAE1*], and 3-ketoacyl-CoA reductase [*KCR2*]), 3 desaturases (stearoyl-ACP desaturase [*SAD*], oleate desaturase [*FAD2*], and linoleate desaturase [*FAD3*]) and diacylglycerol acyltransferase [*DGAT*]; 6 genes is involved in carbohydrate metabolism including ADP-glucose pyrophosphorylase (*AGP*), sucrose transporter 2 (*SUC2*), Glc-6-P translocator (*GPT*), fructose-bisphosphate aldolase 1 (*Aldolase1*), chloroplast pyruvate kinase beta subunit (*PK-β*), and pyruvate dehydrogenase E1 alpha subunit (*PDE1a*); 2 genes are major storage protein genes (*Cruciferin* and *Napin*); and a gene is a Cytochrome P450 gene named *CYP79B5* (*P450*) which is related to glucosinolate biosynthesis.

Statistical analysis

Sources of variance were conducted for silique length, silique weight, 1000 seed weight, and seed storage reserves content. Data were analyzed using Microsoft Excel 2003. Treatments were considered as fixed effects. The relative expression level of each gene in each treatment was calculated using the Mxpro software from the QPCR System (Agilent Technologies, Inc., Santa Clara, USA). Significant differences were determined using student's *t*-test. The software Originpro 8 (OriginLab Corporation, Northampton, USA) and Photoshop (Adobe Systems, Inc., San Jose, USA) were employed to construct graphs.

RESULTS

Effects of GA_3 on seed related traits and reserve accumulation in oilseed rape

As shown in Table 1, the dry weight of the siliques was higher in all GA_3-treated samples compared to the control. The dry weight increased by 12.16 and 16.04% for treated GA_3 at 25 DAF and 35 DAF, respectively. In addition, the length of the siliques increased by 3.46, 4.45, and 8.76% in the 3 respective GA_3-treated samples. However, the 1000 seed weight decreased by almost 7% in the 25 DAF and 35 DAF GA_3-treated samples, while no difference was found for the 15 DAF.

Table 1. Analysis of variance of storage reserve content in seed and seed related traits measured for oilseed rape under GA_3 and control treatments at different stages.

Treatment[§]	-GA[§]	15DAF+GA	25DAF+GA	35DAF+GA
Dry silique weight (mg)[‡]	91.33 ± 3.141	92.61 ± 2.820	102.44 ± 2.143**	105.98 ± 2.494**
Silique length (cm)[‡]	4.72 ± 0.058	4.88 ± 0.086	4.93 ± 0.049**	5.13 ± 0.073**
Thousand seed weight (g)[††]	2.19 ± 0.004	2.16 ± 0.005	2.04 ± 0.009**	2.04 ± 0.008**
Crude fat content (%)[††]	36.58 ± 0.254	38.30 ± 0.360**	37.01 ± 0.170	36.35 ± 0.073
Total protein content (%)[††]	23.58 ± 0.277	22.83 ± 0.146	22.22 ± 0.269**	24.96 ± 0.185*
Total sugar content (%)[††]	10.74 ± 0.097	11.62 ± 0.174**	11.28 ± 0.120*	11.29 ± 0.110*
Glucosinolates content (μmol/g)[††]	42.88 ± 0.944	58.50 ± 0.491**	56.65 ± 0.385**	63.43 ± 0.185**

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. [§]-GA = siliques treated with distilled water (control); +GA = siliques treated with GA_3; DAF = days after flowering. [‡]Each data point is reported as means ± SD of 30 siliques. [††]Each data point is reported as means ± SD of three replicates.

The storage reserves of the seeds (including crude fat, total protein, total sugar, and glucosinolates) also changed in response to GA_3. The crude fat content of the seeds increased by 4.70% in the 15 DAF samples, whereas little change was observed in the 25 DAF and 35 DAF samples. Total protein content decreased by 3.18 and 5.77% in the 15 DAF and 25 DAF samples, but increased by 5.85% in the 35 DAF GA_3-treated sample. Total sugar content increased by 6.77, 4.10, and 5.04% the 3 respective treatments. Glucosinolate content was remarkably enhanced by GA_3-treatment, showing a 36.43, 32.11, and 47.92% increase at 15, 25, and 35 DAF, respectively.

Expression of master seed transcript factors in response to GA_3

The transcriptional levels of 4 genes (*LEC1*, *LEC2*, *FUS3*, and *WRI*) were upregulated

in the 15 DAF samples, with *LEC2* and *WRI* noticeably increasing 101.9 and 104.4% compared to the control (Figure 2). Yet, the expression of *ABI3* was suppressed in the 15 DAF sample. The transcripts of 4 of these genes (excluding *WRI*) were suppressed by GA_3 treatment at 25 DAF, showing a decrease of 51.6-84.1%. In contrast, GA_3 treatment at 35 DAF resulted in the upregulation of the mRNA levels of *FUS3* and *ABI3*, while *LEC1*, *LEC2*, and *WRI* were downregulated.

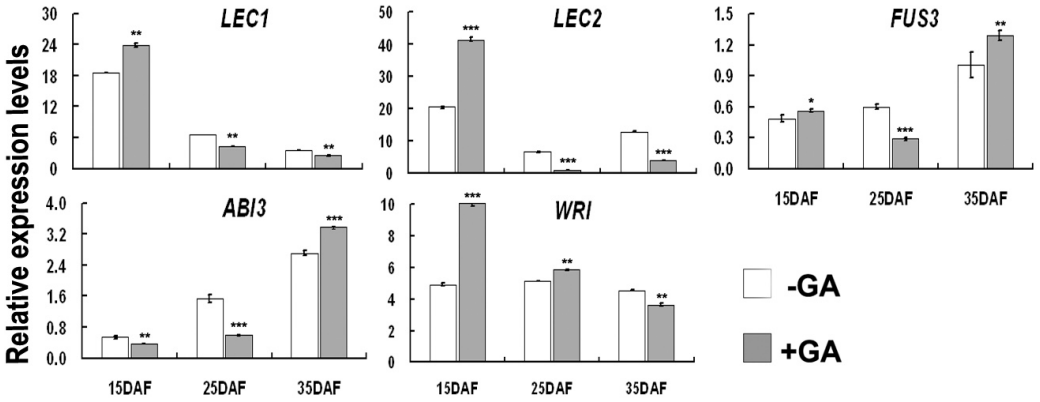

Figure 2. Effects of GA3 on transcript levels of genes encoding master seed transcript factors during siliques maturing. Total RNA was extracted from the 15, 25 and 35 DAF (Days after flowering) siliques treated by GA_3 solution or distilled water (control) for 3 h, and used for Q-PCR analysis. The housekeep gene *Actin 7* was amplified as an internal control. Three replicates were carried out on cDNA template dilutions which obtained from three independent total RNA extractions. The error bars represent the standard deviations. *P < 0.05, **P < 0.01, ***P < 0.001, significant differences between the control and GA treatments. -GA = siliques treated with distilled water (control); +GA = siliques treated with GA_3.

Expression of oil synthesis genes in response to GA_3

It was found that the transcription levels of all these indicated genes altered in response to GA_3 at the 3 different stages during seed filing (Figure 3).

In response to GA_3 at 15 DAF, the transcription levels of 3 genes (*β-CT*, *MCAT*, and *DGAT*) were upregulated, with noticeable increases of 42.0, 55.3, and 108.9%, respectively. Seven genes (*α-CT*, *KASI*, *KASΠ*, *KASШ*, *KCR2*, *FAD2*, and *FAD3*) were downregulated, with noticeable decreases of 30.0, 50.1, 55.9, and 76.3% for the *α-CT*, *KAS I*, *KAS Π*, and *FAD*, respectively.

In response to GA_3 at 25 DAF, the transcription levels of 4 genes (ACC, *β-CT*, *MCAT*, and *FAD2*) were upregulated, while those of four genes (*KASΠ*, *SAD*, *FAD3*, and *KCR2*) were downregulated by more than 20%. Among these, *ACC* and *β-CT* increased by 64.1 and 89.9%, respectively, while *KAS Π*, *SAD*, and *FAD 3* decreased by 41.8, 46.2, and 47.5%, respectively.

In response to GA_3 at 35 DAF, the transcription levels of 4 genes (ACC, *α-CT*, *β-CT*, and *SAD*) were upregulated, while those of 6 genes (*KAS I*, *KAS Ш*, *MACT*, *FAE 1*, *KCR2*, and *FAD3*) were downregulated by more than 20%. The expression levels of *ACC*, *α-CT*, and *β-CT* noticeably increased by 119.7, 138.4, and 201.9%, respectively. Interestingly, genes involved in fatty acid elongation (*KAS I*, *KAS Ш*, *MACT*, *FAE1*, and *KCR2*) showed a noticeable decline of 45.6, 40.1, 51.6, 75.1, and 38.8%, respectively.

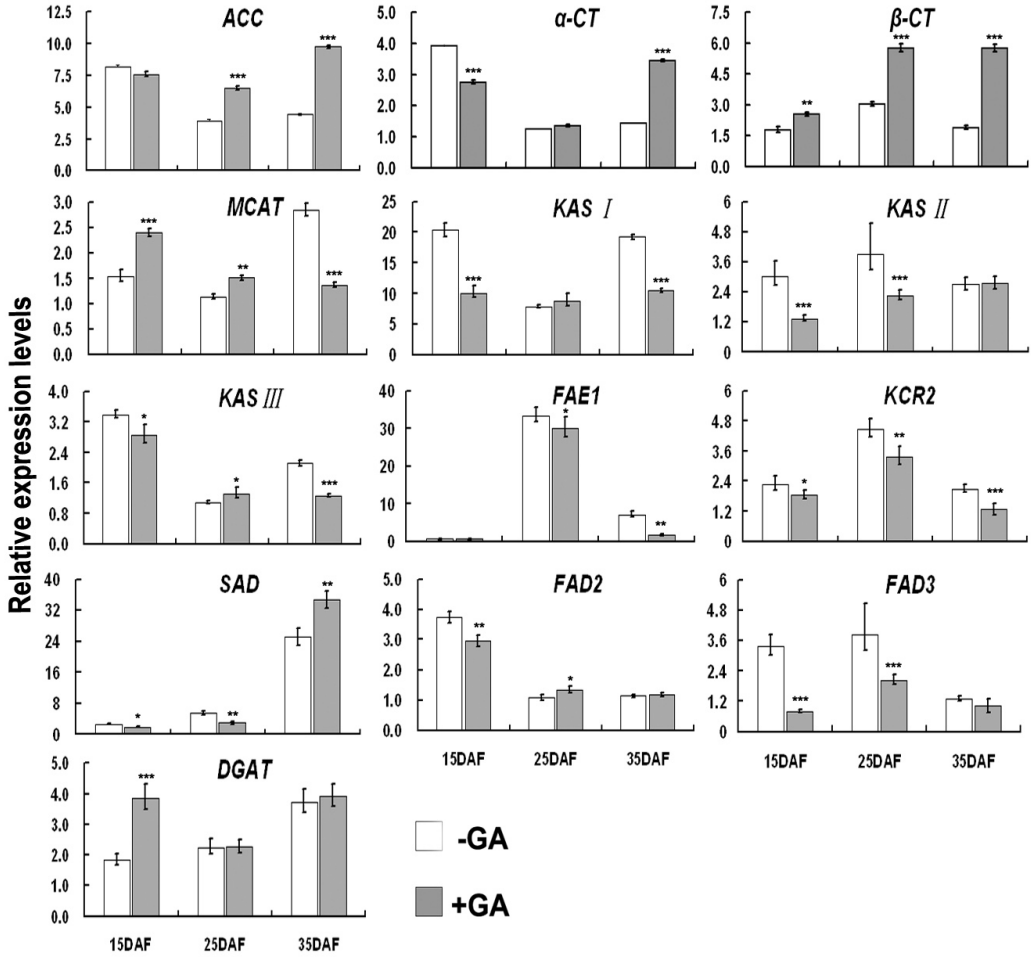

Figure 3. Effects of GA_3 on transcript levels of oil synthesis genes during siliques maturing. Total RNA was extracted from the 15, 25 and 35 DAF (days after flowering) siliques treated by GA_3 solution or distilled water (control) for 3h, and used for Q-PCR analysis. The housekeep gene *Actin 7* was amplified as an internal control. Three replicates were carried out on cDNA template dilutions which obtained from three independent total RNA extractions. The error bars represent the standard deviations. *P < 0.05, **P < 0.01, ***P < 0.001, significant differences between the control and GA treatments. -GA = siliques treated with distilled water (control); +GA = siliques treated with GA_3.

Expression of carbohydrates metabolism-related genes, storage protein genes, and gene *P450* in response to GA_3

As shown in Figure 4, in the 15 DAF samples, *GPT* and *SUC2* were upregulated by 53.4 and 37.2%, *Aldolase1* and *PK-β* were downregulated by 28.3 and 36.9%, and *AGP* and *PDE1a* remained relatively stable. In the 25 DAF samples, the expressions of *AGP* and *Aldolase1* became upregulated by 112.1 and 61.7%, *PK-β* remained downregulated by 37.8%,

and the other 3 genes (*GPT*, *SUC2*, and *PDE1a*) remained relatively unchanged. In the 35 DAF samples, the expression of *PK-β* was upregulated by 34.9%, but *SUC 2*, *Aldolase1*, and *PDE1a* were downregulated by 42.5, 39.4, and 37.1%, respectively. *GPT* was slightly downregulated, and *AGP* remained relatively stable.

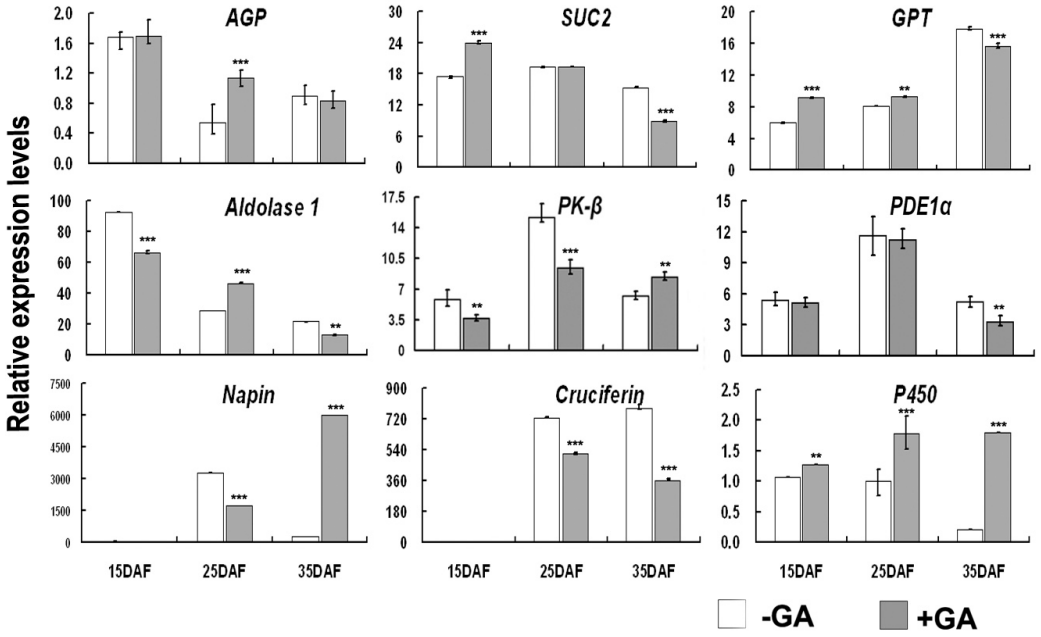

Figure 4. Effects of GA_3 on transcript levels of several genes respectively related with carbohydrates metabolism, storage protein and glucosinolates deposition during siliques maturing. Total RNA was extracted from the 15, 25 and 35 DAF (days after flowering) siliques treated by GA_3 solution or distilled water (control) for 3 h, and used for Q-PCR analysis. The housekeep gene *Actin 7* was amplified as an internal control. Three replicates were carried out on cDNA template dilutions which obtained from three independent total RNA extractions. The error bars represent the standard deviations. *P < 0.05, **P < 0.01, ***P < 0.001, significant differences between GA treatments and the control. -GA = siliques treated with distilled water (control); +GA = siliques treated with GA_3.

The transcription levels of *Cruciferin* and *Napin*, which encode 2 major storage proteins (Cruciferin and Napin) in oilseed rape, were also detected in this study. Both genes showed almost no expression in the 15 DAF and control samples, and were downregulated by 47.6 and 28.8% in the 25 DAF samples. In the 35 DAF samples, the transcription of *Cruciferin* remained downregulated (by 53.8%), whereas that of *Napin* expressed a noticeable 20-fold increase (Figure 4).

It was found that the expression of *P450* was upregulated by 20.0, 77.8, and 759.1% in response to GA_3 at 15, 25, and 35 DAF, respectively (Figure 4). This result is consistent with the increased glucosinolate content recorded in the seeds of GA_3 treated samples (Table 1).

DISCUSSION

GAs are used to improve the productivity of various crops. For instance, it is used to gener-

ate larger fruits in orange and grape crops. In this study, longer and heavier siliques were observed in oilseed rape treated with GA_3 (Table 1). Seed weight is an important agricultural trait that is related to seed yield per plant in oilseed rape (Yang et al., 2012). Previous reports found that seed weight is not affected by GA-deficiency (Barendse et al., 1986). In the current study, we found the 1000 seed weight was not affected by GA_3 treatment at 15 DAF, but that it was reduced by GA_3 at 25 and 35 DAF. The analysis of heavier siliques (containing seeds) (Table 1) indicated that exogenous GA_3 might accelerate nutrient flow to the silique, but not the actual seed at 25 and 35 DAF.

Carbohydrates, proteins, and lipids are considered to be the 3 predominant component storage units of seed reserves. A previous study on the fern *Ceratopteris thalictroides* L. demonstrated that GA stimulates the accumulation of starch in mature leaves (Stein, 1971). In this study, we showed that GA_3 treatment modified the percentages of proteins and lipids in the seeds. In addition, GA_3 stimulated the total sugar and glucosinolates accumulation in the seeds. Previous studies by our research group indicated that the heterologous expression of *AtGA2ox8*, which is a GA inactivation gene, in oilseed rape enhanced photosynthetic capacity (Zhou et al., 2011), siliques, seed yield, and seed oil content, but decreased glucosinolate content (Zhao et al., 2010; Zhou et al., 2012). These findings in combination with the results of the current study indicate that both endogenous and exogenous GAs regulate seed development, seed storage accumulation, and glucosinolate synthesis.

Master seed transcription factors

Previous studies have shown that many elementary seed development processes are regulated by the *LEC1*/(*ABI3/FUS3/LEC2*)-B3 network (Junker et al., 2010). It has been reported that the overexpression of *LEC1* in *Arabidopsis* generates increased levels of major fatty acid species and lipids (Mu et al., 2008). For instance, ectopic *LEC2* expression induces the accumulation of seed storage proteins and oil bodies in vegetative and reproductive organs (Stone et al., 2008). The *Arabidopsis lec2* mutant was found to have 30% less oil and 15% less protein (Angeles-Nunez and Tiessen, 2011). The results of the current study showed that *LEC1* and *LEC2* were significantly upregulated by GA_3 at 15 DAF, but downregulated at 25 DAF and 35 DAF. Based on the deposition of crude fats and total proteins (Table 1), *LEC1* and *LEC2* might regulate lipid synthesis at the early stage and late seed filling stages, and regulate storage proteins during the middle stage.

WRI is a transcriptional activator that is involved in oil accumulation during seed development, by regulating the activation of a subset of sugar-responsive genes and oil synthesis genes (Focks, 1998; Baud et al., 2009). In response to GA_3, this activator was upregulated by 104.4 and 13.4% in the 15 DAF and 25 DAF treatments, but was downregulated by 20.8% in the 35 DAF treatment, which corresponds with the observed changes in seed oil content (Table 1). *WRI* might play an important role in seed oil synthesis in response to GA_3.

ABI3 and *FUS3* regulate the expression of storage protein genes, such as *At2S* and *Cruciferin*, in an ABA (Abscisic Acid) dependent manner (Kagaya et al., 2005b). The two genes are required for *LEC1* to regulate seed storage protein genes (Kagaya et al., 2005a). Here, we showed that the total protein content is highly consistent with the expression pattern of *ABI3* in response to GA_3.

Oil biosynthesis related genes

ACCase is essential for the malonyl-CoA supply of fatty acid biosynthesis (Brownsey

et al., 1997), with high ACCase mRNAs tissue accumulations indicating highly active of fatty acids biosynthesis (Ke et al., 2000a). The Q-PCR results of the current study showed that the changes in expression of ACCase genes (*ACC*, *α-CT*, and *β-CT*) were negatively correlated with changes in oil content in response to GA_3. In comparison, previous studies found that lipid accumulation is not substantially affected by the altered expression of ACCase genes (Roesler et al., 1997; Thelen and Ohlrogge, 2002). Thus, no significant correlation exists between ACCase genes and lipid accumulation.

MCAT, ACP, and KASIII are 3 enzymes considered essential for the initiation of fatty-acid biosynthesis by fatty acid synthase II (FASII) (Prigge et al., 2003). Here, we found that the transcription pattern of *MCAT* was consistent with the observed changes in oil content in response to GA_3. KASI and KASΠ catalyze the fatty acid elongation step from 4:0-ACP to 18:0-ACP in the plastid (Yasuno et al., 2004). Subsequently, FAE extends the chain length of fatty acids from C18 to C20 and C22. The results of the Q-PCR analysis of this study showed that the expressions of 3 fatty acid elongase genes (*KASI*, *KASΠ*, *KASШ*) and 2 genes from the FAE multienzyme complex (*FAE1* and *KCR2*) were mostly downregulated at all 3 stages in GA_3-treated samples, except for *KASI* and *KASШ* in the 25 DAF GA_3 treatment, which were upregulated. This observation indicates that GA_3 partly regulates fatty acid synthesis by suppressing the expression of fatty acid elongase genes.

DGAT plays a key role in lipid accumulation. Seed oil content decreases when *DGAT* is silent (Zhang et al., 2005), and increases when *DGAT* is overexpressed (Taylor et al., 2009). The Q-PCR results of the current study showed that the expression pattern of *DGAT* (Figure 3) was consistent with the observed changes in the oil content (Table 1) of the GA_3-treated samples.

The 3 fatty acid desaturases, SAD, FAD2, and FAD3, are believed to control the production of oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3), respectively, in plants (McKeon and Stumpf, 1982; Browse et al., 1993; Okuley et al., 1994; Nabloussi et al., 2005). The results of the current study showed that all 3 genes were suppressed by GA_3 in the 15 DAF and 25 DAF treatments, but that only *SAD* was noticeably upregulated in the 35 DAF treatment. Overall, GA_3 might contribute towards downregulating the unsaturation of fatty acids during the early stage of seed filling.

Carbohydrate metabolism

AGP catalyzes the formation of ADP-Glc, and ADP-Glc is the substrate of starch synthases; hence, the inactivation of *AGP* would lead to a decline in starch levels and an increase in TAG levels (Li et al., 2010). In our study, the expression of *AGP* remained stable in the 15 DAF and 35 DAF treatments, but was noticeably upregulated by GA_3 in the 25 DAF treatment. This result indicates the absence of a clear relationship between *AGP* expression and total sugar accumulation in GA_3 treated seeds (Table 1).

SUC2 is a major sucrose transporter that is essential for phloem loading (Srivastava et al., 2008). The silencing of *SUC2* might block carbohydrate export from the leaves (Riesmeier et al., 1994). During carbon partitioning, *GPT* contributes to importing glucose 6-phosphate into the plastids of non-green tissues (Niewiadomski et al., 2005). In this study, *SUC2* and *GPT* were upregulated by GA_3 in the 15 DAF treatment, remained almost unchanged in the 25 DAF treatment, and were downregulated in the 35 DAF treatment. These results indicate the strong correlation of these transporters with crude fats deposition (Table 1).

Glycolysis is central to carbon partitioning, because it converts sugars into precursors for the synthesis of storage reserves. Aldolase 1 is a glycolytic enzyme that is involved in the hexose cleavage reaction, with its activity increasing in rice roots treated with GA_3 (Komatsu et al., 2004). Plastidic pyruvate kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate during ATP production, and is involved in the biosynthesis of seed oil (Andre et al., 2007). PDE1a is the alpha subunit of Pyruvate dehydrogenase E1, which plays an important role in acetyl-CoA formation during the synthesis of lipids in seeds (Ke et al., 2000b). The results of our Q-PCR analysis showed that Aldolase 1 was downregulated by GA_3 in the 15 and 35 DAF treatments, but that its transcription levels were enhanced in the 25 DAF treatment. PK-β was suppressed in the 15 and 25 DAF treatments, but was upregulated in the 35 DAF treatment. PDE1a was weakly influenced by GA_3 in the 15 and 25 DAF treatments, but was suppressed in the 35 DAF treatment. A significant correlation was not shown by these 3 genes with either total sugar or crude fat accumulation in the GA_3 treatments.

Storage protein and glucosinolate-related genes

Napin and Cruciferin are the 2 main types of storage proteins in oilseed rape. In this study, *Cruciferin* was downregulated by GA_3 in the 25 and 35 DAF treatments. In contrast, *Napin* was suppressed by GA_3 in the 15 and 25 DAF treatment (not shown in the figure), but noticeably increased in the 35 DAF treatment. We assumed that GA_3 decreases total protein levels by suppressing the expression of these 2 genes during the early and middle stages of seed filling, and that it mainly increases total protein levels by regulating *Napin* expression during the late seed filling stage.

Glucosinolates are associated with the bitter taste of Brassicaceae (van Doorn et al., 1998). A previous study revealed that cytochrome P450 is important in the glucosinolate biosynthetic pathway (Du et al., 1995). In our study, the transcription pattern of *P450* corresponded to changes in the glucosinolate content of seeds treated with GA_3 at the 3 different stages of seed filling (Table 1).

In conclusion, exogenous GA_3 application increases silique weight and length, in addition to the total sugar and glucosinolate content of seeds during seed filling; however, exogenous GA_3 application has variable effects on the weight, crude fat content, and total protein content of seeds at the 3 stages of seed filling. In this study, the expression pattern of transcription factor *WRI* and the 4 genes *DGAT*, *MCAT*, *SUC2,* and *GPT* were correlated with crude fat content in response to GA_3 treatment. In addition, the transcription factor *ABI3* and gene *Napin* were correlated with total protein content. These results provide new insights about the interaction between phytohormones and the synthesis pathways of plant reserve substances.

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Supplementary material

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