The inhibited seed germination by ABA and MeJA is associated with the disturbance of reserve utilizations in Astragalus membranaceus

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ABSTRACT

Astragalus membranaceus is a major traditional Chinese medicinal plant. Here, we investigated the mobilizations of seed reserves during its germination and post-germination growth, as well as the effects of exogenous abscisic acid (ABA) and methyl jasmonate (MeJA). It was found that both starch and protein were rapidly mobilized during the seed germination. However, lipid was mostly utilized during the post-germination. Exogenous ABA and MeJA treatments significantly inhibited the germination and post-germination growth. Meanwhile, the treatments decreased the weight of mobilized seed reserves and seed reserves utilization efficiency, retarded the mobilizations of protein and lipid, and led to excessive consumption of carbon energy. Moreover, the treatments changed fatty acid compositions in cotyledons, with the decreasing of the double bond index and average carbon chain length. This study will help us to understand the inhibition mechanism of exogenous ABA and MeJA on the germination and post-germination growth of A. membranaceus.

Introduction

In the life cycle of a plant, germination is the initial and most crucial stage and determines the establishment and growth of the seedling (Zhang et al. 2010). Energy supply is necessary for the germination and the following seedling growth until it is autotrophic (Eisenstadt and Mancinelli 1974). With the beginning of seed germination, the starch stored in granules and proteins in storage vacuoles are mobilized and respectively converted to soluble sugars and amino acids (Juliano and Varner 1969; Vitale and Hinz 2005). As the other seed reserve, the lipid is stored in oil bodies with the form of triacylglycerol (TAG). Its mobilization is usually posterior to the starch and proteins (Borek et al. 2006; Graham 2008). The TAGs release free fatty acids, and then the free fatty acids sequentially enter the process of β-oxidation and glyoxylate cycle, finally converting to four-carbon organic acids which are transported into mitochondrion and cytosol for the conversion of sucrose (Graham 2008; Erbas et al. 2016). Lipid is known as the most energy-enriched reserve in plant seed. Compared to the hydrolysis of carbohydrates, the complete oxidation of the same weight lipid can produce more than two times of energy (Quettier and Eastmond 2009).

The mobilization of the reserves during seed germination and the following post-germination growth has been well studied in many plant species, including Acrocomia aculeate (Bicalho et al. 2016), Helianthus annuus (Sebei et al. 2007), Jatropha curcas (Lopes et al. 2013), etc. However, the reserves compositions and their metabolic ways are diverse in different genotypes of the phanerogam species (Lopes et al. 2013). For an example, the total protein content and the composition of the lipid showed a significant difference between the two cultivars of H. annuus, DUET CL and TR 3080 (Erbas et al. 2016).

The differences were also found in the utilization of sugars and fatty acid compositions during seed germination and early seedling growth between the cultivars (Erbas et al. 2016). So, the further studies on more plant species and cultivars are needed to better understand the reserves mobilization during seed germination and the post-germination growth.

Besides the inherent genotype, the external environments also effectively altered the reserves utilization during the germination and post-germination growth. It has been reported that the salinity stress induced the delay of the degradation of seed lipids during the first hour after sowing and a precocity of seed protein mobilization in Linum usitatissimum (Sebei et al. 2007). In Vicia ervilia, Na2CO3 treatment declined the seed reserves utilization efficiency (SRUE), which led to the reduction of seed germination (Sepehri et al. 2016). In the study on Triticum aestivum, the weight of mobilized seed reserves (WMSR) was found to be sensitive to drought and salinity (Soltani et al. 2006).

Abscisic acid (ABA), formed by cleavage of C40 oxygenated carotenoids in plastids, plays important roles in the response and tolerance of plant to the environmental stresses, especially to the drought (Arc et al. 2013; Nakashima and Yamaguchi-Shinozaki 2013; Dar et al. 2017). On the other side, ABA also plays a crucial role in the maintenance of seed dormancy and negatively regulates the seed germination (Kermode 2005; Gianinetti and Vernieri 2007; Yu et al. 2016). It has been confirmed that the seed dormancy is imposed by only the ABA produced by the zygotic tissues at late maturation stages (Karssen et al. 1983; Frey et al. 2004). The ABA level decreases during the process of imbibition in dormant and non-dormant seeds of Arabidopsis thaliana. However, the level falls further in non-dormant seeds than in
dormant ones (Millar et al. 2006). The existing evidence shows that the retardation of the reserves degradation might be an important mechanism for the inhibited germination by ABA. Exogenous ABA treatment negatively affected the reserves degradation during the seed germination and post-germination growth, as well as the synthesis and activities of the enzymes which participate the degradation process (Ranjan and Lewak 1995; Shintani et al. 1997; Cercos et al. 1999; Domash et al. 2006; Tonini et al. 2010).

Jasmonic acid (JA) and its methyl ester (methyl jasmonate (MeJA)) are well known to play critical roles in the chilling stress (Koo et al. 2009; Jin et al. 2013). Similar to ABA, JA and MeJA are also involved in the seed germination and the post-germination growth (Ranjan and Lewak 1992; Bogatek et al. 2002; Zalewski et al. 2010). Nevertheless, the regulation of JA on germination is much more complicated. It was found that exogenous JA inhibited the germination of starchy seeds, but did not affect that of oily ones (Ranjan and Lewak 1992). On the other hand, JA stimulated the germination of dormant Pyrus communis seeds, whereas retarded stratified ones (Yildiz et al. 2008).

*Astragalus membranaceus*, a major traditional Chinese medicinal plant, is commonly used to treat a wide variety of diseases and body disorders (Ayueung et al. 2016). At present, it is mainly cultivated in the Northeast and Northwest of China, including provinces of Heilongjiang, Inner Mongolia and Gansu. In the growing regions, drought and low temperature are frequently occurred in early spring (Jiao et al. 2004; Fengmei et al. 2010; He et al. 2011). Just at this time, the cultured *A. membranaceus* is germinating and post-germination growing. According to the regulation roles of ABA and MeJA in environmental stress and seed germination, it is valuable to reveal how the exogenous ABA and MeJA affect germination and post-germination growth of *A. membranaceus*.

In the present study, we investigated the effects of exogenous ABA and MeJA on germination rate, reserves utilization efficiency, content variations of starch, soluble sugar, soluble protein and lipid in cotyledon during the germination and post-germination growth of *A. membranaceus*. Moreover, the fatty acid compositions of the lipid were determined through employing a GC-MS system.

### Materials and methods

#### Seed material and treatments

The seeds of *A. membranaceus* (Fisch.) Bunge were purchased from a seed supplier (Anguo Traditional Chinese Medicine Promotion Station, China). Uniform-sized seeds were selected and surface sterilized with 10% sodium hypochlorite solution for 20 min, and then thoroughly washing with distilled water for three times. Immediately, the sterilized seeds were sowed on petri dishes with two layers of sterile filter paper soaked with distilled water (the control), 10 μM ABA solution (the ABA treatment), or 800 μM MeJA solution (the MeJA treatment). The germination experiments were conducted in a climate incubator. The conditions were maintained with temperature of 25°C/20°C (day/night) and humidity of 75%. The filter papers were renewed every day to keep the treatment concentration. Every treatment was conducted with three replicates and 200 seeds in one replicate.

### Calculations of germination percentage and germination index

Taking the emerging radicle out of the seed coat as the standard of germinated seed, the number of the germinated seeds was counted in 0.5, 1.5, 3, 4 and 5 days after sowing (DAS). The germination percentage was calculated according to the following formula:

\[
\text{Germination percentage (\%)} = \frac{\text{number of the germinated seeds}}{\text{number of the total seeds}} \times 100.
\]

The germination index was calculated with the following formula:

\[
\text{Germination index} = \frac{\sum_{n=0}^{5} \text{number of germinated seeds in day } n}{5}.
\]

#### Determination of seedling length and weight of fresh embryo and dry embryo

The seedling length was measured by a ruler with accuracy of 1 mm. To determine the weight of fresh embryo (WFE) and weight of dry embryo (WDE), the seed coats were removed and the embryos were separated. The fresh embryos were weighed (WFE). Then they were dried in an oven at 70°C and dry weight of embryos was recorded as WDE.

#### Calculations of WMSR and SRUE

WMSR and SRUE were calculated according to the methods of Soltani et al. (2006).

#### Determination of total soluble protein content

The total soluble protein was extracted according to the method of Ribeiro et al. (2011). The extracted solution was used to determine the protein content according to the method of Bradford (1976) with the bovine serum albumin as standard.

#### Determination of starch and total soluble sugar contents

The contents of total soluble sugar and starch were determined using the method of Fu et al. (2014). Briefly, the cotyledons were grounded in sterile water and boiled for 30 min. After a centrifugation at 2000 rpm for 10 min, the supernatant was used to determine the soluble sugar content employing the phenol sulfuric acid assay (DuBois et al. 1956). The residual was re-suspended in 35% (v/v) perchloric acid to quantify the starch content according to the method of Morris (1948).

#### Determination of lipid content and fatty acid compositions

Total lipids were extracted according to the method of Zhou et al. (2013) with minor modifications. 0.5 g fresh cotyledons were fully homogenized in 6 mL chloroform:methanol (2:1,
v/v). Two milliliter 0.1 M KCl aqueous solution was added to the homogenate and mixed with a vortex generator. After a centrifugation for 5 min at 10,000 rpm, the lower lipid phase was collected. The solvent in the lipid phase was evaporated, and the lipid was weighed.

To determine the fatty acid compositions, 0.1 g lipid sample was methyl esterified following the method of Metcalfe and Schmitz (1961). The fatty acid methyl esters were analyzed by GC-MS (QP2010 Plus, Shimadzu, Japan) equipped with a CP-Sil 88 column (100 m length × 0.25 mm diameter and 0.20 μm thickness, Agilent, USA). The initial column temperature was set at 140°C and a holding time of 5 min, followed by a climbing of oven temperature to 240°C with a speed of 4°C/min, and then it was kept constant for 15 min. The ionization energy and ion source were maintained at 70 eV and 200°C, respectively. The scanning range was from 50 to 500 m/z. Identification of the each peak in the sample chromatogram was achieved by comparing their peak retention time to a mixed standard of 37 fatty acid methyl ester (Nestle 37, Nu-Chek Prep, USA). According to peak area normalization, the percentage of fatty acids in the total fatty acid is determined.

**Statistical analysis**

In the present study, there were three replicates arranged in a randomized block design manner for each treatment including the control. All the data were presented as mean ± standard error (SE). The comparison of means and the correlation analysis were performed using SPSS (SPSS 17.0, SPSS Inc., USA). The one-way ANOVA with the Duncan’s post-hoc test was performed to test the difference significance ($p < .05$) of means. The data of fatty acid compositions were standardized to −1 to 1, with −1 representing for the best down-regulation and 1 for the best up-regulation. Then the heap map was made using HemI (Heatmap Illustrator, version 1.0).

**Results**

**Germination and post-germination growth of A. membranaceus and the effects of ABA and MeJA**

Under control condition, the seeds of *A. membranaceus* started to germinated at the 0.5 DAS, and about 70% seeds germinated at the 5 DAS (Figure 1(a)). Both ABA and MeJA treatments completely suppressed the seed germination at the 0.5 DAS. At the 1.5 DAS, the germination percentages were respectively decreased 88% and 95% in ABA and MeJA treatments when compared to the control. Although the germination percentages in both the treatments sharply increased in the following time, they were still far less than that in control. At the end of our experiments (5 DAS), only 60% and 49% seeds germinated in ABA and MeJA treatments, with decreasing of 15% and 32% by contrast with the control. It was not surprising that ABA and MeJA treatments obviously reduced the germination index of seeds of *A. membranaceus* (Figure 1(b)). Moreover, it was found that MeJA had a greater effect on the seed germination than ABA (Figure 1).

It was more than that, both ABA and MeJA treatments also inhibited the post-germination growth of *A. membranaceus* measured with the seedling length, weight of fresh embryo (WFE) and WDE (Figure 2). Throughout the whole experimental period, the lengths of seedling in ABA and MeJA treatments were kept lower than that under control conditions (Figure 2(a)). At the 5 DAS, the length of seedling was reduced 20% and 31% by the ABA and MeJA treatment, respectively. Similar to the seedling length, the WFE and WDE were prominently lowered by the treatments of ABA and MeJA (Figure 2(b,c)). Simultaneously, it could be found that the effect of the treatments on the WFE was greater than that on the WDE. At the 5 DAS, the WFE was reduced 40% and 80% by the ABA and MeJA treatment, but the WDE declined only 11% and 32%.

**Reserves mobilization rate and utilization efficiency during germination and post-germination growth of A. membranaceus and the effects of ABA and MeJA**

As shown in Figure 3(a), the WMSR rapidly increased to about 1.5 mg/seed in the control and both treatments at 0.5 DAS, which was more than 30% of the seed weight. After that, the rising rates of WMSR were obviously slowed down. However, this rate was significantly higher under control conditions than that in ABA or MeJA treatment during 1.5–5 DAS. At the 5 DAS, the ABA and MeJA treatment, respectively, lowered the WMSR by 12% and 18%.

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**Figure 1.** The germination percentage (a) and germination index (b) of *A. membranaceus* seeds and the effects of ABA and MeJA. The sterilized seeds were sowed on petri dish with sterile filter paper soaked with distilled water (CT), 10 μM ABA solution (ABA), or 800 μM MeJA solution (MeJA). The number of germinated seeds was recorded in 0.5–5 days after sowing (DAS). All experiments were performed in triplicate (200 seeds/replicate), and error bars represent SE.
Unlike the parameter of WMSR that displays the amount of mobilized reserves, the SRUE is able to better reflect how much of the seed reserves were turned into substances for the growth of seedling. Under control conditions, the SRUE was about 41% during 0.5–1.5 DAS, and then it continuously increased in the remaining time (Figure 3(b)). The ABA treatment prominently decreased the SRUE during 0.5–3 DAS. Nevertheless, no difference was found between the control and the ABA treatment in SRUE after the 4 DAS. The MeJA treatment showed a greater negative effect on SRUE than the ABA treatment. At the 4 and 5 DAS, MeJA treatment decreased the SRUE lower 18% and 17% than in control.

Protein, sugar and lipid in cotyledons during germination and post-germination growth of *A. membranaceus* and the effects of ABA and MeJA

In the initial 0.5 days, the soluble protein content in cotyledon rose from 0.48 to 0.75 mg/seed under control conditions, with an increase of above 50% (Figure 4(a)). Subsequently, it reached its peak of 0.86 mg/seed at the 4 DAS after sustained gradually increasing. Both ABA and MeJA treatments inhibited the increases of soluble protein content during 0.5–5 DAS, with the exception of ABA treatment at the 5 DAS. Moreover, the greater inhibition was found in the MeJA treatment.

Contrary to the soluble protein, the starch content in cotyledon rapidly declined in control and only 52% of it was found in cotyledon at the 0.5 DAS (Figure 4(b)). Thereafter, it kept constant during 0.5–3 DAS, and slightly decreased at the 4 and 5 DAS. Compared to the control, ABA and MeJA treatments dramatically accelerated the degradation of cotyledon starch at the 0.5 DAS. Furthermore, ABA treatment also promoted the starch degradation at the 3 DAS. However, the obstacles of starch degradation were found in MeJA treatment during 3–4 DAS and in ABA treatment at the 5 DAS.

As the outcome of starch degradation, the soluble sugar content in cotyledon accordingly increased in 0.5 DAS under control conditions (Figure 4(c)). Following this increase, the sugar content continuously declined during 1.5–5 DAS and at the end about 70% of the soluble sugars disappeared in cotyledon. The ABA and MeJA treatments showed different effects on the sugar content depending on the time course. During 0.5–1.5 DAS, both treatments obviously enhanced the consumption of sugars in cotyledon.
Nevertheless, the sugar consumption was suppressed in the two treatments during 3–5 DAS.

The lipid content in cotyledon slightly increased at the initial 1.5 days, and then constantly decreased during 3–5 DAS (Figure 4d). When the experiments were finished, almost 50% of the lipid was consumed. Same to the sugar, the lipid content was differently affected by the ABA and MeJA treatments during the different periods. Comparing with the control, the lipid content was decreased by the treatments during 0.5–1.5 DAS, but was increased during 4–5 DAS. At the 5 DAS, the lipid content detected in cotyledon treated with ABA and MeJA was 1.28 and 1.54-folds of that in control, respectively.

The correlations between germination, post-germination growth and reserves content in cotyledon

To examine the relationships between cotyledon reserves mobilizations and the seed germination as well as the post-germination growth, their correlations were analyzed and the results were shown in Table 1. Under control conditions, all the parameters charactering seed germination and post-germination growth, including the germination percentage, length of seedling, WFE, WDE, WMSR and SRUE, were positively correlated to the content of soluble protein ($p < .05$), but negatively correlated to the content of starch, soluble sugar or lipid ($p < .05$). The correlations of soluble protein and sugar contents to germination percentage, length of seedling, WFE, WDE, WMSR and SRUE were not affected by the ABA and MeJA treatments. However, there was no significant correlation found between the starch content and any parameter charactering germination and post-germination growth in both treatments. Additionally, the negative

### Table 1. The correlations of items charactering germination and post-germination growth with content of the reserves.

| Treatments | Germination percentage | Starch content | Sugar content | Lipid content |
|------------|------------------------|----------------|---------------|---------------|
| Control    | 0.976**                | −0.659**       | −0.974**      | −0.790**      |
| ABA        | 0.890**                | 0.039          | −0.577*       | −0.552*       |
| MeJA       | 0.793**                | 0.083          | −0.544*       | −0.416*       |

*$p < .05.$

**$p < .01.$

Figure 4. The content variations of soluble protein (a), starch (b), total soluble sugar (c) and lipid (d) during germination and post-germination growth of A. membranaceus and the effects of ABA and MeJA. The sterilized seeds were sowed on petri dish with sterile filter paper soaked with distilled water (CT), 10 μM ABA solution (ABA), or 800 μM MeJA solution (MeJA). The contents of soluble protein, starch, total soluble sugar and lipid in cotyledon were determined in 0–5 days after sowing (DAS). All experiments were performed in triplicate (200 seeds/replicate), and error bars represent SE.
correlation of lipid content to length of seedling was changed by the ABA treatment, as well as the correlations of lipid content to germination percentage and SRUE by the MeJA treatment.

**Fatty acid compositions during germination and post-germination growth of A. membranaceus and effects of ABA and MeJA**

In the present study, nine kinds of fatty acid compositions were detected in the lipid extracted from cotyledons, including five saturated fatty acids (palmitic acid, C16:0; stearic acid, C18:0; arachidic acid, C20:0; docosanoic acid, C22:0 and tetraicosanoic acid, C24:0), two monounsaturated fatty acids (oleic acid, C18:1 and eicosenoic acid, C20:1) and two polyunsaturated fatty acids (linoleic acid, C18:2 and linolenic acid C18:3). The nine compositions represented 98.66% of the total fatty acids (Table 2).

In the heat map, the fatty acid compositions in control and treatments were clustered to four groups, according to the changes of their percentages in the lipid during 0–5 DAS (Figure 5). The fatty acids of control were clustered to group I and group II. Those of the ABA and MeJA treatments were clustered to group II–IV. The group I covered palmitic acid, stearic acid, arachidic acid, oleic acid and eicosenoic acid of the control, in which the fatty acid percentages increased during 0.5–1.5 DAS and then decreased during 4–5 DAS. In the group II the changes of the fatty acid percentages were contrary to the group I. Additionally, the percentages of the fatty acids in the group III constantly were increased during germination and post-germination growth. On the contrary, those in the group IV were decreased all the while.

For their important biological functions, the polyunsaturated fatty acids get the most attention. In this study, it was found that the linoleic acid and linolenic acid were clustered to group II in control. However, the ABA and MeJA treatments altered their clustering groups. The linoleic acid in MeJA treatment was clustered to group III and the linolenic acid in both ABA and MeJA treatments were clustered to group IV (Figure 5).

To investigate the changes in the fatty acid unsaturation degree and carbon chain length, the double bond index (DBI) and the average carbon chain length (ACCL) were determined. Under control conditions, DBI decreased in 0.5 DAS and then steadily increased during 1.5–5 DAS (Figure 6(a)). On the side of ACCL, it remained unchanged during 0.5–1.5 DAS, following with a continued rise during 3–5 DAS (Figure 6(b)). ABA and MeJA complicatedly affected the changes of DBI and ACCL during seed germination and post-germination growth. However, it was obvious that both DBI and ACCL were significantly decreased by the treatments of ABA and MeJA at the last two days (Figure 6).

![Figure 5. The heat map of fatty acid compositions. The sterilized seeds were sowed on petri dish with sterile filter paper soaked with distilled water (CT), 10 μM ABA solution (ABA), or 800 μM MeJA solution (MeJA). The fatty acid composition of lipid in cotyledon was determined in 0–5 days after sowing (DAS). The data was standardized to –1 to 1, with –1 representing for the best down-regulation and 1 for the best up-regulation, and the heat map was made.](image-url)
Figure 6. The double bond index (DBI, a) and average carbon chain length (ACCL, b) during germination and post-germination growth of A. membranaceus and the effects of ABA and MeJA. The sterilized seeds were sowed on petri dish with sterile filter paper soaked with distilled water (CT), 10 μM ABA solution (ABA), or 800 μM MeJA solution (MeJA). The DBI and ACCL in 0–5 days after sowing (DAS) were calculated from the data of fatty acid composition.

Discussion

The fresh A. membranaceus seeds generally show a germination percentage of 60% and the commercial cultivation seeds usually germinate in 4–9 weeks after they are sowed (Zhou et al. 2012). It is well known that the requirement of a long time before the germination is due to the seed dormancy mainly caused by the hardness of seed coat, which is the universal phenomenon in Leguminosae (Shibata et al. 1995). However, in the present study, the seeds of A. membranaceus started to germinate in 0.5 DAS and the germination percentage reached 72% at the 5 DAS (Figure 1(a)). Our result was similar to an earlier study reporting the mean germination time of A. membranaceus was only 10 days (Zhou et al. 2012). It was believed that the rapid germination was connected with the suitable environment conditions, such as the light and temperature (Zhou et al. 2012).

Seed germination is tightly controlled by series of internal and external factors, in which the hormones were given to the prominent place (Gazzarrini and Tsai 2015). It has been confirmed that ABA, antagonistically cross-talking with gibberellic acid, promotes seed dormancy and inhibits germination (Pritchard et al. 2002; Gazzarrini and Tsai 2015; Shu et al. 2016). As expected, our result showed that 10 μM ABA treatment intensely inhibited the seed germination of A. membranaceus. At the 1.5 DAS, the germination percentage of the seeds treated with ABA was only 11.7% of that in control (Figure 1(a)). The germination index was lowered 43% by the ABA treatment (Figure 1(b)). Moreover, ABA treatment also suppressed the post-germination growth, measured with the length of the seedling, WFE and WDE (Figure 2). Being different from ABA, MeJA seems to have two sides in regulation of the seed germination. On one side, it can promote the germination of dormancy seeds, and on the other side it can inhibit the stratified ones (Yildiz et al. 2008). In this study, 800 μM MeJA exhibited much stronger inhibition to the seed germination and the post-germination growth than the ABA in A. membranaceus (Figures 1 and 2). This is similar to the reports on Lupinus luteus (Zalewski et al. 2010) and Amaranthus caudatus (Bialecka and Kępczyński 2007). It implied that the seeds used in our experiments were stratified.

As well known, the reserves stored in seed during the maturation will provide carbon and nitrogen skeletons, as well as energy during the seed germination and post-germination growth (Eisenstadt and Mancinelli 1974). With the starting of seed germination, the reserves are promptly degraded and partially utilized to establish the new-growing tissues and organs. It has been reported that the highest loss of dry matters in the cotyledon was observed in the first 24 h during the germination of H. annuus (Erbas et al. 2016) and Brassica oleracea (Taraseviciene et al. 2009). This was confirmed in the present study, for that more than 30% dry weight of the cotyledon was reduced by 30% at the 0.5 DAS in A. membranaceus (Figure 3(a)). The speed of reserves mobilization is closely related to the situations of seed germination and the following seedling growth. There was report demonstrating that the salinity and drought inhibited the seedling growth of T. aestivum, and simultaneously induced the decrease of WMSR (Solitania et al. 2006). Here, we found that both ABA and MeJA treatments significantly reduced the WMSR during 1.5–5 DAS (Figure 3(a)). Furthermore, the inhibition of the SRUE was also observed in ABA and MeJA treatments (Figure 3(b)). So we suggested that the retarded reserves mobilization and the lowered utilization efficiency might play an important role in inhibition of germination and post-germination growth by ABA and MeJA treatments. Coinciding with our suggestion, in A. thaliana, the germination inhibition by ABA was considered being caused by the blocks of energy and building, because the inhibition was effectively alleviated by the applications of sugars and amino acids (Garciarrubio et al. 1997).

The seed reserves of protein, carbohydrate and lipid are mobilized following an inherent schedule during the germination and the early seedling growth. In most cases, the carbohydrate and protein are the firstly utilized compounds. They are, respectively, converted to sucrose for the vigorous respiration and amino acids for the new enzymes and raw materials (Taraseviciene et al. 2009; Bicalho et al. 2016; Paula et al. 2016; Mazzottini-dos-Santos et al. 2017). In this study, we found that the cotyledon starch content decreased by 48% in the initial 0.5 days (Figure 4(b)). Correspondingly, the total soluble sugar increased at the 0.5 DAS (Figure 4(c)), indicating that the starch had been quickly degraded to soluble sugar with the beginning of the seed germination. The same phenomenon has also been observed in Phaseolus vulgaris (Sfaxi-Bousbih et al. 2010). Surprisingly, the ABA and MeJA treatments promoted the starch degradation, but did
not increase the total soluble sugar content at 0.5 DAS (Figure 4(b,c)). It may be caused by the enhancement of carbon consumption for the seed respiration. In the previous studies, the increase of respiration induced by ABA and MeJA has been stated (Hemberg 1978; Chen et al. 2004). Additionally, a prominent increase of the total soluble sugar was induced by ABA and MeJA at the 3 DAS (Figure 4(c)), along with the spurt of germination percentage (Figure 1(a)). We deduced that the increase was caused by the stress signals triggered by ABA or MeJA. After all, lots of evidences have identified that the abiotic stresses can elevate the contents of soluble sugars (Chen et al. 2004; Iordachescu and Imai 2008; Sfaxi-Bousbih et al. 2010; Sami et al. 2016). Furthermore, the ABA and MeJA treatments broke the correlations of starch to every parameters characterizing germination and post-germination growth (Table 1), indicating the ABA and MeJA treatments block the role of starch playing in germination and the early seedling growth.

Besides the starch, the reserved protein was also reported as the firstly mobilized storage compound during seed germination in some plant species (Oliveira et al. 2013; Bicalho et al. 2016). However, a drastically increasing of the soluble protein content in cotyledon was observed in A. membranaceus in the initial 0.5 days after the seeds were sowed. Moreover, the protein content continuously increased during 0.5–5 DAS (Figure 4(a)). Our results coincided with an earlier study on P. vulgaris (Ribeiro et al. 2011). We supposed that the increase of the soluble protein might be achieved by the degradation of the insoluble storage proteins. It has been well known that the seed stores quantity of insoluble macromolecular proteins, such as globulin, prolamin and glutelin, which are degraded to soluble proteins, peptides and amino acids during the germination and the following seedling growth (Fukushima 1991). About the functions of the rapidly accumulated soluble proteins, they were reported to provide parts of the energy for germination and post-germination growth in legume (Bewley 1997). Both ABA and MeJA treatments obviously reduced the increase of the soluble protein content (Figure 4(a)), implying that ABA and MeJA inhibited the degradation of insoluble storage proteins. In earlier studies, ABA and MeJA were reported to directly suppress the activity of proteinase or increased the proteinase inhibitor activities (Croissant-Sych and Bopp 1988; Casaretto et al. 2004).

As a kind of high energy compound, the lipid is usually mobilized posterior to the protein and starch (Bewley 1997; Han and Yang 2015). In several plant species, the lipase activities were not activated at the germination beginning period (Rodriguez-Rosales et al. 1998). In the present study, no significant decrease in cotyledon lipid content was found in the initial 1.5 days, and then a continuous and accelerated decline appeared in the later period (Figure 4(d)). This was consistent with the reports on H. annuus (Erbas et al. 2016), A. thaliana (Graham 2008), A. aculeate (Bicalho et al. 2016) and L. usitatissimum (Sebei et al. 2007). The delay of lipid mobilization is thought to be related with its complex process, which involves the coordination of several biochemical pathways in different subcellular (Borek et al. 2006; Graham 2008). The effect of ABA on lipid mobilization during seed germination is implicated in this process, depending on the plant species and tissues (Penfield et al. 2006). The lipid mobilization was inhibited by ABA in embryos of A. thaliana (Eastmond et al. 2000; Pritchard et al. 2002; Penfield et al. 2004), but this did not happen in endosperms of A. thaliana (Pritchard et al. 2002; Penfield et al. 2004) and Nicotiana tabacum (Manz et al. 2005). In the present study, we found that the lipid content in cotyledon of A. membranaceus was reduced by ABA treatment at the 1.5 DAS. However, it was increased during 4–5 DAS (Figure 4(d)). Our results further exhibited the diversity of the effects of ABA on lipid mobilization during the germination and the post-germination growth. Meanwhile, MeJA treatment affected the lipid mobilization with a similar pattern to ABA (Figure 4(d)). The feedback of the soluble sugar content on the lipid catabolism might be involved in the alteration of lipid mobilization induced by ABA and MeJA. It has been reported that the application of exogenous sugar during germination inhibited the lipid breakdown in A. thaliana (To et al. 2002). In this work, we also found that the lipid catabolism was accelerated when the soluble sugars were over-consumed in ABA and MeJA treatments, and vice versa (Figure 4(c,d)).

The seed lipid greatly varies in fatty acid composition in different plant species (Zhang et al. 2015). In cotyledon of A. membranaceus, nine fatty acids were detected in our study and about 76% of the total fatty acids were unsaturated fatty acid (Table 2). It implied that A. membranaceus originated from the high latitude areas (Zhang et al. 2015), which coincided with its major producing areas. In G. max, the fatty acid composition in cotyledon was only slight changed during its germination (Liu and Brown 1996). However, in some other species, the prominent decrease of oleic acid and increase of linoleic acid were found during the germination (Bush and Grunwald 1972; Rodriguez-Rosales et al. 1998; Fernández-Moya et al. 2000). The similar changes were also observed in the present study (Figure 5). Moreover, we found that the saturated and monounsaturated fatty acids with chain length of 16 to 20 were ultimately decreased, but the polyunsaturated fatty acids of C18 and the very long chain fatty acids of C22 and C24 were increased (Figure 5). ABA and MeJA treatments obviously altered the cotyledon fatty acid composition during germination and the post-germination growth. In the heat map, the changes of the fatty acid percentage in control and the treatments were basically clustered to the different groups (Figure 5). Furthermore, the treatments led to the decreases of the degree of unsaturation (DBI) and the ACCL (Figure 6). It has been considered that desaturation and elongation of the fatty acids were favorable for the fast germination (Munshi et al. 2007). Additionally, the decrease of polyunsaturated fatty acids restricted the lipid catabolism pathway, in which only the fatty acids with unsaturated bond would be converted into small molecules (Porta and Rochasosa 2002; Ju et al. 2016). So we concluded that the ABA and MeJA treatments interfered the fatty acid composition, especially decreased the unsaturation and chain length, finally led to the retardation of seed germination and post-germination growth.

Conclusion

During the seed germination of A. membranaceus, the stored starch and protein in cotyledon were firstly mobilized to provide energy, enzymes and cell architectures matters. Exogenous ABA and MeJA treatments dramatically inhibited the germination and the post-germination growth, through retarding the mobilization of the reserves and lowering
their utilization efficiency. Moreover, the increase of energy consumption in the earlier stage and the disturbance of fatty acid composition also played important roles in the inhibitions of germination and post-germination growth.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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