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DEVELOPMENT OF DORMANCY IN SEEDS OF *SAPIUM SEBIFERUM* (L.) ROXB. DURING MATURATION

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Abstract

Sapium sebiferum seeds were harvested from the marked donor individual at five developmental stages of maturity to study the changes in fresh weight, dry weight, moisture content, germination percentage, endogenous inhibitor activity, and plant growth regulators (PGRs) content. Moisture content of seeds declined from 46.98% (collected on September 1) to 12.00% (collected on 1 November). Fresh weight decreased while dry weight increased significantly during seed development. Germination inhibitor activity in the seed coat and endosperm increased during seed maturation and at the same developmental stage, and inhibitor activity in the seed coat was higher than that in the endosperm. Excised embryos were capable of germinating for seeds collected on 16 September, and maximum germination percentage was observed for seeds collected on October 16. With increasing seed maturation, gibberellins (GAs) content decreased while abscisic acid (ABA) content increased in both the stratified and untreated seeds. Compared to the untreated controls, the stratified seeds were consistently found to have lower ABA content and higher GAs content throughout different developmental stages. During the course of seed development, the GAs : ABA ratio was found to steadily decrease in both the stratified and untreated seeds, with an exceptional observation for seeds collected on October 1 that underwent stratification. With increasing seed maturity, the germination of untreated seeds initially increased but declined after October 1. Stratified seeds showed the same trend, with higher germination percentage than the control at all developmental stages. Results of this study showed that seeds harvested in September were immature and incapable of germinating, whereas those harvested in November were fully mature but showed strong dormancy. This study showed that seeds are ready to harvest on October 1, and they will be naturally dispersed in November. Thus we propose to harvest seeds before the middle of October for rapid and efficient germination.

Key words: ABA, endogenous inhibitor, excised embryo, GAs

INTRODUCTION

Chinese tallow tree (*Sapium sebiferum* (L.) Roxb.; Euphorbiaceae) is a large, deciduous tree species distributed in many regions of China. It has been widely used in landscaping due to its colorful foliage and the decorative fruit in autumn. In addition, the species is also considered as an important economic tree for production of biodiesel (Potts 1946, Ruskin and Eckholm 1983, Yang and Kinghorn 1985).

S. sebiferum is commonly propagated from seeds, but its propagation is seriously impeded by seed dormancy. During seed maturation, embryo growth ceases, food reserve accumulates, the protective tegument differentiates and tolerance to desiccation develops, leading to seed dormancy (Gutierrez et al. 2007).

Studies showed that seed dormancy of many species was due to endogenous inhibitors in tissues surrounding the embryo (Bhattacharyya et al. 1999), e.g., phenolics were identified as the effective inhibitors in seeds of *Acer caesium* (Phartyal et al. 2003). Besides, some plant growth regulators (PGRs), especially abscisic acid (ABA) and gibberellins (GAs) play an important role in regulating the dormancy and germination of seeds (Ritchie and Gilroy 1998, John et al. 2002). ABA is the PGR required throughout the process of seed maturation and regulates diverse processes in plant growth and development (Finch-Savage and Leubner-Metzger 2006, Guo et al. 2008). It was confirmed that ABA affected the dormancy of mature seeds, and a direct correlation between deep dormancy and high ABA levels was revealed in many plant species (Singh and Browning

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1991, Pinfield and Gwarazimba 1992). For example, increasing levels of ABA were found to correlate closely with the gradual establishment of dormancy in seed embryos of apple (Bouyon and Bulard 1986). By contrast, GAs released seed dormancy, promoted germination and counteracted inhibitory ABA effects (Kucera et al. 2005). More recently, researchers proposed that seed dormancy was mainly regulated by the GAs : ABA ratio, and a high ratio would improve seed germination (El-Araby et al. 2006, Sudhakar et al. 2011).

The freshly collected *S. sebiferum* seeds resulted in poor germination (Li et al. 2012), and it was revealed that the dormancy in it was mainly caused by the endogenous inhibitors presented in the endosperm and seed coat (Li et al. 2011). However, quantitative assessment of seeds dormancy for seeds in different stages of maturation has not been investigated. Therefore, the aim of our investigation was to study the changes in contents of endogenous inhibitors during seed maturation of *S. sebiferum*.

MATERIALS AND METHODS

Plant material

S. sebiferum usually starts to flower in early July, and seeds mature in late October with natural dispersal in November. Experiments in this study were performed with seeds collected from a natural stand of 10-15-year old plant at Xuanwu Lake Park in Nanjing, China. Fruits were collected every half-month from September 1 (85 days after anthesis) until the natural dispersal of seeds. After collection, the capsulae wall was carefully removed to release the seeds. The fresh weight, dry weight, and moisture content were measured for seeds with the white waxy aril. All seeds used for other analyses were washed in 1% NaOH for 1 h to remove the white waxy aril. Subsequently, the seeds were rinsed with tap water, cleaned with paper and dried at room temperature for 1 day, stored in sealed plastic bags in a refrigerator at 0-5°C until November 10. The storage time was 71, 56, 41, 26, and 9 days corresponding the date of each collection.

Seed fresh and dry weights

Four replicates, each containing 50 seeds with the white waxy aril, were weighed (to 0.001 g accuracy) to obtain the fresh weight for each harvest. The dry weight was determined after drying at 80°C for 32 h to a constant weight and cooling in a desiccator containing silica gel (Pinfield and Gwarazimba 1992).

Seed moisture content

The moisture content of seeds was determined by means of a constant-temperature oven method according to the International Seed Testing Association Rules (ISTA 2011). Three replicates, each with 20 seeds, were dried in a drying oven at 103°C for 17 h, then cooled in a desiccator with silica gel, and reweighed for estimation of the moisture content on a fresh weight basis.

Germination test with excised embryos

Seeds at the different developmental stages were soaked for 2 days in running water. After removing of seed coat and endosperm, the embryos were placed in plastic boxes containing wet absorbent cotton and incubated at 25°C with an 8 h photoperiod. During the incubation, the embryos were watered every 2 days to maintain the moisture level. The germination percentage of the excised embryos was calculated after 16 days. Germination tests were done in four replicates, each containing 50 seeds.

Effect of extracts from the seed coat and endosperm on germination of cabbage seeds

Inhibitors were extracted from the coats and endosperms of *S. sebiferum* seeds with 80% methanol. The activity of each extract was determined by the germination percentage of the non-dormant seeds of cabbage (*Brassica rapa* L. Chinese Group) on filter paper moistened with the extracts. The method was according to Phartyal et al. (2003) with some modifications. First, seed coats and endosperms were separately excised from seeds harvested on September16, October 16, and November 1. Then they were separately ground into powder in liquid nitrogen, and the powder was extracted with methanol in Soxhlet apparatus. Samples (5 g) of each tissue (20 seeds were used for the seed coat extracts and 10 seeds used for the endosperm extracts) were extracted three times for 24 h, each with 100 ml of 80% (v/v) aqueous methanol at 5°C. After filtration, supernatants from three extractions for each tissue were combined and evaporated under vacuum at 37°C to remove the methanol. The final volume was made up to 50 ml by distilled water.

Germination of cabbage seeds was estimated in three replicates, each containing 100 seeds. Seeds in each replicate were soaked in 5 ml of the extract solution for 3 h, and these of controls were soaked in distilled water. Seed germination was tested in a chamber with temperature maintained at 25°C and an 8 h light photoperiod. Seedling emergence was counted daily after incubation of 6 days and a seedling was recorded as emerged when the cotyledons were visible according to ISTA rules (ISTA 2011).

PGRs content of seeds

The contents of GAs, ABA, and the ratio of GAs : ABA were measured for the freshly harvested seeds (untreated) and seeds that were treated with cold stratification (at 0-5°C) for 30 days. PGR contents were determined using an indirect enzyme-linked immunosorbent assay (ELISA) technique (Yuan et al. 2009, Bai

et al. 2011) with some modifications. In detail, 500 mg of seeds were ground into powder in liquid nitrogen and extracted in cold 80% (v/v) methanol containing 1 mM butylated hydroxytoluene as an antioxidant. The extracts were incubated at 4°C for 4 h under agitation. Samples were centrifuged using a Allegra 25R centrifuge (Beckman Coulter, Inc.) at 3500 r min^{-1} for 8 min at 4°C and the supernatant was recovered through Chromosep C_{18} columns (C_{18} Sep-Pak Cartridge, Waters Corp., Millford, MA, USA), then prewashed with 1 ml of 80% (v/v) methanol. The PGRs fractions from the columns were deluted with 5 ml of 100% (v/v) methanol and 5 ml ether. The supernatant was air-dried to remove residual methanol. The dried supernatant was dissolved in 2 ml phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin for the ELISA assay.

The concentrations of GAs and ABA were determined using a competitive ELISA method. ELISA was performed using a 96-well ELISA plate. Each well was filled with 50 µl of extract. All samples were run in three replicates, and GAs or ABA standards were included in each run for construction of a standard curve. Standard and sample absorbency was recorded at 490 nm using a microplate reader, and GAs or ABA content was measured by interpolation from the standard curve of Weiler et al. (1981).

Germination test at standard conditions

Seeds of *S. sebiferum* from each harvest were immersed in Milli-pore filtered water for 24 h at room temperature on the same day. After soaking, the seeds were placed in plastic boxes with moistened sand and stored in a refrigerator at 0-5°C for 0 or 30 days. Seeds soaked in water for 24 h with no cold stratification were used as the control. Two months later, the final germination percentage was calculated as the proportion of normal seedlings relative to the total number of seeds sown as recommended by ISTA (2011). In the germination test, there were ungerminated seeds that remained firm and viable until the end of the test, which were defined as fresh seeds (ISTA, 2011). Besides germination percentage, we also recorded the percentage of fresh seeds that was relative to the total number of seeds sown.

The inhibitory effect of the seed coats was evaluated by calculating the ratio: ((germinated embryos % - germinated seeds %) / germinated embryos %)×100 (Shang et al. 2012).

Statistical analysis

Data from the different experiments were analyzed separately. The significance of treatments was tested by one-way Analysis of variance, and Duncan's multiple range test was used to identify significant differences between means.

RESULTS

Fresh weight, dry weight and moisture content of the seed

The seeds showed a significant decrease in fresh weight from 5.240 g to 4.128 g ($p < 0.05$) from September 1 to November 1, and a significant increase in dry weight from September 1 to October 16 ($p < 0.05$), with only a small increase in dry weight observed on November 1. The moisture content of seeds decreased throughout the maturation period. By the time the seeds attained full maturity, the moisture content had declined from a maximum of 46.98% to 12.00% on the basis of fresh weight (Table 1).

Germination of excised embryos

Embryos excised from seeds collected on 1 September were immature, so they failed to germinate. The germination of excised embryos from seeds collected on September 16 was still low (42.5%) and most embryos were incapable of developing into normal seedlings. From seeds collected on October 1, when the fruit was still green, about 90% of the excised embryos were capable of germinating. The germination of embryos excised from seeds collected on subsequent dates showed no significant difference from those collected on October 1 (Table 2).

Germination of seeds with increasing maturity and cold stratification treatment

In general, with increasing seed maturity, the germination of the untreated seeds increased at the beginning,

Table 1. Fresh and dry weights and moisture content of *Sapium sebiferum* seeds on each harvest date.

Collection date	Fresh weight ± SE (g)	Dry weight ± SE (g)	Moisture content ± SE (% FW)
September 1	5.240 ± 0.098 a	2.435 ± 0.093 d	46.98 ± 1.83 a
September 16	4.907 ± 0.111 b	2.868 ± 0.064 c	42.59 ± 0.76 b
October 1	4.697 ± 0.113 c	3.089 ± 0.110 b	35.55 ± 0.53 c
October 16	4.507 ± 0.084 c	3.553 ± 0.090 a	30.18 ± 3.04 d
November 1	4.128 ± 0.112 d	3.654 ± 0.085 a	12.00 ± 0.21 e

The means ± standard error (SE) within a column followed by the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$.

Table 2. Germination of excised embryos at different collection time.

Collection date	Germination ± SE (%)
September 1	0.0 ± 0.0 c
September 16	42.5 ± 3.2 b
October 1	89.0 ± 2.9 a
October 16	94.3 ± 4.3 a
November 1	93.3 ± 6.5 a

The means ± standard error (SE) within a column followed by the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$.

Table 3. The inhibitory effect of the seed coats at different collection time.

Collection date	Inhibitory effect ± SE (%)
September 1	0.0 ± 0.0 a
September 16	33.1 ± 18.6 bc
October 1	25.6 ± 14.1 b
October 16	54.0 ± 8.2 c
November 1	80.3 ± 7.9 d

The means ± standard error (SE) within a column followed by the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$.

and was up to 66.0% for seeds harvested on October 1. Then germination declined, and was only 18.5% for seeds harvested on November 1 (seeds naturally dispersed) (Fig. 1A). This revealed that the good germination ability present at earlier developmental stages was gradually lost as the propagules approached maturity, which was caused by increasing seeds dormancy in mature seeds. With increasing seed maturity, the percentage of fresh seeds increased from 0.0% to 75.5% for the untreated seeds (Fig. 1B), indicating also that seeds harvested on November 1 possess strong dormancy.

Germination of the stratified seeds showed similar trends as that of the control. However, for seeds harvested on the same date, germination of the stratified seeds was higher than that of the control, e.g., for seeds collected on September 16, the germination was 28.8% in the control, while it was 39.5% in the stratification treatment (Fig. 1A). Seeds collected on September 1 were incapable to germinate in both the control and stratification treatment. This result is consistent with that of the germination test with the excised embryos described above. The percentage of viable seeds (germinated and fresh seeds) on September 16 was about 40% (Fig. 1A, B), which was much lower than that of seeds collected on subsequent dates. With increasing maturity, the inhibitory effect of the seed coats was changed. The minimum ratio was observed on October 1 (25.6%), and then increased to 54.0% and 80.3% on October 16 and November 1, respectively (Table 3).

Effect of seed coat and endosperm extracts on germination of cabbage seeds

Extracts from the seed coat and endosperm of *S. sebiferum* can significantly reduce the germination of cabbage seeds ($p = 0.05$). For seeds collected on different dates, seed coat extract had a stronger inhibitory effect than the endosperm extract, and the inhibitory effect intensified with increasing seed maturity. For example, the germination of cabbage seeds was 78.3% when treated with seed coat extract from seeds collected on 16 September, but it decreased to 25.7% when treated with seed coat extract from seeds harvested on 1 November. Thus, naturally dispersed seeds were supposed to contain a high content of inhibitors (Table 4).

PGRs content in seeds

No significant difference was found in GAs content for seeds harvested on September 16 and October 1 in the control (Fig. 2). However, on the subsequent harvest dates, the GAs content decreased from 17.86 ng g^{-1}

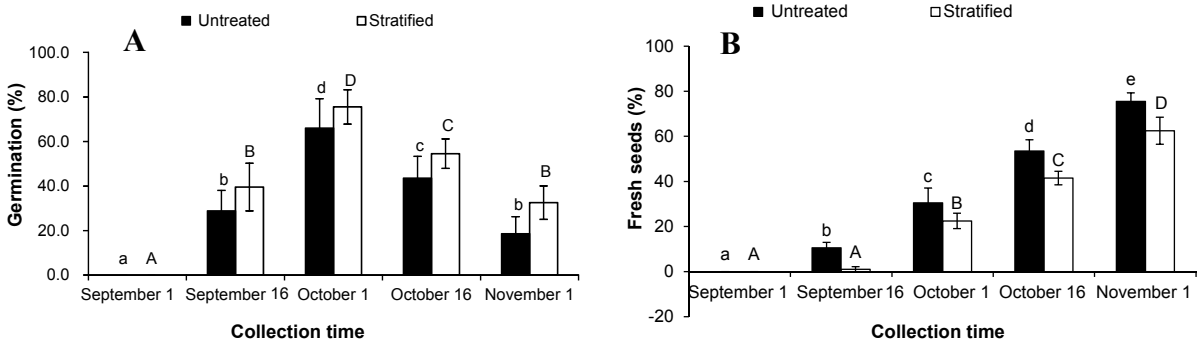

Fig. 1. A) The seeds germination for different collections without treatment and with cold stratification. B) The percentage of fresh seeds for different collections without treatment and with cold stratification.
The means ± standard errors (bars) with the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$. The statistical differences in the stratification experiment are indicated with capital letters and that in the untreated experiment are indicated with lowercase letters.

Table 4. Effect of seed coat and endosperm extracts from *Sapium sebiferum* seeds, collected on different dates during seed maturation, on germination of cabbage seeds.

Collection date	Germination ± SE (%)		
	Control	Treatment 1	Treatment 2
September 16	100	78.3 ± 2.6 a	89.3 ± 1.0 a
October 16	100	38.0 ± 1.5 b	67.7 ± 3.2 b
November 1	100	25.7 ± 2.2 c	41.7 ± 1.7 c

Note: Control, treatment 1, and treatment 2 were the experiments that cabbage seeds were soaked for 3h in water, extracts from the seed coat, and extracts from the endosperm, respectively.
The means ± standard error (SE) within a column followed by the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$.

(on October 1) to 9.57 ng g^{-1} (on November 1) in the control. Compared with the control, the stratified seeds always had a higher GAs content throughout different developmental stages (Fig. 2).

For both stratified and the control seeds, free ABA levels at the early stages (before October 1) were comparatively low. An appreciable increase in seeds ABA content was observed from October 1 to October 16, and then slight increase was recorded on November 1. The ABA content in stratified seeds was much lower than that in the control throughout the different harvest dates (Table 5).

With increasing seed maturity, the GAs : ABA ratio of seeds in the control showed a steady decrease from 0.18 to 0.04 (Fig. 3). For the stratified seeds, this ratio also declined except for that of seeds harvested on October 1. The ratio varied more significantly from 0.31 to 0.13 for the stratified seeds than the control (Fig. 3), and the lowest GAs : ABA ratio was observed in seeds harvest on November 1 for both the cold stratification treatment and the control.

DISCUSSION

Table 5. Abscisic acid (ABA) content of *Sapium sebiferum* seeds collected on different dates during seed maturation.

Collection date	ABA content ± SE (ng g^{-1})	
	Untreated	Stratified
September 16	96.54 ± 3.06 a	85.09 ± 2.61 b
October 1	107.11 ± 2.19 b	79.99 ± 0.82 a
October 16	211.99 ± 1.40 c	154.18 ± 1.93 c
November 1	223.28 ± 2.85 d	170.94 ± 2.23 d

Note: Untreated corresponds to seeds without cold stratification. Stratified indicates the seeds treated with cold stratification for 30 days.
The means ± standard errors (SE) within a column followed by the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$.

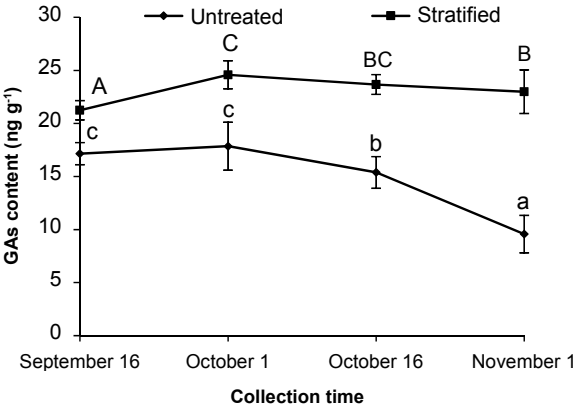

Fig. 2. Gibberellins (GAs) content of *Sapium sebiferum* seeds collected on different dates during seed maturation
Note: The curve labeled with "Untreated" corresponds to seeds without cold stratification. The curve labeled with "Stratification" stands for seeds treated with cold stratification for 30 days.
The means ± standard errors (bars) with the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$. The statistical differences in the stratification experiment are indicated with capital letters and that in the untreated experiment are indicated with lowercase letters.

Changes in inhibitor activity during seed maturation

Previous studies have revealed that chemical inhibitors play a major role in suppressing seed germination of

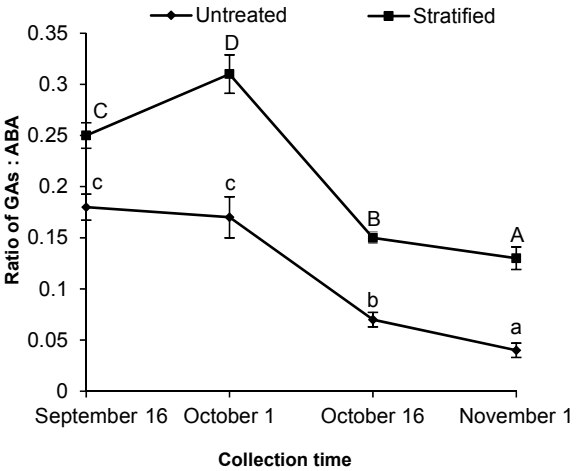

Fig. 3. Gibberellins : abscisic acid (GAs : ABA) ratio of *Sapium sebiferum* seeds collected on different dates during seed maturation
Note: The curve labeled with "Untreated" corresponds to seeds without cold stratification. The curve labeled with "Stratification" stands for seeds treated with cold stratification for 30 days.
The means ± standard errors (bars) with the same letter are not significantly different according Duncan's multiple range test at $p \leq 0.05$. The statistical differences in the stratification experiment are indicated with capital letters and that in the untreated experiment are indicated with lowercase letters.

Acer caesium (Phartyal et al. 2003). In the present study, we observed that at the same developmental stage, the activity of inhibitors in the seed coat was higher than that in the endosperm, as indicated by their inhibitory effects on cabbage seed germination. However, in an individual seed, these results did not necessarily mean that the activity of inhibitors in the seed coat was higher than that in the endosperm, because different quantities of seeds were needed to obtain the two extracts. On the other hand, with increasing seed maturity, the germination of cabbage seeds decreased significantly. Given that the decrease in cabbage seed germination reflected inhibitor activity, it can be concluded that inhibitor activity increased with seed maturation. This may be correlated with strong dormancy of mature seeds of *S. sebiferum*. However, this result differed from those of Zhang (2008) for *Fraxinus mandshurica* Rupr. He found that the intensity of seed dormancy did not reflect inhibitor activity. The different conclusions in the two experiments indicate that the factors contributing to seed dormancy differ among plant taxa.

Changes in GAs and ABA content during seed maturation

The previous studies indicated that dormancy and germination are controlled by the action and interplay of the PGRs (White et al. 2000). Among these PGRs, ABA is a positive regulator of dormancy induction and a negative regulator of germination (Kucera et al. 2005), while GAs are involved in the induction of germination (Brady and McCourt 2003, Bai et al. 2011). The present study showed that free ABA levels of the control seeds were low during seed development, but reached maximum levels at seed maturity. On the same harvest date, the ABA content of stratified seeds was lower than that of control seeds (Table 5), which was in accordance with the higher germination percentage of stratified seeds compared with the control (Fig. 1A).

The GAs content of the control seeds showed the opposite trend to that of ABA. This trend may reflect a decline in the synthesis and deposition of seed reserves, and the general retardation of metabolism due to seed desiccation (Bewley and Black 1978). After seeds were treated by cold stratification, the GAs content was markedly higher than that of the control seeds, which is presumed to reflect the breaking of dormancy by stratification.

Mature seeds contained a lower GAs : ABA ratio than immature seeds. GAs : ABA ratio was found to be negatively correlated with the intensity of seed dormancy. These results are in agreement with those of Amen (1968). In summary, seed coat and endosperm extracts and free ABA were correlated with the observed germination, which may suppress the germination of *S. sebiferum* seeds. For example, 66.0% of seeds harvested on October 1 germinated (Fig. 1A), whereas 89.0% of embryos excised from seeds collected on the same date were capable of germinating (Table 2). However, whether the embryos excised from dormant seeds are more sensitive to free ABA remains unresolved in this study.

Optimal time for seed harvest

Seed dormancy represents a complex sequence of events controlled by the physiological or structural properties of a seed and the external conditions (Pawłowski 2009). As *S. sebiferum* seeds matured, several phenomena appeared to be closely correlated: the capsule walls turned brown, the seed coat turned black, the fresh weight declined, the dry weight increased steadily but then increased more gradually in advanced stages of maturation, the moisture content decreased (Table 1) and germination percentage declined after October 1 (Fig. 1A), indicating that seed dormancy gradually occurred.

With the development of *S. sebiferum* seeds, excised embryos showed a dramatic increase in germination percentage, which was more than 89.0% for seeds harvested on and after October 1. By contrast, germination of excised embryos from seeds collected in September remained quite low, and they were not fully developed in size. Thus seeds collected in September were immature and not ready for harvest. Germination tests showed that the seeds collected in October had high germination percentage, while contained less inhibitors than those harvested in November. Thus we propose that tallow tree seeds should be harvested in October before the occurrence of natural dispersal, and would better be collected before the middle of October. Although they were not fully ripened by the time, seeds demonstrated excellent germination efficiency if treated with cold stratification. This proposal is in accordance with that for *F. mandshurica* (Zhang 2008).

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REFERENCES

Amen R. D. (1968). A model of seed dormancy. Botanical Review, 34: 1-31.

Bai T. H., Yin R., Li C. Y., Ma F. W., Yue Z. Y., Shu H. R. (2011). Comparative analysis of endogenous hormones in leaves and roots of two contrasting *Malus* species in response to hypoxia stress. Journal of Plant Growth Regulation, 30: 119-127.

Bewley J. D., Black M. (1978). Physiology and bio-

chemistry of seeds, vol.1, Springer-Verlag, New York, 306 pp.

Bhattacharyya S., Das B., Ghose T. K., Bhattacharya S. (1999). Investigation on seed germination of *Nyctanthes arbor-tristis* (Oleaceae) in relation to the total phenol content. Seed Science and Technology, 27: 321-327.

Bouyon C., Bulard C. (1986). Heterogeneity of dormancy in apple embryos: a link with chlorophyll formation and content of abscisic acid. Journal of Experimental Botany, 37: 1643-1651.

Brady S. M., McCourt P. (2003). Hormone cross-talk in seed dormancy. Journal of Plant Growth Regulation, 22: 25-31.

El-Araby M. M., Moustafa S. M. A., Ismail A. I., Hegaz Z. A. (2006). Hormone and phenol levels during germination and osmopriming of tomato seeds, and associated variations in protein patterns and anatomical seed features. Acta Agronomica Hungarica, 54: 441-457.

Finch-Savage W. E., Leubner-Metzger G. (2006). Seed dormancy and the control of germination. New Phytologist, 171: 501-523.

Guo J. J., Zeng Q. N., Emami M., Ellis B. E., Chen J. G. (2008). The GCR2 gene family is not required for ABA control of seed germination and early seedling development in arabidopsis. PLoS One, 3: e2982.

Gutierrez L., van Wuytswinkel O., Castelain M., Bellini C. (2007). Combined networks regulating seed maturation. Trends in Plant Science, 12: 294-299.

ISTA, International Seed Testing Association (2011). International Rules for Seed Testing. Zurich, Switzerland.

John V. J., David W. P., Andre T. P., Richard P. R., Lewis N. M. (2002). Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. Physiologia Plantarum, 115: 428-441.

Kucera B., Cohn M. A., Leubner-Metzger G. (2005). Plant hormone interactions during seed dormancy release and germination. Seed Science Research, 15: 281-307.

Li S. X., Liu J. J., Tian S. X., Yin T. M. (2011). Study on the causes of *Sapium sebiferum* seed dormancy and the methods for dormancy breaking. Journal of Nanjing Forestry University, 35: 1-4 (in Chinese).

Li S. X., Gu H. B., Mao Y., Yin T. M., Gao H. D. (2012). Effects of tallow tree seed coat on seed germination. Journal of Forestry Research, 23: 229-233.

Pawłowski T. A. (2009). Proteome analysis of Norway maple (*Acer platanoide*s L.) seeds dormancy breaking and germination: influence of abscisic and gibberellic acids. BMC Plant Biology, 48: 1-13.

Phartyal S. S., Thapliyal R. C., Nayal J. S., Joshi G. (2003). Seed dormancy in Himalayan maple (*Acer caesium*) II: Bioassay of inhibitors. Seed Science & Technology, 31: 13-20.

Pinfield N. J., Gwarazimba V. E. E. (1992). Seed dormancy in *Acer*: The role of abscisic acid in the regulation of seed development in *Acer platanoides* L. Plant Growth Regulation, 11: 293-299.

Potts W. M. (1946). The Chinese tallow tree as a chemurgic crop. The Chemurgic Digest, 22: 374-375.

Ritchie S., Gilroy S. (1998). Gibberellins: regulating genes and germination. New Phytologist, 140: 363-383.

Ruskin F. R., Eckholm E. (1983). Firewood crops: shrub and tree species for energy production. Vol. 2, National academy press, Washington, D.C. 92 pp.

Shang X., Xu X., Fang S. (2012). Identification and quantitative analysis of germination inhibitors in the pericarp of *Cyclocarpya paliurus* (Batal) Iljinskaja. Propagation of Ornamental Plants, 12: 195-201.

Singh Z., Browning G. (1991). The role of ABA in the control of apple seed dormancy, reappraised by combined gas chromatography-mass spectrometry. Journal of Experimental Botany, 42: 269-275.

Sudhakar N., Nagendra-Prasad D., Mohan N., Bradfor H., Gunasekaran M., Murugesan K. (2011). Assessing influence of ozone in tomato seed dormancy alleviation. American Journal of Plant Sciences, 2: 443-448.

Weiler E. W., Jordan P. S., Conrad W. (1981). Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by a specific and highly sensitive solid-phase enzyme immunoassay. Planta, 153: 561-571.

White N. W., Proebsting W. M., Hedden P., Rivin C. J. (2000). Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. Plant Physiology, 122: 1081-1088.

Yang P. Q., Kinghorn A. D. (1985). Coumarin constituents of the chinese tallow tree (*Sapium sebiferum*). Journal of Natural Products, 48: 486-488.

Yuan Y., Liu Y. J., Huang L. Q., Cui G. H., Fu G. F. (2009). Soil acidity elevates some phytohormone and β-eudesmol contents in roots of *Atractylodes lancea*. Russian Journal of Plant Physiology, 56: 133-137.

Zhang P. (2008). The dormancy and germination physiology of Manchurian ash seeds in different development stage. PhD Dissertation, Northeast Forestry University, 97 pp. (in Chinese).