Effect of Environmental Factors and Storage on Germination of Syzygium Jambos Seeds

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Abstract. The morphological characteristics of fruit and seed of Syzygium jambos were investigated in this paper. Effects of sowing matrix, illumination conditions, soil cover, storage temperature and duration on seed germination were also investigated. The berry was 4.99 ± 0.45 cm in length, 4.71 ± 0.30 cm in width, 4.42 ± 1.07cm in thickness, 38.96±7.35g in weight, and the seeds were usually 2.28 ± 0.10cm in length, 2.13±0.13cm in width, 2.24±0.15cm in thickness, and 6.67 ± 0.76g in weight. The germination percentage of fresh seeds decreased with delaying of storage time when being preserved at room temperature in sealing condition, and the seeds lost germinability after 6 months, while the seeds could prolong the life of seeds at 3°C and lost germinability after 10 months, so the seeds were likely to be recalcitrant. The seeds could reached higher germination percentage in sand, yellow soil and peat soil, but could not germinate in the nutrient soil used in this experiment. The change of illumination had no significant effect on the seed germination. Soil-buried seeds at a depth of 1 cm had a lower emergence percentage than those with only 2/3 of the volume buried in the soil.

Syzygium jambos is an large evergreen tree of Myrtaceae family, typically 6 to 15 meters high, with a tendency to low branching. There are many varieties of it worldwide. In Thailand the commonest cultivated variety bears green berried, but Malaysian variety generally bears red ones. In many regions its fruit is a shade of pale yellow, often with a slight blush[1-3]. There seems to be considerable variation in flavour if such a description has any merit; in South Africa for example, there is no noticeable bitter aftertaste, but the bouquet is decidedly assertive[4-5]. In Southeast Asian countries, its fruit is frequently served with spiced sugar, its wood is dense and accordingly used as a source of charcoal. Because of rich vitamin C, its fruit can be eaten raw or used in various regional recipes. And because of variously rich tannin that are of some antimicrobial interest, it is also used in regional traditional medicine[6-7].

It always grows in all kinds of soil, mostly grow naturally in waterfront and valley wetlands, and can grow well in sand and soil, especially fertile, deep and moist soil. It is native to Southeast Asia and Hainan Province in China, but now widely cultivated in Southern China be cultivated as a windbreak, good fruit and garden greening tree in humid tropics and south subtropics[8-10]. But it has become established and invasive in several regions. Concern has been expressed on the threat to several ecosystems, including those on several Hawaiian islands, Réunion, the Galápagos Islands, parts of Australia and the warmer regions of the Americas. However, in Hawaii, it has been almost wiped out by the introduced rust Puccinia psidii[8-9].

Because this plant have the ornamental and greening value, edible and medicinal value, so it has great research and development potential. Under natural conditions, the plant can be propagated only by seeds. The study on the seed morphology and the germination characteristics of Syzygium jambos under the different environmental conditions will be helpful for the propagation of this tree. An understanding of how different environments affect seed germination will enhance our ability to predict fluctuations in population dynamics in natural habitats. Therefore, we conducted this study to determine the seed germination pattern under different storage conditions, incubation temperatures and light regimes.
Materials and Methods

The fruits were collected in Meijiang Country Garden in May 2017 in Meizhou City, South China, where enjoys a warm and humid subtropical monsoon climate with abundant sunshine, plenty and concentrated rainfall. After being extracted the maternal trees, the seeds were picked out. After being washed, the unfilled and damaged seeds were removed, the healthy and intact ones were kept in plastic bags for use in the following experiments after air drying for about 24 h. These tests lasted for 10 months in May 2017 - March 2018.

Seed Morphology, Size, Weight and Moisture Content

The shape and color of seeds were observed and recorded. 3 replicates of fresh seeds, 20 seeds each, were weighed with electronic balances, and 20 seeds were measured with vernier calipers (accuracy 0.01mm), to obtain fresh seed weight (accuracy of 0.001g), seed MC (moisture content) and seed size. MC was measured according to the Rules of ISTA (1985).

Seed Germination and Emergence Tests

In order to investigate the fresh seeds’ germinability, a small portion of them were taken out to carry into germination test at 25°C in light. In other germination and emergence tests, 3 samples of 10 seeds each were disinfected for 10 min with 0.2% sodium hypochlorite, then washed with distilled water, and finally assigned to 90 mm-diameter petri-dishes with 0.8-1% agar. Germination and emergence were defined as the appearance of a radicle over 0.5 cm in length and that of seedling height over 0.5 cm above ground. Seed germination in light was observed every day, GP and GI were calculated, and the germination experiment lasted 28-30 d.

Germination at various temperatures Seeds were germinated in incubators at 5°C, 10°C, 20°C, 25°C, 30°C, 35°C and AT (18-32°C) with 12 h daily photoperiod (12 h in light of 800-1000 lx and 12 h in full dark).

Germination under different illumination conditions Seeds were germinated at 25°C at 12 h daily photoperiod from 8:00 AM to 20:00 PM, or in dark 24 h a day. In the dark, 16 10-seed samples were packed with an air-permeable black cloth, and these bags were then put into a closed closet. 3 samples were taken out to germinate every 7 d. The fresh seeds were placed in Petri-dishes with agar (the mixing ratio of agar and water was 1 g: 110 ml). Germination tests were carried out outdoor, indoor under natural light and in dark, 3 replicates of 10 seeds were placed in each dish. The number of germinated seeds was observed every day.

Seed germination experiments in different sowing substrates 3 replicates of 10 fresh seeds were placed in pots containing fine sand, yellow soil, nutritional soil (to buy on the market) and chaff soil respectively. All the seeds were covered with a thin layer of various soil, and only a small portion of the seed was exposed to the soil surface. The soil was fully watered, and then placed on the laboratory platform.

Seedling emergence tests of sowing depth treatment 3 replicates of 10 fresh seeds were sowed in sandy soil in outdoor flowerpots, about two-thirds of the volume of each seed was exposed to the soil surface, and the other part was buried in soil. As a control, 3 replicates of 10 fresh seeds were buried with a 1cm thick sandy soil in other flowerpots.

Seed Storage Tests

The fresh seeds were sealed with plastic bags and stored at ambient temperature (18-32°C) and low temperature refrigerators (constant temperature 3°C). Some seeds were taken out every 2 months for germination tests as mentioned above which lasted for 15d.

Statistical Data Analysis

The GP (germination percentage) and EP (emergence percentage), and GI (germination index) and EI (emergence index) were calculated from the data collected:
GP (%) = number of germinated seeds/total number of seeds per sample × 100
GI = \( \sum (G_t/D_t) \), \( G_t \) means numbers of germinated seeds after \( t \) d.
GP were transformed into angular values in data analysis. ANOVA in WPS Excel Version 2016 (Kingsoft Office Corporation, 2008–2018) was used to test if treatments were significant, and LSD test was used to determine if there were significant differences between individual treatment means.

Results

Morphological Characteristics of Mature Fruits and Seeds
The flowering period was March to April, and the fruits mature from May to Jane. The fully matured fruit was yellow, spherical and fleshy. A fruit usually contained 1 or 2 seeds. Above 95% of seeds were round, and the rest was irregular shape of convex and concave. The fresh fruits were randomly selected to measure the length, width, thickness and weight. The fruit was 4.99 ± 1.05 cm in length, 4.70 ± 1.03 cm in width, and 4.42 ± 1.07 cm in thickness, 38.96 ± 7.35 g in weight respectively. The seed was 2.28 ± 0.10 cm in length, 2.13 ± 0.13 cm in width, 2.24 ± 0.15 cm in thickness. The seeds had 1-2 or multi-embryo. and 6.67 ± 0.76 in weight respectively. The MC of fresh seeds was 25.06 ± 8.24%.

Effect of Illumination on Seed Germination
The GP of seeds outdoor and indoor was both 96.67 ± 5.77%, while the GP in dark was slightly lower, which was 93.33 ± 11.55% (Table 1). Under the 3 illumination conditions, there were no significant differences in GP and GI. But there were differences in growth under the different illumination conditions, the seedlings grew greener and stronger under strong light than under weak illumination. Per seed could grow more than 1 buds, which means that the seed has a multi-embryo phenomenon.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GP(%)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outdoor</td>
<td>96.67±5.77a</td>
<td>1.56±0.08a</td>
</tr>
<tr>
<td>Indoor</td>
<td>96.67±5.77a</td>
<td>1.46±0.18a</td>
</tr>
<tr>
<td>Dark</td>
<td>93.33±11.55a</td>
<td>1.42±0.23a</td>
</tr>
</tbody>
</table>

Note: Data show that contain the same lowercase letters after treatment between the difference was not significant (P > 0.05), and vice versa significant difference. The same as below.

Effects of Different Seeding Substrates on Seed Germination
The seeds germinated faster and reached higher EP in sand than in other substrates. In sand, the final EP was 90%, slightly higher than 86.67% in yellow soil, significantly higher than 67.67% in peat. The EP in peat after 5 d was higher than that in yellow soil, but in peat soil was overtaken that in yellow soil, it was the lowest in nutritious soil and 0%, and the EI was the same. The EI was 1.65 in sand, 1.36 in yellow soil, 0.99 in peat soil and 0 in nutritious soil (Table 2).

<table>
<thead>
<tr>
<th>Substrate category</th>
<th>GP(%)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>90.00±10.00a</td>
<td>1.65±0.12a</td>
</tr>
<tr>
<td>Yellow soil</td>
<td>86.67±5.77a</td>
<td>1.36±0.46b</td>
</tr>
<tr>
<td>Peat soil</td>
<td>66.67±5.77b</td>
<td>0.99±0.23c</td>
</tr>
<tr>
<td>Nutritious soil</td>
<td>0.00±0.00</td>
<td>0.00±0.00d</td>
</tr>
</tbody>
</table>

Effect of Sowing Depth on Seedling Emergence
The seeds with shallow sowing (i.e., only about 2/3 of the seed volume was covered in the soil) emerged 3 d earlier than those with deep sowing (seeds buried about 1cm below the soil), and the
seeds can be seen 3 d earlier. The EP of deep-sowed seeds was significantly higher than that of shallow-sowed ones, and the EI of deep-sowed seeds was 1.41 ±0.16 and significantly that of shallow-sowed ones respectively (Figure 1).

![Graph showing EP (%) and EI for different mulching modes](image)

**Mulching mode**

Figure 1. Effect of sowing depth on seedling emergence.

**Effect of Storage at Ambient Temperature on Seed Germination I**

After storage at ambient temperature for 2 months, MC of seeds was 20.16%, GP reached 66.67%, GI was 1.07. After 4 months, the MC was decreased to 15.33%, the GP decreased significantly to 16.67 %, GI to 0.40, while after 6 months, the MC was decreased to only 8.52%, and no seeds could germinate (Table 3). The storage duration at ambient temperature resulted in the decrease of seed MC, GP and GI.

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>Seed MC (%)</th>
<th>GP (%)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>20.16±5.37a</td>
<td>66.67±5.09a</td>
<td>1.07±0.24a</td>
</tr>
<tr>
<td>4 months</td>
<td>15.33±3.66b</td>
<td>16.67±1.93b</td>
<td>0.40±0.12b</td>
</tr>
<tr>
<td>6 months</td>
<td>8.52±0.76c</td>
<td>0.00±0.00c</td>
<td>0.00±0.00c</td>
</tr>
</tbody>
</table>

**Effect of Storage at Low Temperature on Seed Germination**

After storage at 3°C for 2 months, the MC was 24.23 ±7.31%, the GP was 86.67%, GI was 1.36. The MC, GP and GI of seeds were decreased continuously during the 10th month of storage at 3°C (Table 4). The rate of decrease was slower in the first 6 months, but faster in the last 4 month. Compared with the storage at room temperature, the low temperature storage could maintain the MC of seeds more effectively, prolong the time needed for inactivation, the seed still have a relatively high GP and GI for a long time.

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>Seed MC (%)</th>
<th>GP (%)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>24.23±7.31a</td>
<td>86.67±7.25a</td>
<td>1.36±0.44a</td>
</tr>
<tr>
<td>4 months</td>
<td>22.99±6.26a</td>
<td>76.46±7.03a</td>
<td>1.29±0.44a</td>
</tr>
<tr>
<td>6 months</td>
<td>20.06±5.24a</td>
<td>53.34±8.26b</td>
<td>1.03±0.26a</td>
</tr>
<tr>
<td>8 months</td>
<td>15.68±3.56b</td>
<td>22.48±2.25c</td>
<td>0.44±0.12b</td>
</tr>
<tr>
<td>10 months</td>
<td>9.64±0.77c</td>
<td>0.00±0.00d</td>
<td>0.00±0.00c</td>
</tr>
</tbody>
</table>
Discussion and Conclusion

Some studies have shown that light can stimulate or inhibit the germination of some plant seeds[^10]. However, light may not have any effect on seed germination. Tong et al (2012) found that the seed germination of *Penthorum chinense* seeds was light-dependent, Yang *et al* (2003) showed that some plant seeds had light dormancy[^11-12]. According to the sensitivity of germination to light, plant seeds can be divided into light-sensitive seeds and insensitive seeds. Seed germination is insensitive to light sensitivity, which is related to the presence of a substance called phytochrome in the seed. There was no significant difference in germination rate between fresh and stored seeds under different light conditions, which indicated that the seeds were light-insensitive.

Different plants have different requirements for environment, and their needs and affordable soil environment are also different[^13-15]. Yellow soil refers to the zonal soil formed under subtropical humid bioclimatic conditions, its main soil formation process is desilisitization and alloying of iron and aluminum oxide and clay minerals are mainly vermiculite, followed by kaolinite and hydromica, with a pH value of 4.5-5.5. Its content of surface organic matter, nitrogen, phosphorus, potassium and other nutrients were higher than red soil and lighter in texture. Nutrient soil is generally loose, permeable, fertility, lightweight, and low-salt, no disease, insects and weed seeds. Peat soil is strong in fertility, water absorption and ventilation, which contains a certain amount of humic acid, low decomposition, less nitrogen and ash content, but also has light weight, soft, high porosity and other good properties. Huang *et al*. (2012) found that before sowing, it must be paid attention to the reasonable preparation of nutrient soil, in order to obtain suitable physical properties, so as not to cause damage to plants[^16]. The GP of *Syzygium jambos* seeds in nutrient soil was 0% either indoor, outdoor or in dark conditions, which indicated that the germination of this plant seeds might be related to the excessive humic acid in nutrient soil (purchased on the market), the less strict disinfection and the higher salt content of pathogens (specific reasons to be expected). Further study showed that the seed GP in sand soil, yellow soil and peat soil was almost the same, which confirmed that *Syzygium jambos* had strong adaptability and could be planted in most natural soil substrates.

Some studies have shown that with the increase of sowing depth, seed germination rate will gradually decrease or increase first and then decrease[^13]. Cao *et al*. (2015) found that with the increase of sowing depth, the consumption of nutrients will increase, resulting in the decrease of seedling emergence rate and seedling vigor[^14]. Huang *et al*. (2005) found that the GP of *Psammochloa villosa* seeds decreased with the increase of sowing depth in sand[^16]. The deeper the seeds were sowed, the more seeds would be forced into dormancy, and the worse aeration and lower temperature at the deeper soil depth were also one of the reasons for the decrease of GP. Cao *et al*. (2015) found that the seedling EP of maize seeds decreased with increase of sowing depth, and the time of germination gradually delayed[^15]. Li *et al*. (2017) found that the GP of *Rhododendron simsii* seeds was reduced by inappropriate mulching treatment. The seed GP of *Syzygium jambos* in this experiment was lower than that of semi-mulched seeds when the soil was completely covered with 1 cm depth, so the results of this study are very similar to those of previous studies.

The external environment is an important factor on seed lifespan, which has significant effect on seed MC, temperature and humidity of preservation[^10, 15]. The seed vigor in this study has been decreasing with storage duration, and slowed first and then become faster and finally slower. Seed ripening has already entered the process of senescence. Cryopreservation can effectively reduce the respiration of seeds to slow down seed senescence, but it is only slowing down. The seed viability is still decreasing continuously. Even under the low temperature of national germplasm bank (-18°C), the seed viability will decrease slowly[^17-18]. The previous experimental results showed that, because of the different MC, the variation of respiration of seed with temperature was different, and the lower the MC, the smaller the variation[^17-18]. But to some
extent, prolonging drying duration and decreasing MC can slow down the decrease of GP. The sealed storage of plastic bags in the refrigerator obviously slowed the senescence process of seed, even though the humidity in the refrigerator was greater than that in the room, thus prolonging the seed life. From the aforementioned results of storage experiments, seeds are likely to be recalcitrant seeds.

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References


