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XANTHOCERAS SORBIFOLIUM (YELLOWHORN) SEED GERMINATION AND PHYSIOLOGICAL INDEX TEST

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ABSTRACT

Biodiesel is renewable energy which is also a high-quality diesel substitute. Because of the increasing demand of biodiesel, *X. sorbifolium*, as a good oil raw material, is taken seriously. However, seed germination is a problem for the nursery production of *X. sorbifolium*, which is difficult because of the hard, dense and poor water permeability seed coat which hinders regular germination of these seeds.

For the purpose of improving the seed germination percentage of *X. sorbifolium* seed (collected in September 2009), we studied the seeds quality, also the effects of the water, mechanical and chemical scarification on breaking the seed coat, incubated at constant temperature of 30^{o}C, and a relative humidity of 30%, with light from 7am to 11pm. Different methods were used in this research: measure the thousand kernel weight (TKW), seed viability, crude fat and fatty acid content to analyse the quality of the seeds; water treatment by soak seeds in water which initiation temperature of 80^{o}C, naturally cool down under room temperature 22^{o}C for 24hours; mechanical treatment by cut or remove seeds coat, and chemical scarification by soaking in concentrated sulphuric acid (H_2SO_4) for 2, 4 and 6hours. By measure their germination rate, germination energy, germination index and vigour index, and statistic analysis by SPSS determined how a treatment affected the *X. sorbifolium* seed germination.

Our results showed by removing seeds coat can increase the seed germination rate, which accomplished germination in 10 days with 97% germination percentage (P=0.000), 5.45cm average primary root length and 5.28 average vigour index. Water and chemical treatments also show potentials, by soaking seeds in 80^{o}C water for 24hours (22%), or in H_2SO_4 for 4hours (33%).

From the present study, it can be concluded that the quality of *X. sorbifolium* seed sample, is that the seeds are in medium size, which were plump, firm and highly endosperm content, also is highly oil content seeds, which is secondary grade oil. Therefore *X. sorbifolium* seed is qualified for making biodiesel. For promoting *X. sorbifolium* seed germination, the method of removing the seeds coat is recommended, however, mechanical treatment could be labour intensive for mass nursery production, so chemical treatments or the combination of water and mechanical treatments could be adopted as an alternative.

Further investigation is necessary to support this finding and to investigate more efficient remove seed coat method. Also more research is needed to improve seed germination. Further studies could handle the issue of *X. sorbifolium* seeds which it was not in the scope of this research, such as environmental effects (e.g. temperature, light).

Key words: *Xanthoceras sorbifolium (yellowhorn), seed germination, germination index, seed coat, seed dormancy*

CHAPTER 1 INTRODUCTION

Xanthoceras sorbifolium (yellowhorn), the sole species in the genus *Xanthoceras,* is a flowering plant in the family Sapindaceae. It is an oil bearing crop with resistance to drought or cold weather and barrenness, as a large shrub or small tree. It is original grown in northern China (Xu *et al*., 2006).

Nowadays, petroleum resource becomes increasingly deficit. Biodiesel is renewable energy which made from oil-bearing crops, oil plants, or animal fats and discarded restaurant oil, and is a high-quality diesel substitute. Also biodiesel is a typical "green energy", and development of biodiesel has great significance on sustainable economic development, which also promotes alternative energy, in order to reducing the pressure of environment, and control the urban air pollution (Han, *et al.*, 2002).

China as the representative of a large number of developing countries is accelerating the process of industrialization. With increased the demand for energy, people clearly realize that oil is no longer the inexhaustible resources of the underground (Liu, 2009).

In China Forestry Bureau publication of developing biological power source/energy (Anon, 2007), *X. sorbifolium* is defined as the only suitable trees to develop biological energy in north of China. According to the Chinese Academy of Science (CAS) research result, the conversion rate of *X. sorbifolium* oil to biological kerosene/diesel fuel is more than 94%, and it has high energy content (Anon, 2009b). Therefore as a biological oil raw material, Chinese government starts to build *X. sorbifolium* resource base, to produce biological kerosene, in order to change Chinese energy resource structure, to reduce the dependency on petroleum.

In recent years, a lot of research of *X. sorbifolium* mainly focuses on its nursery production technique (Qun, 2009). Zhao (2009) introduced the *X. sorbifolium* seedlings with production in the container could increase its survival rate up to 90% or more. He proposed the *X. sorbifolium* reproduction should promote container nursery production.

Seed germination capability is essential to the seed quality, also a key to the large acreage planting. However, there is little research about the improvement of *X. sorbifolium* seed germination, which is difficult because of the hard, dense and

poor water permeability seed coat of *X. sorbifolium* seeds (Appendix 5: Photo 1). Chen (2004) proposed that in order to increase *X. sorbifolium* rate of seed emergence, pre-treatment is an essential step before planting the seeds, 20 days to 3 months. With the traditional agriculture method, storage in sand and put under a cold temperature could improve the germination percentage, but this method has long nursery period, complex procedure and hard to control the condition. Xu *et al*., (2006) using water plus mechanical treatment to break the *X. sorbifolium* seed dormancy, obtained some positive effect.

Research on the species is being undertaken at Beijing Academy of Agriculture and Forestry sciences, in China. In this research we put forward different methods and new approaches to increase the germination percentage of *X. sorbifolium* and measure the germination percentage and physiological index, in order to provide a scientific reference for further research of the species and to promote its use as an alternative biological fuel.

CHAPTER 2 RESEARCH OBJECTIVES AND QUESTIONS

2.1 THE OBJECTIVES OF THE RESEARCH

Through this research, we aim to find out which treatment gives the best germination percentage at the most suitable conditions for seed germination in order to provide a scientific reference for further research and use of *X. sorbifolium.*

2.2 RESEARCH QUESTIONS

Main question: What/which treatment improves the seed germination percentage of *X. sorbifolium?*

Sub questions:

1) What is the quality of a fresh batch of *X. sorbifolium* seeds?

2) Are there any differences in the germination percentage, germination energy, vigour index and seedling morphology index when comparing seeds soaked in distilled water under the following conditions:

a) Control treatment: without any treatment.
b) Initiation temperature of 80^{o}C, one time soak and naturally cool down for 24 hours.
c) Initiation temperature is 80^{o}C, two times soak, and every 12 hours change the water with an initiation temperature of 80^{o}C (total soak time is 24 hours)
d) Initiation temperature is 80^{o}C, three times soak, and every 8 hours change the water with an initiation temperature of 80^{o}C (total soak time is 24 hours)

3) Are there any differences in the germination percentage, germination energy, vigour index and seedling morphology index when comparing seeds after mechanical treatment under the following conditions:

a) Control treatment: without any treatment.
b) Use knife slightly cut the seed top. Only cut one time.
c) Use knife slightly cut the seed side face. Each side cut one time.
d) Remove all the seed coat of the seed.

4) Are there any differences in the germination percentage, germination energy, vigour index and seedling morphology index when comparing seeds soaked in 96% concentrated sulphuric acid (H_2SO_4) under the following conditions:

a) Control treatment: without any treatment.
b) Soak seeds in 96% H_2SO_4 for 2 hours.
c) Soak seeds in 96% H_2SO_4 for 4 hours.
d) Soak seeds in 96% H_2SO_4 for 6 hours.

CHAPTER 3 MATERIALS AND METHODOLOGY

3.1 RESEARCH MATERIAL

Twenty kilogram seeds sample: brought from Henan San Men Xia ecological forest Co., LTD. (Ee fengjiang lu No.2, 472000, Henan, China.). These are fresh seeds which were harvested from the mother plant in September 2009. After we receive these seeds, we keep the seed under 4^{o}C in a cold storage.

3.2 RESEARCH METHODS

3.2.1 PREGERMINATION TESTS

Before the seed treatment in this research, we want to know the general quality of the seed sample. So we choose to test the following index of seeds: thousand kernel weight (TKW), seed viability, crude fat and fatty acid content.

1) Thousand kernel weight (TKW)

TKW is an indicator of the seed plumpness and seeds size. The larger the number is the fuller, bigger and better quality seeds contained in the samples, also the seed TKW differ with seed varieties and seed size. There are three grades of *X. sorbifolium* seeds TKW (Xiang, 2009): big (more than 1000 grams TKW), medium (between 700-1000 grams TKW) and small (less than 700 grams TKW).

Procedure as follows: Use the method of quartering (Guo *et al.*, 2009), randomly select seeds sample, equally divide into four parts, from each part randomly select 100 seeds and weigh, calculate the mean of four groups and multiply the number by ten, then it is TKW of total seeds.

2) Seed viability test

Seed viability is an indicator of the percentage of live seeds in the total seeds sample. The method of this test is staining, which is based on living cells having different permeability of protoplasm (Qi *et al.*, 2008). The red ink cannot stain the live seeds embryo but dead seeds only (Appendix 5: Photo 2 and 3).

Procedure as follows: Randomly select 50 seeds (for each four replications) and remove seed coat. Use knives carefully cut the embryo from its seed, stain with red ink and leave for 10 min. Count the coloured embryos and non coloured or slightly pinkly embryos, and calculate the seed viability as follows:

Viability (%) = (number of no stained embryo/seed sample) x 100%

3) Crude Fat content test

Any plant seeds or pulp which contains more than 10% crude fat has the value of making oil (Xiang, 2009). The fat content of oil plants is graded into two levels: high oil content (equal or more than 30%) and low oil content (around 20%).

Procedure as follows (Anon, 2008): Take 5g seeds (W) as test sample (6 replications), put in a test tube, add 8 ml distilled water, blend and add 10mL hydrochloric acid. Put the test tube in 75^{o}C water-bath kettle for about 40-50 minutes. Add 10ml ethanol and mix it up. After cooling down, remove it to another tube, mix with 25mL ether and shake tube for 1 minute. Release the gases, and then put the cap back. Wait for 12 minutes, and use petroleum ether to flush the fat which attached on the inside surface of tube. After 10-20 minutes, use pipette to take out the supernatant in a weighed tapered bottle (W0), then put the bottle in an oven set at 95-105^{o}C drying out for 2 hours. After cooling down, weigh it (W1).

Formula: $\text{Fat}\ (\%) = \frac{W1-W0}{W} \times 100\%$

W: sample weight (g)
W1: bottles and fat weight (g)
W0: bottle weight (g)

4) Fatty Acid content test

According to the research which carried out by Chang *et al*. (2009), the content of fatty acids is an important index for oil crops. Also Wei, (2008), present that *X. sorbifolium* seed contains over 13 kinds of different fatty acids and the quality of *X. sorbifolium* seed oil is suitable for biodiesel raw material.

Fatty acid content value (Zhang, 2004): under 0.5 is high quality oil; 0.5-1 is secondary grade oil.

Procedure as follows (Pei *et al.*, 2009): Take 5g seeds (6 replications), with 3mL 95% ethanol and grind it. Put into a test tube with another 22mL ethanol, and then put the test tube in 70^{o}C water-bath kettle for 30mins. Put into centrifugal machine for 10min and use pipette to take out the supernatant. Then use activated carbon filter to decolorize the supernatant. Then take 10mL filter liquor of supernatant add in triangle bottle, add 2 drops of phenolphthalein reagent. Then start titration with 0.05mol/L NaOH, carefully drop the NaOH into the supernatant and observe the change in colour. Stop the titration when the colour turns red and will not fade in one minute. Take record how many millilitre of NaOH been used; use this number to calculate the fatty acid content.

$$\text{Fatty acid content} = \text{Used NaOH (mL)} \times \frac{25}{10} \times \frac{1}{\text{seeds weight}}$$

3.2.2 GERMINATION TREATMENTS

There are three treatments methods, each treatment method contains 4 different treatments, and each treatment has 3 replications. Every replication use 30 seeds sample which are randomly selected from the total *X. sorbifolium* seed population.

1) Water treatments

Soak replications in distilled water which the initiation temperature is 80^{o}C, in a prepared container and then let it naturally cool down under room temperature 22^{o}C. Make sure submerge all the seed in the water.

W1: Without any treatment (control treatment).
W2: Initiation temperature is 80^{o}C, one time soak and naturally cools down for 24 hours.
W3: Initiation temperature is 80^{o}C, two times soak, and every 12 hours change the water, total soak time is 24 hours.
W4: Initiation temperature is 80^{o}C, three times soak, and every 8 hours change the water, total soak time is 24 hours.

For group W3 and group W4, at the time of the water, will put in new water which initiation temperature is also 80^{o}C.

2) Mechanical treatments

Mechanical treatment is artificially use a knife slightly cut the seed coat.

M1: Without any treatment (control treatment).
M2: Use knife slightly cut the seed top. Only cut one time. (Figure 1)
M3: Use knife slightly cut each side of seeds once. (Figure 1)
M4: Remove all the seed coat of the seed, only select the complete, no damage seed.

FIGURE 1: THE SKETCH OF M2 AND M3 TREATMENTS (TAKEN BY LIU YUHAN, 03-2010)

3) Chemical treatments

Soak seeds in 96% concentrated sulfuric acid (H_2SO_4) for various lengths of time. Each treatment has three replications. For each replication, randomly select 30 seeds from the total *X. sorbifolium* seed sample.

C1: Without any treatment (control treatment).
C2: Soak seeds in 96% H_2SO_4 for 2 hours.
C3: Soak seeds in 96% H_2SO_4 for 4 hours.
C4: Soak seeds in 96% H_2SO_4 for 6 hours.

Each replication after every treatment will be placed in disinfected petri dishes which are lined with sand. Moisten the sand then put the lid on and place in an incubator.

The incubator's condition during this research is: lighting time from 7am till 11pm (light intensity unknown), constant temperature of 30°C and a relative humidity of 30%. During germination, we will monitor the relative humidity and observe a number of germination factors (see 3.3).

3.3. OBSERVATIONS AND RECORD

3.3.1 GERMINATION TESTS

Germination testing time is 30 days. The germination percentage, germination energy (after 10 days and 20 days), vigour index and primary root length of the seeds will be monitored and calculated, in order to get a clear and comprehensive understanding of *X. sorbifolium* seed germination.

According to the general principles laid down in the International Seed Testing Association (ISTA) Handbook on Seedling Evaluation (Don, 2003), we will observe when the length of radical elongation is equal to or longer than the radius of seed length as the selection standard of successfully germinated seed.

1) Germination percentage (GP)

GP= (n/ N) x 100 %

n: the final number of germinate seeds

N: the total number of seed samples

2) Germination energy (GE) after 10 days and 20 days

During the germination tests, after 10 days and 20 days, calculate the germination energy. This indicates the uniformity of the seed germination and the germination rate.

Calculate by using the following formula (Wang, 2008):

GE = (n/ N) x 100 %

n: the number of germinated seeds after the appointed time

N: the total number of seed samples

3) Primary root length

Radical growth could increase the absorption of nutrients, and provide to the seedling, which guarantees healthy seedling growth (Xi and Kou, 2009). After 30 days, measure the primary root length (cm).

4) Vigour index

Vigour index is an index combining the seedlings growth with seed germination ability. Calculate by using the following formula (Wang and Sang, 2008).

Formula: Vigour Index (VI) = S x (GP)

S: the average length of primary root.

GP: Germination percentage.

3.4 STATISTICAL ANALYSIS

SPSS and Excel software will be used to analyse data. Comparisons of means will be performed by using the ANOVA, LSD and S-N-K's multiple range tests at 0.05 probability.

CHAPTER 4 RESULTS AND DISCUSSION

4.1. PRE-GERMINATION TESTS

As can be seen from Table 1, the mean TKW of our entire sample is 989.58g. According to the TKW grading (Xiang, 2009), our seeds sample is stand at a middle level of *X. sorbifolium* seeds size. This value of TKW (Qi *et al.*, 2008) indicates the seeds are large, plump and firm with a high endosperm content, which means it contain more nutrient for its future seedling growth.

TABLE 1: THE RESULTS OF PRE-GERMINATION TESTS OF *X. SORBIFOLIUM* SEEDS*

Pre-germination factors	**Test results**
TKW (g/1000seeds)	989.58 g
Seed viability (%)	79%
Crude fat content (%)	42%
Fatty acid content	0.6

* Data represent mean values of six replications.

The seed viability result, shows at least 79% seeds are live (viable) seeds, and have the potential to germinate successfully. This number could contain some error of measurements, affected by the error of selection of coloured embryos during the pre-treatment (Figure 2).

X. sorbifolium as an oil plant has crude fat content of 42% which is far more adequate than the standard of crude fat content of oil plants as it is belongs to the group of high oil content. Further, from the result of fatty acid test, we can see that the average of our *X. sorbifolium* seed samples' oil is secondary grade oil, therefore qualified to be a raw material of biodiesel (Wei, 2008).

FIGURE 2: THE COMPARE VIEW OF *X. SORBIFOLIUM* SEED VIABILITY TEST RESULT (TAKEN BY LIU YUHAN, 03-2010)

4.2. GERMINATION TREATMENTS

4.2.1 WATER TREATMENTS

As can be seen from the Table 2, there is no significance difference between the four water treatments (Appendix 1) on the germination percentage (P=0.262). The effects of W2, W3 and W4 treatments on the germination percentage, is decreased as the increase of time of change hot water in 24 hours, and the W1 (control treatment) gives the lowest germination percentage of 9%. This shows the water treatment has certain promoter action to the germination percentage, because by using hot water soaking treatment could soften the seed coat (Li, *et al.,* 2006). Meanwhile, hot water treatment also improves the seed coat permeability, in order to accelerate water absorption and break the seed dormancy of the seeds.

TABLE 2: THE EFFECTS OF DIFFERENT WATER TREATMENTS ON THE GERMINATION OF *X. SORBIFOLIUM* SEEDS*

Methods	Treatments	Germination percentage (%)	Germination Energy-10d (%)	Germination Energy-20d (%)	Primary root Length(cm)	Vigour Index
Water	W1	9[a]	1[a]	8[b]	1.68[a]	0.19[a]
	W2	**22[a]**	**4[a]**	**18[a]**	**3.63[a]**	**0.84[a]**
	W3	21[a]	3[a]	16[a]	2.47[a]	0.75[a]
	W4	10[a]	1[a]	7[b]	2.81[a]	0.24[a]

*Data represent mean values of three replications (30 seeds per replication). The same superscript letters indicate the treatments are not significant different from each other at P=0.05.

There is no significant differences on germination energy (Table 2) after 10 days (P=0.58), but there is a significant difference on the germination energy after 20 days (P=0.22). This also followed the similar trend as the germination percentage, showed us the *X. sorbifolium* germination rate under the different water treatments is hard to distinguish. However, after 10 days, the W2 and W3 starts to increase the germinated seeds number, and showed a comparable difference to other treatments. When comparing the primary root length and vigour index, there is no significance difference between the four water treatments on both two factors (P=0.694 and P=0.319) respectively. But we still

can find the root length of W2, W3 and W4 are both higher than W1. Perhaps the water treatments promote the radical elongation of the *X. sorbifolium* seeds.

The result suggest that when using a hot water treatment, is important to think about the hot water soaking time, and may be could also use longer time but lower temperature water.

4.2.2 MECHANICAL TREATMENTS

As can be seen from the Table 3, there are significant differences between the germination percentage, germination energy, and vigour index (all P=0.000) between treatments (Appendix 2). We expected M4 to be the best germination group, and indeed the results of the M4 group, are the best. M4 was able to greatly increase the *X. sorbifolium* seeds germination percentage and germination energy (97%).

TABLE 3: THE EFFECTS OF DIFFERENT MECHANICAL TREATMENTS ON THE GERMINATION OF *X. SORBIFOLIUM* SEEDS*

Methods	**Treatments**	**Germination percentage (%)**	**Germination Energy-10d (%)**	**Germination Energy-20d (%)**	**Primary root Length(cm)**	**Vigour Index**
Mechanical	M1	9^{c}	1^{b}	8^{b}	1.68^{a}	0.19^{b}
	M2	24^{b}	3^{b}	22^{b}	3.93^{a}	1.08^{b}
	M3	7^{c}	0^{b}	6^{b}	3.23^{a}	0.37^{b}
	M4	**97^{a}**	**97^{a}**	**97^{a}**	**5.45^{a}**	**5.28^{a}**

*Data represent mean values of three replications (30 seeds per replication). The same superscript letters indicate the treatments are not significant different from each other at P=0.05.

Li *et al.* (2007) reported that the causes of low seed germination rate are related to the hard seed coat and seed dormancy. To improve seed germination and also to ensure orderly and same emergence, we must break the seed dormancy by break the hard testa. So, M4 facilitates the *X. sorbifolium* seeds water absorption, and promotes rapid seed germination. M4 group starts to germinate on the second day after treatment, and within 10 days, maximum germination is accomplished (Figure 3).

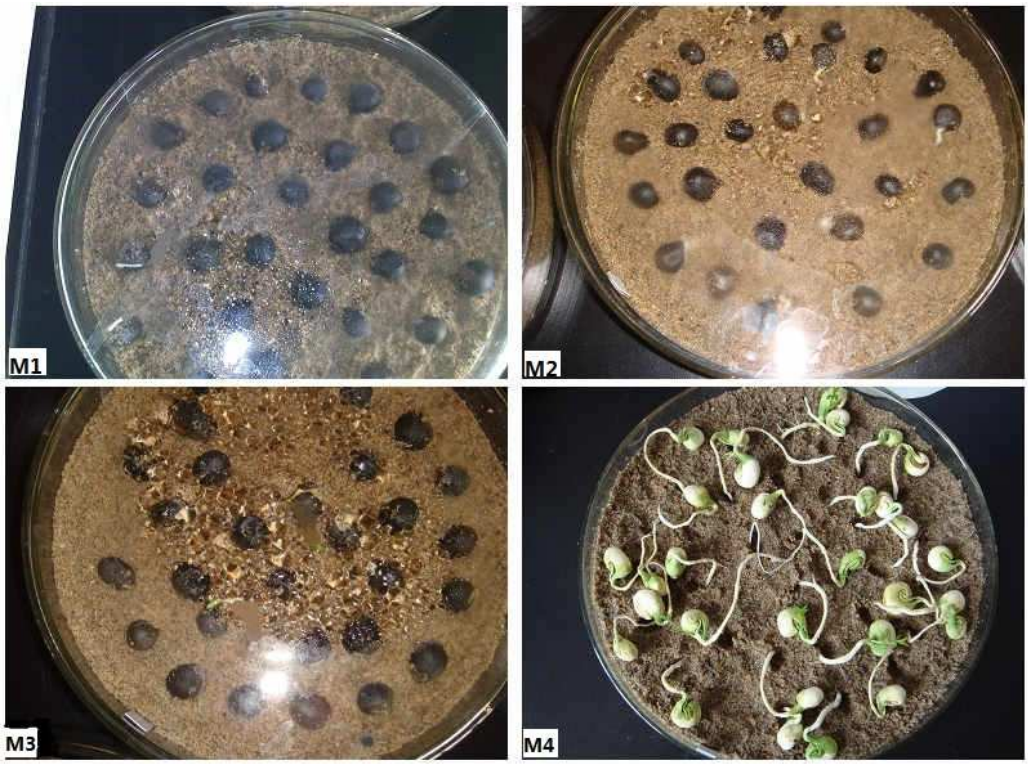

FIGURE 3: THE CONTRAST PICTURE OF GERMINATION ENERGY AT 10TH DAY WITHIN MECHANICAL TREATMENTS OF *X. SORBIFOLIUM* SEEDS (TAKEN BY LIU YUHAN, 03-2010)

When comparing M2 and M3, there is a significant difference between the two groups germination percentage. M2 has a much better result in germination percentages than the M3 treatment (24% and 7% respectively). Zhang *et al.* (2009) reported that, because the seed coat which is around the radical parts is slightly thinner than others, so manually piercing or mechanical treatments around the radical of the seed, could have a high probability of success of breaking the seed dormancy, and improve seed germination. As Figure 1 showed, the M2 cutting place is on the seeds outside, on the top seed and along with the direction of radical, which offers the seed a better way to absorb enough water to germinate, which also makes the radical emerge quicker (Wang, 2008). Although in this research, the germination rate of M2 is not very good, but I think it is possible to combine the M2 with other treatment, for example M3. This perhaps could improve the effects together on the seed, to improve a better germination percentage of *X. sorbifolium* seeds.

According to the results, there is no significant difference of mechanical treatment on the primary root length (P=0.283). However, results indicate M4 increases primary root length, because M4 starts germinated very early compare to others.

The results suggest that for seeds which have a hard coat like *X. sorbifolium* seeds, it better to remove seed coat in the nursery production to get a good germination rate. However, removes the seed coat of *X. sorbifolium* is time and labour consuming, so for an alternative way, the results also suggest that perhaps could use mechanical with other treatments.

4.2.3 CHEMICAL TREATMENTS

Table 4 shows that there is only one factor which has a significant difference within the chemical treatments (Appendix 3), which is the germination percentage (P=0.008). The number increased with the soaking time: the percentage of germination with 4 hours immersion was highest (33%), and the germination number starts to decline at somewhere between the C3 (4 hours) and the C4 (6 hours) treatment.

H_2SO_4 corrodes the seed coat and soften it which is conducive to the exchange of water and oxygen, make it more breathable (Li, 2006). As was mentioned earlier, water is an important factor for seed germination. The germination of seeds starts during imbibitions, by absorbing water, increasing free water content, to promote various physical activities inside. However, from the results, after 4 hours treatments, it shows that after an appropriate time of chemical treatments, the H_2SO_4 could starts to have a negative effect on the seeds germination. This also been proved by Wang *et al.* (2007), who showed that too long within the H_2SO_4 treatments will burn the embryo and cotyledons of seed, resulting in lower germination rate, or the loss of seed vigour.

TABLE 4: THE EFFECTS OF DIFFERENT CHEMICAL TREATMENTS ON THE GERMINATION OF *X. SORBIFOLIUM* SEEDS*

Methods	Treatments	Germination percentage (%)	Germination Energy-10d (%)	Germination Energy-20d (%)	Primary root Length(cm)	Vigour Index
Chemical	C 1	9^{b}	1^{a}	8^{a}	1.68^{a}	0.19^{a}
	C 2	21^{b}	4^{a}	21^{a}	0.81^{a}	0.19^{a}
	C3	$\mathbf{33^{a}}$	$\mathbf{11^{a}}$	$\mathbf{33^{a}}$	$\mathbf{3.06^{a}}$	$\mathbf{1.14^{a}}$
	C4	9^{b}	4^{a}	9^{a}	1.85^{a}	0.18^{a}

*Data represent mean values of three replications (30 seeds per replication). The same superscript letters indicate the treatments are not significant different from each other at P=0.05.

We found the seeds after chemical treatment seems easily get mildew (brown and green mildew) on the seed. It is perhaps because the seeds after H_2SO_4 treatments had damaged the seed coat, and do not have any protection from the seed shell, so they are more susceptible than both water and mechanical treatments. Because of the mildew infection, the germination and their primary root elongation were all influenced even damaged in different ways (Figure 4).

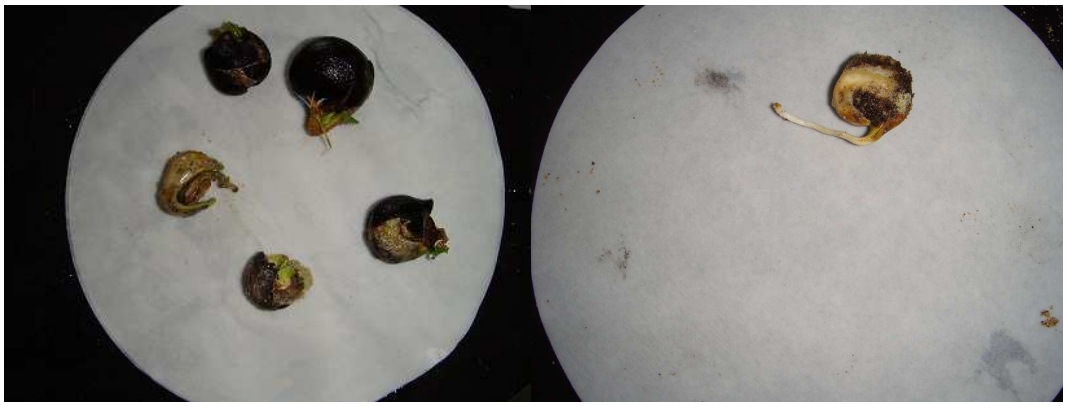

FIGURE 4: THE MILDEW INFECTION OF *X. SORBIFOLIUM* SEEDS GERMINATION AND EFFCET ON PRIMARY ROOT DEVELOPMENT IN MECHANICAL TREATMENTS (TAKEN BY LIU YUHAN, 03-2010)

4.2.4 THE OVERVIEW OF TREATMENTS

In Table 5, there are selected 4 good treatments from this research; the M4 is the best overall treatment. However, it is labour and time intensive, and there is no machinery to remove the seed coat yet so no other mechanical alternative. So it is not ready to be a good and efficient treatment for mass production. Hence, it is useful to come up with an alternative solution to improve the germination percentage.

As can be seen from Table 5, there are significant differences in each germination factor when comparing the 4 best treatments (Appendix 4) expect primary root length (P=0.000 for germination percentage, germination energy and vigour index; P=0.434 for primary root length) showing M4 to have the most significant effect. There is no significant difference between M2, W2 and C3 group. However, we still think besides M4, the M2, W2 and C3 have potential to improve the seed germination, like when comparing their root elongation to other treatments, W2, M2 and C3 all have longer root lengths than the others, which indicates that the treatments could potentially promote seed germination and provide more nutrients for the seedlings. It is also shown to be a way to improve germination quality.

TABLE 5: THE EFFECTS OF 4 SELECTED GOOD TREATMENTS FROM WATER, MECHANICAL AND CHEMICAL TREATMENTS ON THE GERMINATION OF *X. SORBIFOLIUM* SEEDS*

Treatments	**Germination percentages (%)**	**Germination Energy-10d (%)**	**Germination Energy-20d (%)**	**Primary root Length(cm)**	**Vigour Index**
W2	22^{b}	4^{b}	18^{b}	3.63^{a}	0.84^{b}
M2	24^{b}	3^{b}	22^{b}	3.93^{a}	1.08^{b}
C3	33^{b}	11^{b}	33^{b}	3.06^{a}	1.14^{b}
M4	97^{a}	97^{a}	97^{a}	5.45^{a}	5.28^{a}

*Data represent mean values of three replications (30 seeds per replication). The same superscript letters indicate the treatments are not significant different from each other at P=0.05.

What's more, it could be possible to combine the W2 and M2 treatments together for seedling production. First apply W2 treatment, and then use M2 to help seeds to break the dormancy. Using this method could complement each other with their strength and improve their effect, and could also avoid the danger of using acid treatments.

CHAPTER 5 CONCLUSION

According to the present study, it can be concluded that the quality of *X. sorbifolium* seed sample, is that the seeds are in medium size, which were plump, firm and highly endosperm content, also is highly oil content seeds, which is secondary grade oil. Therefore, *X. sorbifolium* seed is qualified to be a raw material of biodiesel, with a great potential for future use as an alternative biological fuel.

Our results indicated the significant effect of removing the seeds coat, and the potential improvement effect of other treatments. The physical effect seems to be more efficient in reducing seed dormancy. This method can be effectively used for treating *X. sorbifolium* seed samples in the laboratory. As sulphuric acid treatment may be dangerous, hot water soaking combined with mechanical treatment by cutting could be adopted as an alternative. However, further investigation is necessary to support this finding and to investigate more efficient remove seed coat method. Also more research is needed to improve seed germination. Further studies could handle the issue of *X. sorbifolium* seeds which it was not in the scope of this research, such as environmental effects (e.g. temperature, light).

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APPENDICES

APPENDIX 1 GERMINATION FACTORS ANALYSIS RESULTS OF WATER TREATMENTS

1.1 SPSS ANALYSIS OF GERMINATION PERCENTAGE WITHIN WATER TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

ANOVA

W.GR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.046	3	.015	1.609	.262
Within Groups	.077	8	.010		
Total	.123	11			

1.2 SPSS ANALYSIS OF GERMINATION ENERGY WITHIN WATER TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
ge20	4.406	3	.022	12.25000	3.4024	21.0976

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
ge10	3.000	3	.058	2.25000	-.1368	4.6368

1.3 SPSS ANALYSIS OF PRIMARY ROOT LENGTH WITHIN WATER TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

ANOVA

W.RL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.920	3	1.973	.498	.694
Within Groups	31.731	8	3.966		
Total	37.651	11			

1.4 SPSS ANALYSIS OF VIGOUR INDEX WITHIN WATER TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

W.VI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.036	3	.345	1.374	.319
Within Groups	2.010	8	.251		
Total	3.046	11			

APPENDIX 2 GERMINATION FACTORS ANALYSIS RESULTS OF MECHANICAL TREATMENTS

2.1 SPSS ANALYSIS OF GERMINATION PERCENTAGE WITHIN MECHANICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

M.GR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.621	3	.540	109.520	.000
Within Groups	.039	8	.005		
Total	1.660	11			

M.GR

	M.Treatment	N	Subset for alpha = 0.05		
			1	2	3
Student-Newman-Keuls[a]	M3	3	.0667		
	M1	3	.0867		
	M2	3		.2467	
	M4	3			.9667
	Sig.		.736	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

2.2 SPSS ANALYSIS OF GERMINATION ENERGY WITHIN MECHANICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

mge10

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1751.583	3	583.861	875.792	.000
Within Groups	5.333	8	.667		
Total	1756.917	11			

mge20

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1456.917	3	485.639	253.377	.000
Within Groups	15.333	8	1.917		
Total	1472.250	11			

2.3 SPSS ANALYSIS OF PRIMARY ROOT LENGTH WITHIN MECHANICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

M.RL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22.131	3	7.377	1.517	.283
Within Groups	38.900	8	4.863		
Total	61.031	11			

2.4 SPSS ANALYSIS OF VIGOUR INDEX WITHIN MECHANICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

ANOVA

M.VI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51.792	3	17.264	38.787	.000
Within Groups	3.561	8	.445		
Total	55.353	11			

APPENDIX 3 GERMINATION FACTORS ANALYSIS RESULTS OF CHEMICAL TREATMENTS

3.1 SPSS ANALYSIS OF GERMINATION PERCENTAGE WITHIN CHEMICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

C.GR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.124	3	.041	8.370	.008
Within Groups	.040	8	.005		
Total	.164	11			

C.GR

	C.Treatment	N	Subset for alpha = 0.05	
			1	2
Student-Newman-Keuls[a]	C1	3	.0867	
	C4	3	.0900	
	C2	3	.2133	.2133
	C3	3		.3333
	Sig.		.130	.070

3.2 SPSS ANALYSIS OF GERMINATION ENERGY WITHIN CHEMICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

One-Sample Test

	Test Value = 0					
					90% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
cge10	2.357	3	.100	5.00000	.0078	9.9922
cge20	3.019	3	.057	17.75000	3.9146	31.5854

3.3 SPSS ANALYSIS OF PRIMARY ROOT LENGTH WITHIN CHEMICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

C.RL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.710	3	2.570	.943	.464
Within Groups	21.796	8	2.725		
Total	29.506	11			

3.4 SPSS ANALYSIS OF VIGOUR INDEX WITHIN CHEMICAL TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

C.VI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.026	3	.675	2.638	.121
Within Groups	2.048	8	.256		
Total	4.074	11			

APPENDIX 4 GERMINATION FACTORS ANALYSIS RESULTS OF OVERVIEW TREATMENTS

4.1 SPSS ANALYSIS OF OVERVIEW TREATMENTS OF X. SORBIFOLIUM SEED GERMINATION TESTS

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
gp	Between Groups	1.119	3	.373	50.356	.000
	Within Groups	.059	8	.007		
	Total	1.178	11			
vi	Between Groups	41.061	3	13.687	21.099	.000
	Within Groups	5.190	8	.649		
	Total	46.251	11			
rl	Between Groups	9.380	3	3.127	1.019	.434
	Within Groups	24.541	8	3.068		
	Total	33.921	11			

APPENDIX 5 *XANTHOCERAS SORBIFOLIUM* (YELLOWHORN) SEED GERMINATION RESEARCH PHOTOGRAPHS

PHOTO 1: THE SEED OF *X. SORBIFOLIUM*, TAKEN BY LIU YUHAN, 03, 2010.

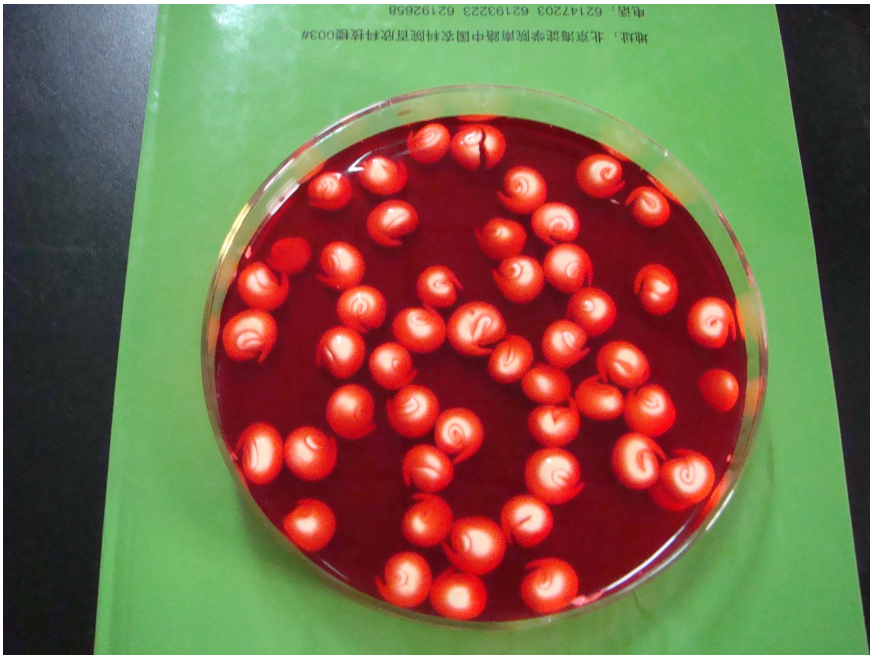

PHOTO 2: THE VIABILITY TEST OF *X. SORBIFOLIUM* SEEDS, TAKEN BY LIU YUHAN, 03, 2010.

PHOTO 3: THE SELECTION OF VIABILITY TEST OF *X. SORBIFOLIUM* SEEDS, TAKEN BY LIU YUHAN, 03, 2010.

PHOTO 4: THE MATURE SEEDLING OF *X. SORBIFOLIUM* FROM LAST YEAR, IN THE GREENHOUSE, TAKEN BY LIU YUHAN, 03, 2010.

PHOTO 5: THE PRIMARY ROOT LENGTH MEASUREMENT OF *X. SORBIFOLIUM* SEEDS, TAKEN BY LIU YUHAN, 03, 2010.

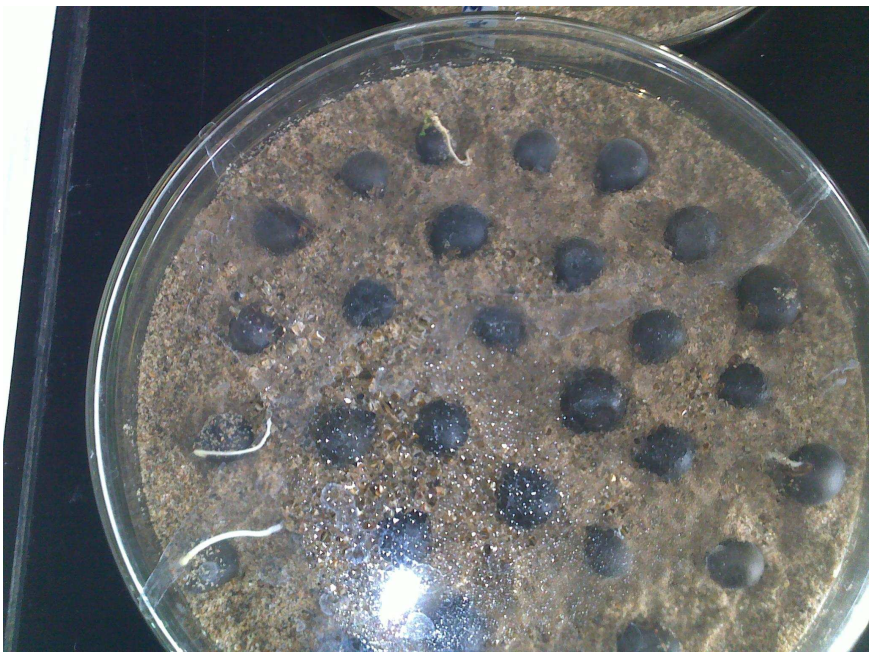

PHOTO 6: THE GERMINATION TEST OF *X. SORBIFOLIUM* SEEDS-WATER TREATMENTS, TAKEN BY LIU YUHAN, 03, 2010.

PHOTO 7: THE COMPARE OF *X. SORBIFOLIUM* SEEDS AFTER WATER TREATMENTS, TAKEN BY LIU YUHAN, 03, 2010.

[i]The Photo of title page sourced from: http://210.14.113.18/gate/big5/earth.cpst.net.cn/smycydt/zjsm/st/2009_10/256691523.html