Deutscher Tropentag 2002
Witzenhausen, October 9-11, 2002
Conference on International Agricultural Research for Development

**Pre-emergence Effeect to Imbibition of Soybean Seeds**

Sangtiwa Suriyong*, Suchada Vearasilp*, Elke Pawelzik**, Nattasak Krittigamas*, Sa-nguansak Thanapornpoonpong*
*Agronomy Department, Faculty of Agriculture, ChiangMai University, Thailand
**Institute of Agricultural Chemistry , Georg-August University Goettingen, Germany

**Abstract**

*Soybean (Glycine max L.) is grown in the irrigated areas in upper-north of Thailand. The most widely planted cultivar is Chiangmai 60 (CM60) which has cultivation limitation in that seed may not germinate or seed rot may occur if planted in clay loam soil and water logged condition. Therefore, this experiment was done to identify factors influencing on water absorption or imbibition degree and investigate imbibition pattern before seed germination process. 5 varieties of soybean seeds, seed coat thickness before soaking as well as protein and lipid content of the seeds were determined. Seed weight before and after soaking were measured. After soaking, the coats from the seeds were picked out and dried and re-soaked for 12 hours for imbibition and then seed weight were measured. Measurements were also made to evaluate changes in seed length due to cell expansion after imbibition in closed and un-closed seed. This study found that the thickest seed coat occurred in SJ5 variety whereas that of CM60 was slightly thinner. At first stage of imbibition (within first 5 hours of soaking) water enters the seed in relatively high and steady rate. At the following stage (between 5 and 12 hrs.), the imbibition process slow down and seed reaches almost full capacity of hydration. In the initial stage of imbibition ,the water absorption rates were similar in every line but CM60 variety had the highest concentration of water. This evidence probably result in higher protein contents in CM60 compared with others varieties which absorbed more water for metabolism process before germination. Moreover, its thicker seed coat allowed better water absorption. In this study, hilum and micropyle were found as important parts of the seed for the water entry in soybean seed.*

## Introduction

*Glycine max* L. Merr is an oil crop popularly grown in irrigated area after rice planting in northern Thailand. The popular variety is Chiang Mai 60 (CM60) because it can respond well to both high and low fertilizer application. Furthermore, it well adapts to a wide range of environment and provides high yield (Department of Agriculture, 1996). CM60 has cultivation limitation in that seed may not germinate or seed rot may occur if planted in clay loam soil and water logged condition.

Generally, the factors affecting seed germination are water, oxygen and temperature. When seed is undergoing the osmosis process, its cell will enlarge and seed coat becomes softened allowing oxygen diffusion in seed respiration. Moreover, water is a solvent of protoplasm and it also stimulates enzyme activity for any metabolic process of seed germination and it is used for food digestion and translocation. In each seed, the amount of water needed for seed germination depends on variety such as rice needs around 32-35 %, soybean needs around 51.1 %(Copeland, 1995). However, it was found that some seed varieties do not need much water and over watering gave low germination percentage due to lower oxygen absorption (Gulliver

and Heydecker, 1973). Therefore, seedling may die as a result of inadequate water or seed germination failure can occurs due to seed rot resulted from over watering.

The process of water osmosis into seed is called ”imbibition”. It includes adsorption and absorption processes, depending on the following factors:-

- **Seed component**; especially seed coat characteristics for water permeability, which is different among varieties. For example, the thick seed coat is slower in absorbing water than thinner one such as rice which can absorb water slower than soybean because its seed comprises pericarp, seed coat and hull whereas soybean has only thin seed coat .
- **Seed chemical composition**; Difference in chemical composition of seed determines water absorption properties. Particularly, carbohydrate has the highest quality for water absorption and followed by protein, and lipid is the poorest for water absorption.
- **Seed age;** Old age seed has higher speed of water absorption than the newly harvested seed due to deterioration of its cell membrane to keep mineral and loss of turgor pressure of cell.
- **Others factor;** Others factor are the fluctuation of water such as attachment area of adsorption between seed and soil concerning with seed size and shape, soil structure and bulk density. Moreover, Sliming, small and flat seed coat is better for attachment than rough seed coat.

At the end of seed imbibition, moisture content absorption depends on seed variety and this process occurs around the seed coat. But, in legume species, hilum and micropyle were found as important parts of the seed for the water entry.

Each seed tissue has a varying property for imbibing. Normally, embryonic tissue absorbs water faster than the supporting tissues such as endosperm and cotyledon because it always accumulates the protein and wants more water for stimulating of enzyme activity whereas most of other supporting tissues are reserve carbohydrate and lipid. Not only seed coat adsorbs to water but some seeds have also specific entrance. For legume species, John and Larsten (1987) identified that water comes into seed by seed coat especially palisade epidermis and the closed point like hilum and micropyle similar to the result of Pietrzak (2000). In addition, Gober *et al* (1997) found that rate of water absorption in soybean seed and the amount of water are different among soybean seed variety.

0 DWHULDOV HWKRGV

Five varieties of soybean seeds were used: - Chiang Mai 60 (CM60), Sukothai2 (ST2), SJ4, SJ5 and Chiang Mai 2 (CM2)). Seed qualities were then investigated until their moisture content decreased to 13 %. Seed viability was determined by Seed Germination testing (ISTA, 1993). The thickness of seed coat was determined by micrometer. Moreover, seed chemical composition was analyzed for protein content (Kjdahl method (Hach *et al*, 1985) and lipid content (AOSA 1995). In each variety, soybean seed was studied for water imbibition by the following procedure:

a) Seed weights before and after soaking for 12 hours were measured
b) After soaking, the coats from the seed were picked out and dried and re-soaked for 12 hours for imbibition and then seed weights were measured.
c) Measurements were also made to evaluate changes in seed length due to cell expansion after imbibition in closed and un-closed hilum and micropyle of seed.

**Statistical analysis:** Analysis of Variance (ANOVA) was used for analyzing the seed thickness and protein and lipid content in each variety and comparison among mean was done by least significant difference (Steel and Torie, 1960).

**Results**

***Physiological and chemical of seed****:* From table 1, it was revealed that the thickest seed coat occurred in CM2 and SJ4 variety at 10.82 and 10.64 micron respectively, and SJ5, ST2 and CM60 were slightly thinner, at around 8.9-9.26 micron. For chemical composition, there was no statistical difference in protein content but CM60 had the highest protein**.**

Table 1**.** The thickness of various soybeans seed coats and percentage of protein and lipid content**.**

| **Variety** | **Thickness of seed coat (micron)** | **Protein content(%)** | **Lipid content(%)** |
|---|---|---|---|
| SJ4 | 10.64a | 39.04 | 18.66a |
| SJ5 | 9.26b | 40.39 | 20.10ab |
| ST2 ) | 8.90b | 40.54 | 20.66bc |
| CM2 | 10.82a | 40.24 | 18.88a |
| CM60 | 9.18b | 41.55 | 22.11c |
| F-test(0.05) | ** | NS | * |
| LSD(0.01) | 1.12 | - | 1.56 |
| C.V(%) | 20 | 4.30 | 4.25 |

***Water imbibition:*** At first stage of imbibition(5 hours), water absorption rates were similar in every line then the rates declined during 5-10 hours and CM60 variety had the highest concentration of water. After that, at 10 hours it was the end point of imbibition due to the amount of water in seed was stable.

***Imbibition of re-soaked seed:*** Weight measurement of re-soaked seed indicated that CM60 had the highest point of water imbibition. And, 7 hours were needed for the end point of imbibition. But in the picked out seeds coat case it needed less time only 3 hours to reach saturation point. (Figure 2 and 3).

***Length of closed and un-closed seed:*** The study found that the length of each soybean variety that was put plug to close hilum and micropyle as closed seed had slight increase in starting time (3 hours) with a slope value at 1.5 (Figure 4), In the case of un-closed seed imbibition , the slope value was 1.8. CM2 variey has a higher length than the others.

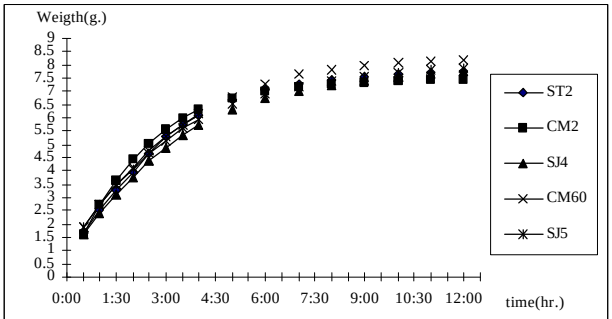


Fig 1. Weight of imbibition soybean seed

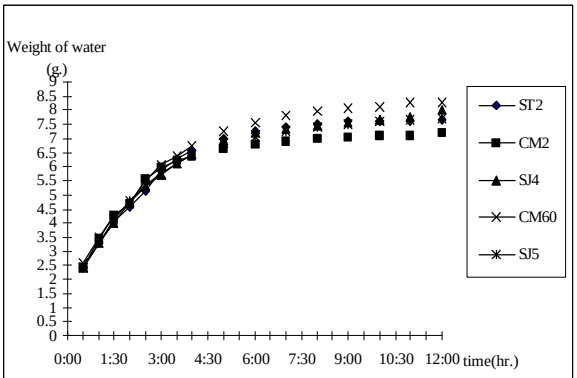


Fig 2. Water weight of re-soaked seed

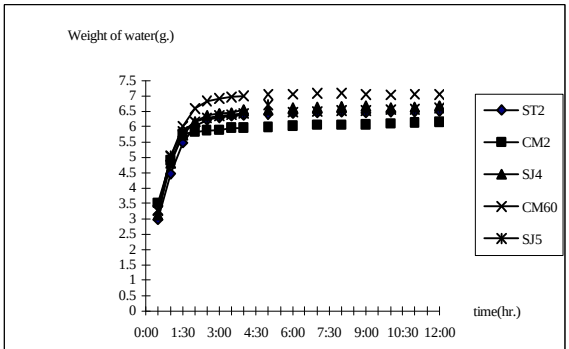


Fig3. Water weight of resoaked and picked out seed

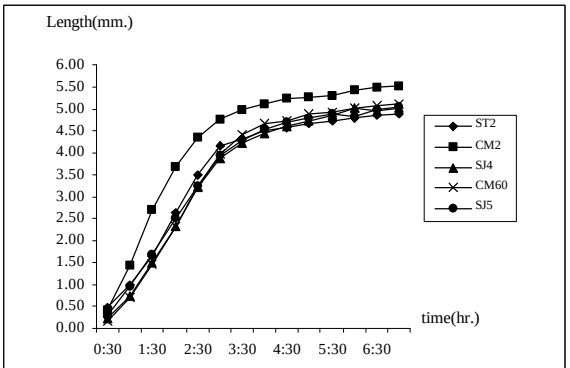


Fig. 4 Length of soaked and closed soybean seed

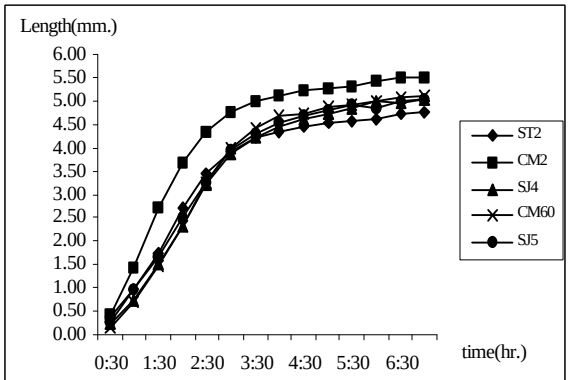


Fig. 5 Lenght of soaked and un-closed soybean seed

**Discussion and Conclusion**

Five varieties of soybean seed were measured for viability by germination test and it was found having high performance to be vigor seedling. For seed coat, CM2 and SJ2 have a thick seed coat at 10.36-10.82 micron, while CM60, ST2 and SJ5 have a thin seed coat at 8.9-9.2 micron. Although, there were no statistical difference among varieties in the protein and lipid quantity, CM60 has the highest amount of protein at 41.5 % and lipid 22.11 %. It mean that CM60 has more protein which allows more water absorption ability since to protein is the major composition sensitive to water imbibition (Copeland, 1995). At the first step of imbitition, of dry soybean seed, protein helps in protein synthetic and enzyme activity necessarily for germination process. CM60 soybean seed variety has an imbibition rate similar to the others, but it had the highest saturation point of water absorption. This evidence suggested that higher protein content in CM60 absorbed more water for metabolism processes before germination. (Fig.1) And when it is grown in the wet condition, it has a higher anaerobic process than the others due to more seed rot.

Soybean seed imbibition was divided into 3 stages. At the first 5 hours, seed rapidly absorbed water due to the difference of water potential between seed and water. Then, second stage, the amount of water absorption slightly increased in 5-10 hours and at third stage, imbibition rate declined and saturation of seed was stable in 12 hours (Fig 1). CM60 seed has a highest saturated point of water compared to others. This evidence was similar to the investigation of seed soaked for 4 hours, then dried and re-soaked seed again, whereas the imbibition was slightly slow and seed absorption rapidly absorbed for the first 4 hours and slightly decreased in 5-8 hours. It means that the seed imbibition process for 4 hours could be a reversible process because to re-soaked seed can re-imbibite and germinate again and spend much less time than normal seed. Meanwhile, every variety of the soaked seed and seed coat to be was picked out and dried and re-soaked used as less time as 3 hours for saturation to water imbibition and CM60 still absorbs the highest water content. This evidence shows that the high protein content in the cotyledon of CM60 affects performance of water absorption and seed coat is nescessary for allowig the water absorption ability. Moreover the determination of length of closed and un-closed micropyle and hilium of soybean seed (Fig 4, 5), revealed that CM2 has more enlargement in length than the others because to thick seed coat allowed better water absorption. In this study, hilum and micropyle were found as important parts of the seed for the water entry in soybean seed.

**Conclusion**

-The thick seed coat of CM2 has high expanding and allowing for water absorption

-During 12 hours of soybean seed hydration, mostly of water enters the seed through micropyle and hilum.

-Inside the seed, protein in cotyledon might be related to the seed imbibition and it is the cause of anaerobic process causing seed rot.

**References**

AOAC Official Methods 991.19.1995 Chapter 41. **Oils and Fats**. AOAC Official Method of Analysis(1995) Supplement March 1996: p.41.1-41.53.

Department of Agriculture.1996.**Document of agronomy plantation**. Department of Agriculture.
Ministry of Agriculture.Bangkok.

ISTA (International Seed Testing Association). 1993. **International Rules for Seed Testing Rules** 1993. Seed Sci. & Technol. 21:1-288.

John.B.C and N.R.Lersten.1987.**Reproductive morphology**.*In* J.R.Wilcox(ed.)Soybean: Improvement, Production and Uses Madison.American Society of Agronomy.Wisconsin.U.S.A.

Pietrzak L.N., J.Fregeau-Reid, B.Chatson and B.Blackwell.**Determination of water movement in soybean during imbibition process using nuclear magnetic resonance**.

htttp://res2.agr.ca/ecorc/pietrzak/pietrzak199061.pdf
Steel, R.G.D and J.H.Torrie, 1960. **Principle and procedure of statistics**. Mc. Graw- Hill Comp, Inc. New York