UNIVERSITI PUTRA MALAYSIA

STORAGE AND VIABILITY OF HEVEA SEEDS

SAKHIBUN BIN MOHAMAD HUSIN

STORAGE AND VIABILITY OF HEVEA SEEDS

by

SAKHIBUN BIN MOHAMAD HUSIN

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ABSTRAK

Satu pemerhatian telah dijalankan mengkaji tabiat pokok berbunga, pembentukan buah dan bijibenih pada pokok *Hevea brasiliensis* Muell Arg. klon RRIM 600. Didapati pokok berbunga lebih galak di bawah keadaan terdedah, dengan tanaman yang jarang dan di tanah pamah. Perubahan rekabentuk dan struktur buah getah berterusan dari masa mula berbuah hingga masa buah gugur. Buah-buah cukup matang dan mula gugur di antara 23 dan 24 minggu selepas berbunga. Daya hidup bijibenih adalah paling tinggi sebelum buah-buah gugur. Pada peringkat ini berat kering buah dan bijibenih adalah paling tinggi dan kandungan kelembapannya paling rendah.

Terdapat perbezaan nyata pada peratus percambahan bijibenih-bijibenih yang berbeza peringkat kematangannya dan setelah disimpan dalam jangka masa yang berlainan. Bijibenih yang diperolehi dari buah yang berumur 19 dan 20 minggu dari masa mula berbunga telah menghasilkan hanya 39% dan 52% percambahan masing-masing. Berat kering, peratus percambahan dan kesuburan anak benih bertambah pada bijibenih yang lebih matang. Bijibenih dari buah yang berumur 22 minggu dari mula berbunga telah menghasilkan peratus cambahan yang paling tinggi, tetapi bijibenih yang paling tahan lama disimpan adalah dari buah yang berumur 21 minggu. Warna buah boleh digunakan sebagai panduan untuk menganggarkan kematangan bijibenih semasa memungut buah.

Kajian kajian telah dijalankan untuk menilai kesan campuran tiga suhu bilik simpanan yang berbeza dan rawatan kimia keatas daya hidup bijibenih *Hevea brasiliensis*. Bijibenih ini yang telah disimpan dalam bilik sejuk 5°C disertakan dengan bahan kimia polietailin glikol (PEG 1500), natrium klorid (0.08 molar) dan asid absisik (25 bsj) telah menghasilkan keputusan yang sederhana dalam kemampuan memanjangkan nyawa bijibenih dalam simpanan. Kesemua rawatan dalam bilik simpanan berhawa dingin 21°C memberikan keputusan yang kurang memuaskan kecuali rawatan polietailin glikol. Dengan mencampurkan polietailin glikol dan natrium klorid pada bahan penyimpan (abuk gergaji) telah dapat melanjutkan umur bijibenih dalam simpanan setelah disimpan dalam bilik biasa dengan suhu 26°C. Di bawah suhu ini daya hidup bijibenih telah dapat dilanjutkan sahingga enam bulan dengan rawatan polietailin glikol.

Satu kaedah segera untuk menganggarkan keupayaan hidup bijibenih *Hevea* dengan menggunakan ujian tetrazolium telah disiasat. Bijibenih yang telah diberi pra-rawatan lebih lama selama 16 jam telah menghasilkan warna yang lebih baik. Bijibenih yang telah direndam dalam satu peratus 2, 3, 5 - trifenil tetrazolium klorid pada suhu 40°C selama dua jam telah menghasilkan warna merah serata yang paling baik pada embryo dan endosperma. Dengan menggunakan teknik pewarnaan ini didapati keputusan ujian tetrazolium berhubungan rapat dengan keputusan ujian percambahan bagi bijibenih yang kualitinya berbeza-beza.

Dengan membuang kulit bijibenih *Hevea* telah dapat menggalakkan percambahan yang lebih awal, tetapi kerosakan akibat serangga dan penyakit juga tinggi. Dengan hanya meretakkan kulit bijibenih percambahan bijibenih telah bertambah baik sebanyak 21% dibandingkan dengan bijibenih tanpa rawatan sebagai pengawal bandingan (control), dua minggu sesudah disemai. Anak benih dari bijibenih yang telah diretakkan kulitnya telah menghasilkan bahan kering yang lebih tinggi. Tidak terdapat kesan perbezaan yang nyata penghasilan bahan kering dan peratus percambahan di antara anak benih yang diperolehi dari bahan cambahan pasir dan abuk gergaji.

ABSTRACT

Observation on the flowering habit, fruit set and seed development of *Hevea brasiliensis* Muell Arg. was carried out on Clone RRIM 600. Flowering appeared to be more frequent under exposed, sparsely planted conditions and on flat terrain. Morphological and structural changes continued from fruit set until dehiscence. The fruits ripened and dehisced from 23 to 24 weeks after anthesis. Seed viability was highest before fruit dehiscence. At this stage the dry weight of the fruit and the seed were highest and their moisture content was lowest.

Germination percentage of seeds of different maturity and of different periods of storage was significantly different. Seeds extracted from fruit 19 and 20 weeks after anthesis gave only 39% and 52% germination respectively. Dry weight, percentage germination and seedling vigour increased in seeds that were more mature. Seeds from fruits 22 weeks after anthesis gave the highest germination percentage, but storage life was longest for seeds from 21 week old fruits. Fruit colour can be used for estimating seed maturity during fruit harvest.

Studies were carried out to evaluate the combination effect of three different storage temperatures and chemical treatments on the viability of seeds of *Hevea*. Chilling at $5^{\circ}C$ and addition of polyethylene glycol (PEG 1500), sodium chloride (0.08 molar) and abscisic acid (25 ppm) produced moderate response in prolonging the seed life in storage. All treatments in air-conditioned storage at $21^{\circ}C$, except polyethylene glycol,

gave poor results. The incorporation of polyethylene glycol and sodium chloride into the storage medium (sawdust) was found to improve the storage life of the seeds at ambient temperature (26°C). Under this storage temperature, the viability of the seeds had been extended to six months with polyethylene glycol treatment.

Tetrazolium test as a rapid method of estimating the viability of *Hevea* seeds was investigated. Preconditioning of the seeds for 16 hours gave good staining results. Seeds immersed in one percent 2, 3, 5 - triphenyl tetrazolium chloride at 40°C for two hours gave the best uniform red staining of the embryo and endosperm. Using this staining technique, it was found that the tetrazolium test results closely correlated with those of germination test for different quality seeds.

Removing the testa of *Hevea* seeds promoted early germination, but incidence of pest and disease was also high. Merely cracking the seed coats improved the germination of the seed by 21% at two weeks after sowing compared to the seeds with testa untreated as the control. Seedlings produced from seeds with cracked testa were higher in dry matter content. Seedlings obtained from sand and sawdust media showed no significant difference in dry matter content and percentage germination.

STORAGE AND VIABILITY OF HEVEA SEEDS

CHAPTER 1 INTRODUCTION

The rubber tree, *Hevea brasiliensis* Muell Arg. is indigenous to the Amazon basin in South America. It was first introduced into this country in 1876. However, none of the seedlings from this first shipment survived. Only during the second shipment in 1877, 22 seedlings were successfully grown. The tree is now widely cultivated as commercial plantation crop. Due to favourable ecological condition and freedom from endemic diseases the *Hevea* trees grow better here than their country of origin. Malaysia is now the leading natural rubber producing country accounting for about 42 - 45% of the world production. Achievement towards this end was largely due to continuous research and the good organisation in the industry.

The aspect of latex physiology, yield responses and other fundamental end-use research of *Hevea* have been well studied. However, the basic knowledge of the Botany of *Hevea* is still lacking. Little attention in particular was given to the study of the seeds.

Although *Hevea* is normally propagated vegetatively by bud-grafting, the seeds still play a vital role for the production of seedling root-stocks. The seeds are also directly used as planting material or as in the form of clonal seedling stumps.

Apart from the conventional usage as planting materials, there are other uses of *Hevea* seeds. The seeds can be utilized for oil production and seed cake. *Hevea* seed oil belongs to the same class as linseed oil (GEORGI *et al.*, 1932). It posseses drying properties and can be used for the manufacture of paints, alkaloid, resin, soap and fatty acids. The seed cake can be used as cattle feed (KOW, 1970) or as manure (MOHD. NOOR *et al.*, 1976).

The climatic condition in this country characterised by high temperature and high humidity allows the crop to be grown all year round; as such there is a demand for seeds throughout the year. The need for ready sources of stored seeds is therefore becoming increasingly important (CHIN, 1971). With government implementation of large scale land development projects for rubber cultivation together with intensive rubber replanting programme by Rubber Industry Smallholders Development Authority (RISDA), demand for *Hevea* seeds is expected to increase tremendously. Nursery operations which supply propagated materials named as budded stumps, polybag buddings and maxi stumps, depend solely on the availability of seeds for rootstocks. Besides RISDA, other land development authorities like Federal Land Development Authority (FELDA), Federal Land Consolidation and Rehabilitation Authority (FELCRA), State Economic Development Corporation (SEDC) and state land development agencies Southern Kelantan Regional Development Authority (KESEDAR), Johor Regional Development Authority (KEJORA), Trengganu Regional Development Authority (KETENGAH) and Pahang

Tenggara Development Authority (DARA) are also actively involved in rubber cultivation. These agencies require enormous quantity of seeds for the preparation of planting materials. When the requirement of planting materials by the smallholders and land schemes are in excess of what are available in the nurseries, shortage of planting material is a problem. Seeds are therefore uragently required to replenish the exhausted materials by establishing new batches of rootstocks. When seedfall does not coincide with the scheduled nursery and field planting, seed storage become essential for seeds to be readily available.

A storage system that prolongs seed viability is also essential when seeds have to be transported over long distance. It is felt that an efficient packing system as a form of seed storage in transit should be investigated so that any delay in transit does not affect seed viability badly.

About a hundred years ago when Wickham first introduced *Hevea* to this region, it was brought in as seedling and the number was limited, only 22 seedlings survived. There was no chance of bringing in seeds as they would all be dead during the long period of shipment. With the limited seedlings that survived they formed the basis of genetic material of our *Hevea* crop to-day.

The genetic resources of crop plants are the basic materials for crop improvement. Plant breeders are almost exhausted of this genetic resources and are concerned over the limitation of the narrow genetic base of *Hevea* material that they have to work with. They are also concerned on the reports of constant threat to the survival of *Hevea* trees in the Amazon River Valley due to rapid agricultural development and urbanisation. As a result the International Rubber Research Development Board (IRRDB) had organised an expedition to collect planting materials from Brazil.

During the first mission of the joint IRRDB/Brazil expedition early 1981, 64,734 seeds of only one species *Hevea brasiliensis* were collected from Acre, Rodonia and Matto-Grosso, Brazil. These seeds were then sent to Manaus where selection and treatment were carried out. The selected seeds were then despatched to Malaysia Rubber Producers' Research Association (MRPRA) in London for further selection and treatment before they were assigned to Malaysia and Ivory Coast (75% to Malaysia and 25% to Ivory Coast). The reception centre in Malaysia received 24,129 seeds out of which only 65% germinated. It took a month from time of seed collection in Brazil to the time when the seeds arrived in Malaysia. The seeds were placed in polythene bags and packed in boxes. No storage medium was used. Hence adequate storage facilities are essential to maintain a high percentage of viable seeds when they arrived their destination.

Hevea seed is a tropical recalcitrant seed (ROBERTS, 1973) and it has a short life span. Even short term storage of the seeds resulted in measurable decline in viability. Short life span of stored recalcitrant seeds may be contributed to many factors viz., intolerance to dehydration, chilling injury, microbial contamination and germination during storage (KING and ROBERTS, 1980).

As demand for high quality seeds increases, more information on factors affecting seed viability, conditions of storage, method of germination and testing seed viability are required. Studies on fruit and seed formation may also provide clues to understand seed viability. Hitherto, information on fruit and seed development of *Hevea* is lacking. Morphological and physical changes during fruit and seed growth have not been recorded. Information on the relationship of seed development, maturation and seed viability may contribute towards identifying some of the problems of seed storage, and seed quality. Seed maturity may influence seed viability. There is little information on the stages of fruit and seed development on seed viability. A study is therefore carried out to establish a relationship between fruit and seed development and seed viability by harvesting the fruits at different stages of maturity.

Rubber smallholders and planters in this country may be keen to know the viability status of seed lots purchased or collected. Hence a rapid method of determining seed viability is useful. Currently *Hevea* seed viability can only be assessed by the conventional method of seed germination (DIJKMAN, 1951; EDGAR, 1958). A germination test normally takes two to three weeks to complete. Therefore, a faster and fairly reliable method of testing seed viability should be useful for those involved in seed production and seed trade. Hence, a biochemical method of determining seed viability of *Hevea* is evaluated here.

Hevea seeds are normally sown in raised germination bed 100 cm wide and about 15 cm high. The length of the bed varies according to the quantity of seeds to be germinated. This method of germination is based on a simple and practical procedure of germinating seeds. Germination media used are those that are easily available. Other methods of germination including seed treatment and subtrata for sowing the seeds have not been fully investigated.

The aim of this project is to study the various aspects of *Hevea* seeds in relation to their viability and germinability as presented in the following chapters:-

I - Fruit and seed development in *Hevea*

II - The effect of seed maturity on storage of *Hevea brasiliensis* seeds

III - Treatment and storage of *Hevea brasiliensis* seeds

IV - Viability test for *Hevea* seeds by tetrazolium method

V - The effect of testa treatment and different subtrata on the germination of *Hevea* seeds

CHAPTER 2 LITERATURE REVIEW

2.1 - Flowering and Development of fruit and Seed

2.1.1 - Flowering in *Hevea*

Flowering in *Hevea* normally occurs after wintering. The flowers are monoecious inflorescences, appearing along with new flushes of leaves, mainly at the end of the branches (FEWERDA, 1969). They are of two types, male and female; the latter being larger than the former and are confined to the tips of the branches. GEORGE (1967) observed that wintering and flowering were found to be late on young tree. He also reported that the sex ratio varied between clones. The general ratio of male to female flowers is 77 : 1.

The inflorescence matures over a period of one to two weeks. FEWERDA (1969) reported that mature inflorescence consists of strongly scented flowers having no petals but only five-lobed perianth tubes. The female flowers are larger and are rounder in shape. The male flowers are slender, each flower contains ten stamens arranged in two series of five lying above each other around a central column.

It was observed that small insects acts as pollinators (MAAS,1919). Later it was suggested that midges in the family Heleidae play an important role in transfering *Hevea* pollen (WARMKE, 1951 & 1952). However, under Malaysian conditions, RAO (1961) reported that only three types of midges in the family Ceratopogonoidae were responsible for pollination in *Hevea* flowers.

2.1.2 - Fruit development

Some *Hevea* clones are known to be self-incompatible (DIJKMAN, 1951). However, it was generally accepted that *Hevea* shows no preference for cross-pollination over self-pollination (MOHD NOOR, 1980). After fertilization, the percentage fruit set is generally very low. FEWERDA (1969) quoted that not more than five percent of the initial number of female flowers develop into mature fruits, while MOHD NOOR *et al.* (1976) reported that natural fruit set in *Hevea* is only approximately one percent. Premature fruit shedding in *Hevea* is also common (GEORGE, 1967).

The *Hevea* fruit is a large three-lobed capsule which usually contains three walnut-size seeds (FEWERDA, 1969). Under normal circumtances, the fruit takes about five to five and half months from anthesis to full maturity (EDGAR, 1968; PREMAKUMARI, 1975). The *Hevea* fruit is a hard woody capsule which dehisces violently when ripe throwing the three seeds to a distance away from the plant (ENOCH, 1980).

2.1.3 - Seed development

A seed is defined as a fertilized and ripened ovule (BATNAGAR and JOHRI, 1972). Seeds are normally enclosed in a fruit which is a ripened ovary.

The initial step in the formation of fruit and seed is the blooming of flower buds which signified sexual maturity. After fertilization some morphological and physical changes take place in the ovule and the ovary which ultimately form a mature seed capable of reproducing another plant.

At anthesis, the moisture content of the ovary and unfertilized ovule is in the region of 80 - 90%. KERSTING *et al.* (1961) working with sorghum and later LEININGER & URIE (1964) studying safflower seeds found that the initial moisture of unfertilized flowers was 90% (wet basis). Moisture usually increases after fertilization for a short while. With further seed development its moisture decreases until an equilibrium is established with the field environment (DODDS and PELTON, 1967).

During the process of cell division, cell expansion and differentiation of structures, POLLOCK and ROOS (1972) reported that the size of the seed increases, but its moisture content remains constant and high. ABDUL-BAKI and BAKER (1973) showed that seed size increases further until a maximum is reached together with the attainment of maximum fresh weight at rather high moisture content. There is actual loss of moisture as the seed matures.

2.2 - Seed maturity

As the fruit reaches the end of its growth period and development, it undergoes, some characteristic qualitative changes. This last phase of seed growth and development is called maturation (NITSCH, 1965; NITSCH, 1970).

ABDUL-BAKI and BAKER (1973) reported that seed maturation begins when seed reaches maximum fresh weight and terminates at seedfall. Earlier, LEOPOLD (1964) differentiated maturation from fruit ripening. He described maturation as the processes associated with a fruit reaching its full size, while ripening refers to the processes which qualitatively transformed