Fruit and Seed Development in Hevea *(Clone RRIM 600) in Malaysia*

SAKHIBUN BIN MOHAMAD HUSIN, H.F. CHIN* AND Y.L. HOR*

Observation of 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 twenty-three to twenty-four weeks after flowering. Seed viability was highest before fruit dehiscence. At this stage the dry weights of the fruit and the seed were highest and their moisture content was lowest.*

Research on *Hevea* seed has received little attention. Most published accounts[1] deal with rubber seed oil research. Reports on fruit and seed development have been very limited. Dijkman[2] reported the variation in the time of seedfall. In Java seedfall is normally between January and March, while in Sumatra and Malaya it is between October and November. Edgar[3] briefly described the general morphology of *Hevea* flowers. He postulated that the low percentage of seed set in *Hevea* might be the result of too few insects of the right kind necessary for pollination of the flowers. Even after seed set, fruit abortion may occur. Ross[4] observed that a heavy drop of immature fruits occurs approximately midway between pollination and fruit dehiscence.

Investigation on the viability of *Hevea* seeds at various stages of maturity was initiated by Premakumari[5]. She studied the problem of rotting of the capsule in India by analysing the seed viability with green capsules harvested at various stages of maturity. Seeds collected from the capsules two weeks before seedfall gave a good percentage of germination. *Hevea* seed development has not been studied in this country. This paper outlines the results of a study made on the morphological and structural changes of the fruits and seeds from flowering to seedfall. Changes in moisture content and dry weight at various stages of seed maturity and their relationship with seed viability were also studied.

*Universiti Pertanian Malaysia, Serdang, Selangor

MATERIALS AND METHOD

Flowering

Field observation of flowering was conducted in *Field 45* and *Field 31* of the RRIM Experiment Station, Sungei Buloh, Selangor. One hundred clonal trees (RRIM 600), forty-eight trees in *Field 45* and fifty-two trees in *Field 31* were marked. The trees in *Field 45* were sparsely planted (7.5 x 3.6 m with a density of 358 trees per hectare) along the road boundaries on flat terrain, while the trees in *Field 31* were planted closer to one another (9.0 x 2.4 m, with a density of 445 trees per hectare) along the hill contour. Recording of the date of flowering and observation of the frequency of flowering were conducted once every two weeks.

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Inflorescences were tagged with a record of the date of flowering. General observations on the morphological changes of the developing fruit were made once every two weeks. Green capsules were harvested at fortnightly intervals beginning at four weeks after flowering. From the eighteenth week till dehiscence, harvesting of capsules was done weekly and ten capsules were collected randomly each time. The capsules were opened and the seeds were removed. The testa, endosperm and the embryo were separated and in each case the stages of development were recorded.

Changes in Moisture Content and Dry Weight, and their Relationship to Seed Viability

Fifty capsules each containing three seeds were harvested from the tree at random. The harvesting was also done at fortnightly intervals beginning at fourteen weeks from the opening of flowers. From eighteen weeks till seed dehiscence, harvesting was done weekly. The fresh weights of the capsules, fruit-wall, seeds and the various parts of the seed were recorded. These parts were dried in a ventilated oven at 105°C for 16 hours. The dry weight was recorded and the percentage moisture (wet basis) was determined. The analyses of moisture content and dry weight were done in ten replicates of one capsule each, followed by germination tests to determine the viability of the seeds. The seeds were sown on moist sand and evaluated at fourteen and twenty-one days. This test was conducted in four replicates of twenty-five each.

RESULTS AND DISCUSSION

Flowering

Among the 100 trees under observation, inflorescences were seen on forty-eight trees in *Field 45*. The inflorescences, though not in abundance, were present continuously from February to September. Usually *Hevea* trees flower twice a year[6,7]. The first or main flowering season occurs after the annual leaf-shedding (wintering) aroung February to April. Inflorescences of the second season appear after the primary seedfall in August to October.

Clone RRIM 600 is a prolific seeder[8]. These clonal trees in *Field 45* were planted along the road boundaries and were fully exposed to sunlight. Possibly under such conditions, and besides being good seeders, these trees bloomed more frequently. Of the remaining trees which were planted along the hill contour in *Field 31*, the canopies of the trees were close to each other, and they only flowered during the main season between February and April.

Development of Fruits and Seeds

Fruit development. The unfertilised female flowers withered and dropped but in the fertilised flower, the ovary began to grow, unless damaged or diseased it continued to grow and form the fruit. A fruit normally contains three seeds. But occasionally four and five seeds may be found in each capsule. A young fruit gradually increased in size during the first four weeks. Afterwards, the increase became more rapid *(Figure 1)*. The fruit reached its maximum size at twelve weeks after

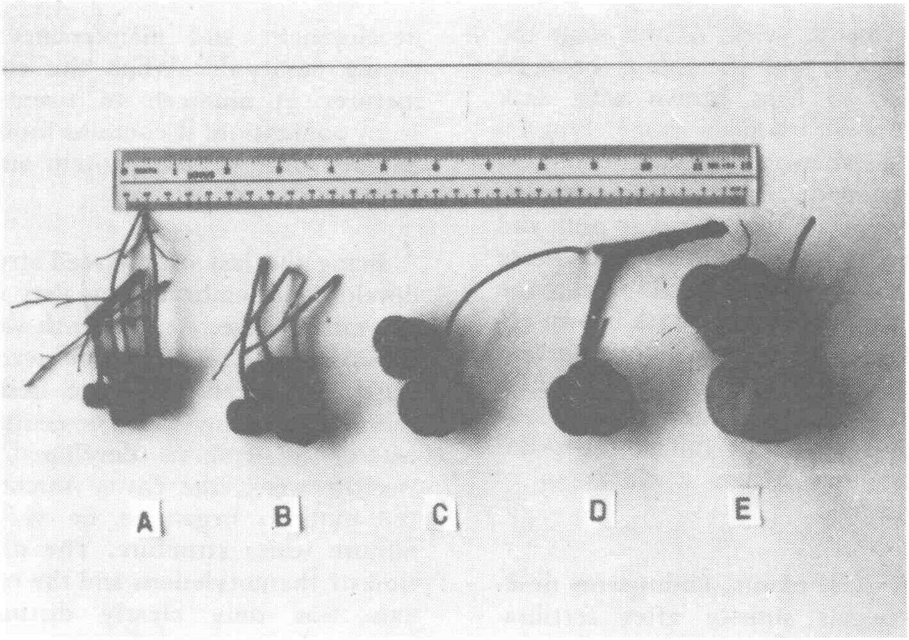

Figure 1. Various stages of fruit development in Hevea. *(A) 4 weeks, (B) 6 weeks, (C) 8 weeks, (D) 10 weeks and (E) 12 weeks.*

flowering. The fruit-wall is a protective structure made up of the outer layer the epicarp and the thicker inner layer, the endocarp. During early development of the fruit, the endocarp is very soft and dull white in colour. The epicarp is light green and at this stage latex oozed out profusedly when pricked.

Once the fruit had attained its maximum size, its external structure remained unchanged until it matured, except for minor colour variation of the epicarp from light green to dark green. When the capsule was fourteen weeks old, the endocarp hardened and became woody at the sixteenth week. The green colouration of the epicarp faded when it was twenty weeks old. At this stage there appeared to be no latex in the epicarp. Finally the epicarp dried up and shrivelled. When sufficiently dried the capsule dehisced at about twenty-three to twenty-four weeks from flowering. During fruit dehiscence the stalk of the inflorescence (the peduncle) remained attached to the tree while the dry fruit opened suddenly with noticeable explosion liberating the seeds and dispersing the woody endocarp a distance away from the tree.

Seed development. At ten weeks after tagging, the seed appeared as a whitish pendulous structure on the placenta in each loculus of the tricarpellary syncarpous ovary. As the seed grew, several structural changes took place.

Ten weeks after flowering, the testa appeared as a whitish fragile protective tissue. As the seed grew the testa increased in size and attained its full size at the fourteenth week. At this stage the testa hardened and its colour changed from white to light brown with dark brown spots of irregular shape. Progressive colour changes and hardening of tissues continued after this until the eighteenth week when it changed to pink and the tissues became harder and more fibrous. At the nineteenth week, the colour of the testa turned dark brown exhibiting the characteristic colour pattern of the seed of RRIM 600 *(Figure 2)*. Thereafter no more structural or colour changes took place. At full maturity the testa fibre may attain a thickness of 800 microns[9].

In most seed plants, endosperm development begins shortly after fertilisation[10, 11]. In *Hevea* seed, it was observed that at four weeks after flowering, the endosperm was already formed, but was still soft and watery. At the tenth week, the endosperm appeared as a white mass of fleshy tissue enveloping the undifferentiated embryo. By the fourteenth week, the endosperm soon reached its full size. From this stage onwards, an analysis of weight changes of the endosperm showed that there was a progressive increase in dry weight but its fresh weight remained constant. Increase in dry weight probably indicates an increase in the activity of accumulating food reserves. Pollock and Roos[12] reported that deposition of reserve materials continues in most dicotyledonous seeds after the seed has attained maximum size so that the dry weight increases more rapidly than the fresh weight. When fully matured at twenty-two weeks from tagging, the firm endosperm appeared as a thick mass of pale cream structure. In *Hevea* seed, the endosperm persists as a storage organ. Its role is to provide nutrients for the development and maintenance of the young embryo[13]. When the endosperm matures at nineteen to twenty weeks from pollination, it contains food reserves in the form of oil, protein and carbohydrates[14].

Being the last of the seed structure to develop, the embryo grew very slowly at the initial stage. At the tenth week from flowering the embryo sac was already filled completely with the fleshy endosperm which enveloped a central cavity where the embryo developed. At the twelfth week, the cavity thickened and the embryo began to be visible as a minute white structure. The differentiation of the cotyledons and the root/shoot axis was only clearly distinguishable within the vacuole after the fourteenth week. The embryo then grew rapidly to form two large flat cotyledons lying parallel to the upper and lower surfaces of the seed with a prominent root/shoot axis. By the sixteenth week, the cotyledons attained the maximum size. At the eighteenth week it was still suspended within the vacuole and could be easily isolated from the endosperm. At the nineteenth week the embryo was fully formed and the cotyledons became fused to the endosperm. During this stage it was difficult to separate the cotyledons from the endosperm. The cotyledons in *Hevea* seed are somewhat fleshy; they do not contain the main food reserves. In seed germination they act as digesting and absorbing structures that transfer food reserves from the endosperm to the growing point of the embryo[15]. The arrangement of the cotyledons in the endosperm was observed to be variable. In a cross-section of the endosperm, the cotyledons formed a central band which was sometimes wavy or V-shaped. In

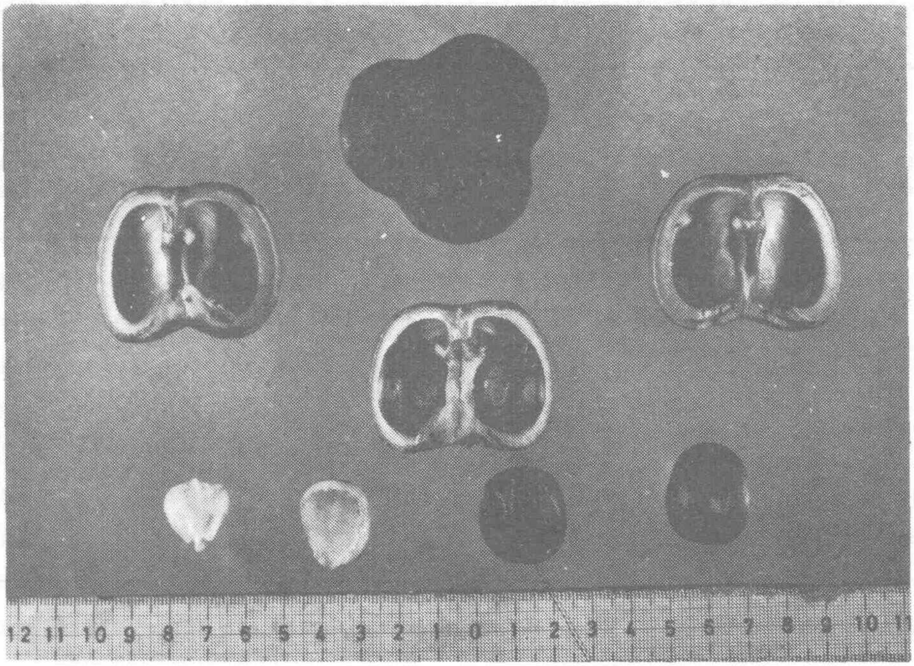

Figure 2. Hevea *fruits, seeds, seed structures (endosperm and embryo) nineteen weeks from flowering.*

fresh seeds, the cotyledons lie close together, but in dry seeds they separate leaving a hollow space in between. Enoch[16] suggested that the air space between the cotyledons is due to shrinkage of the endosperm as the seeds dry up.

Changes in Moisture Content and Dry Weight in Relation to Seed Viability

Changes in moisture content. Young *Hevea* fruits and seeds have very high moisture content. At the sixteenth week the moisture content of the capsule and fruit-wall was above 70%, and the moisture content of the seed was 66%. At this time, the various parts of the seed also have more than 80% moisture except the testa which have only 39% moisture *(Table 1)*.

With further fruit and seed development, the moisture content decreased. As the fruit and seed matured, the loss of moisture in the fruit, seed, fruit-wall, testa, endosperm and embryo followed almost the same pattern. The decrease in moisture content was slow and steady until the eighteenth week. From then on until the twenty-first week the moisture content remained almost constant. This constant phase was clearly indicated by a non-significant decrease in the mean percentage moisture of the fruit, fruit-wall, seed and various parts of the seed *(Table 1)*. Twenty-one weeks after flowering there was a rapid and significant decrease in the moisture content indicating that fruit and seed ripening and desiccation of the fruit-wall had occurred. Pollock and

TABLE 1. MEAN PERCENTAGE MOISTURE OF *HEVEA* FRUIT, SEED AND VARIOUS PARTS OF THE SEED AT DIFFERENT STAGES OF MATURITY

Weeks from flowering	Mean moisture content (%)						
	Pod	Fruit -wall	Seed	Testa	Endo-sperm	Coty-ledons	Root/ shoot axis
16	74.7[a]	78.6[a]	66.3[a]	39.0[a]	84.0[a]	80.6[a]	82.8[a]
18	65.2[b]	67.7[b]	57.4[b]	28.7[b]	70.2[b]	57.5[b]	66.7[b]
19	62.4[bc]	66.9[bc]	46.5[c]	25.6[b]	59.3[c]	51.7[bc]	48.3[c]
20	61.4[c]	66.5[bc]	41.5[c]	15.1[c]	56.9[c]	51.7[bc]	49.3[c]
21	60.0[c]	65.1[c]	42.0[c]	19.5[d]	54.9[cd]	48.7[c]	60.7[b]
22	25.6[d]	18.5[d]	35.9[d]	13.6[d]	49.1[d]	51.2[bc]	49.8[c]
Seedfall	–	–	24.2[e]	13.8[d]	30.8[e]	30.1[d]	34.2[d]

Values within columns having similar subscripts are not significantly different at 1% level.

Roos[12] suggested that as most large seeds mature, the moisture in the seed decreases until an equilibrium is established with the field environment (at about 20% moisture content). At seedfall the moisture in *Hevea* seed seemed to establish an equilibrium with the field environment. At this stage the moisture content of the cotyledons and endosperm was 30%. This value is close to the result of cotyledon analysis by Ang[17]. He estimated the moisture content of the kernel of fresh rubber seed to be about 35%.

It took ten weeks for the moisture content of *Hevea* seeds to decrease from 70% when they were young to 24% at seed dehiscence. Dodds and Pelton[18] reported that atmospheric conditions partially affect the rate of reduction of seed moisture in wheat. Field observation showed that there was variation in the drying time of *Hevea* fruits. Such variation within the same inflorescence may affect the pattern of seedfall.

Changes in dry weight. The dry weight of pod, seed and various part of the seed increased steadily with age until they were eighteen weeks old. Weight increase from sixteen weeks to eighteen weeks appeared to be faster *(Table 2)*. However, there was no significant weight increase of the testa at this stage. With further development, the dry weight continued to increase slowly, reaching their maximum at twenty-one weeks. At seedfall there was a slight decrease in the dry weight of the endosperm. The loss of weight could be due to mobilisation of reserves by the cotyledons to the embryonic tissue to keep it alive during the quiescent stage.

Seed viability. In most cases seed viability is associated with seed maturity.

TABLE 2. MEAN DRY WEIGHT OF FRUIT, SEED AND VARIOUS PARTS OF THE SEED OF *HEVEA* AT DIFFERENT STAGES OF MATURITY

Weeks from flowering	Mean dry weight						
	Pod (g)	Fruit-wall (g)	Seed (g)	Testa (mg)	Endo-sperm (mg)	Coty-ledons (mg)	Root/ shoot axis (mg)
16	15.1^{a}	9.7^{a}	1.8^{a}	1 298.5^{a}	435.9^{a}	60.8^{a}	2.5^{a}
18	23.8^{b}	16.2^{b}	2.5^{b}	1 388.3^{abcd}	999.4^{b}	142.0^{b}	4.1^{b}
19	25.3^{bc}	16.4^{b}	2.9^{c}	1 374.6^{abc}	1 346.8^{cd}	174.8^{bc}	5.3^{c}
20	25.5^{bc}	16.4^{b}	3.0^{c}	1 463.1^{bcd}	1 276.5^{c}	178.8^{bc}	3.8^{b}
21	27.3^{c}	17.4^{b}	3.3^{d}	1 543.1^{d}	1 543.9^{d}	218.7	5.0
22	25.2	16.4	3.0	1 522.2	1 227.6^{c}	209.0	4.7
Seedfall	–	–	2.9	1 308.5	1 383.7^{cd}	224.8	5.3

Values within columns having similar subscripts are not significantly different at 1% level.

Maturation is attained when the seed reaches its maximum fresh weight[19], or at the time when there is no further increase in the dry weight[20]. During the process of fruit and seed development, a stage was reached when their moisture content remained constant. At this stage there was also no significant increase in dry weight. Results of the germination test showed that at the sixteenth week there was no germination and at the eighteenth week only 2% germination (twenty-one days after sowing). At nineteen weeks from flowering, 39% of the seed samples germinated *(Figure 3)*. This indicated the beginning of seed germinability. The percentage germination increased as the dry weight increased, while the moisture content decreased. When the fruit moisture content decreased. When the fruit moisture content declined to 25%, it was sufficiently dry and ready to dehisce. During this stage (twenty-two weeks from flowering) the germination percentage was the highest (97%). Bartel[21] found that in wheat and barley the more mature the seeds the higher the potential vigour and germinability. Similarly in *Hevea* seeds, the highest germination rate was at full maturity.

It may be inferred that *Hevea* seed become germinable when the decrease of moisture content is constant and the increase of dry weight is not significant. Maximum germinability is attained before seed dehiscence when the fruit is at full maturity. Therefore, it is recommended that seeds from dry capsules be harvested before seed dehiscence so that the

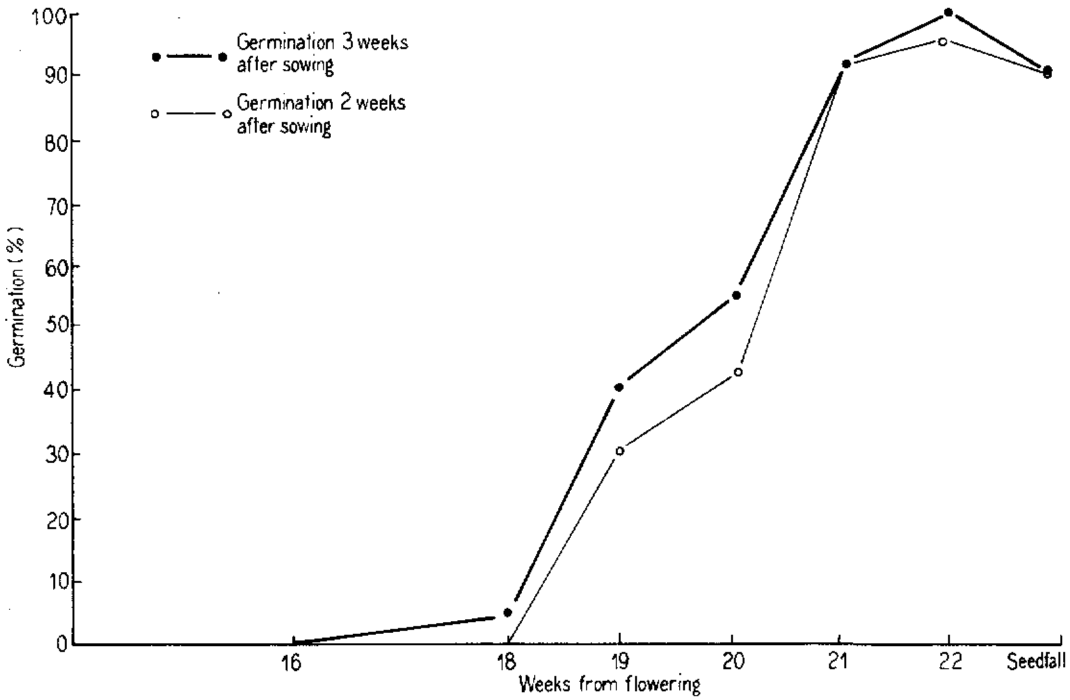

Figure 3. Mean germination percentage of Hevea *seeds at different maturity.*

seed samples would give the highest germination percentage. Harvesting of capsules from the tree can be done either by tree climbing or plucking the fruit using a long pole with a hook or small sickle attached to its end. Fruit harvesting may not be economical as it incurs high labour cost. However, fruit harvesting may have to be carried out if samples of high quality seeds are required for breeding and other experimental purposes.

CONCLUSION

Flowering in *Hevea* trees (clone RRIM 600) appears to be more frequent under low density planting on flat terrain and in trees exposed to full sunlight.

Fruit development begins shortly after pollination. Progressive fruit growth occurs up to the twelfth week after pollination. Once the fruit has attained its maximum size no further external development occurs until it reaches maturity it finally dries up and is ready for dehiscence.

As the fruit grows, the seed and its associated structures develop and grow. The testa and endosperm are among the early structures to be formed, while the embryo is the last to develop and is only visible after fourteen weeks from pollination.

During the early stages of fruit and seed development, the moisture contents are generally high and dry weight low. As development progresses, their moisture contents decrease while dry weights increase. The relationship between these values shows that at physiological maturation the dry weights are highest and moisture contents lowest. At this stage, seed germination is at its maximum.

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