DETERMINATION OF SEED TESTING STANDARDS - MOISTURE CONTENT OF *HOPEA ODORATA* SEEDS

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KRISHNAPILLAY, B., MARZALINA MANSOR, YAP, S.K., CHUA, L.S.L., ANG, K.C., SITI ASHA ABU BAKAR & ZAITON SALLEH. 1991. Determination of seed testing testing standards - moisture content of *Hopea odorata* seeds. Studies carried out to determine the optimum sample size for moisture content determination of *Hopea odorata* seeds showed that around 20 to 25 seeds were sufficient to give significant results at confidence probability of 0.95 and at a desired precision level of 1.0%. Tests carried out on different oven drying temperatures showed that drying at 103°*C* for 20 *h* or 90°*C* for 24 *h* gave consistent moisture content results when seeds were cut either into halves or quarters.

Key words: *Hopea odorata* - recalcitrant - seed testing - sample size - moisture content

Introduction

The International Seed Testing Association (ISTA 1985) rules devised for sample size and moisture content determination are mainly for orthodox seeds. There are no standard methods of seed testing available yet for tropical recalcitrant seeds. Since most tropical recalcitrant seeds are fairly large and the number of seeds available for testing may be limited, it is not possible to follow the sampling rules recommended by ISTA for orthodox seeds. It must, however, be borne in mind that a sample size recommended for use in any test has to be both economical and statistically significant. Various sample sizes have been used for moisture determination (Yap 1986, Tompsett 1987, Rees 1963). Chin (1988) and Berjak (1989) have suggested the use of a minimum of 20 seeds for moisture determination. However, it appears that this recommended minimum sample size has not been adequately defined.

At the 1986 ISTA Congress held in Brisbane, Australia, the working group on recalcitrant seeds recommended that recalcitrant seeds be dried at 103°*C* until constant weight was achieved. Many researchers were of the opinion that this temperature may be too high for some species especially those with high oil content. Hence, it was suggested that for each species, a range of temperature and corresponding times be evaluated. The recommended temperatures for testing were 70, 80, 90, 103 and 130° *C*.

The work reported in this paper was carried out to obtain precise information on required sample size and suitable drying temperatures for consistent moisture

content determination in *Hopea odorata*, a tropical timber species belonging to the family Dipterocarpaceae.

Materials and methods

During the 1990 fruiting season, mature seeds of *H. odorata* were collected from three populations at different locations within the compound of the Forest Research Institute Malaysia (FRIM). From each location, a total of 1500 seeds was collected. The seeds were brought back to the laboratory on the same day.

Seed size and weight

To determine variability in seed size and weight from the three populations, two replicates of 25 seeds each from the three populations were taken at random. For each seed, the length and width of the seed, wings and their respective weight were tabulated.

Estimation of sample size

For the estimation of sample size, four replicates of 25 seeds each were taken at random from each population and dewinged. The moisture content of individual seed was determined in each replicate. Each seed was marked, weighed and oven dried at $103 \pm 1°C$ for 16 *h*. The oven dried seeds after cooling in a desiccator over silica gel were then weighed and the moisture content expressed as a percentage of the fresh weight. The variability of moisture content expressed as standard deviation for the three population was used for the calculation of the sample size.

The sample size for moisture content determination was calculated using the formula developed by Cochran (1953) which is as follows:

$$n = \left| \frac{ts}{d} \right|^2$$

where n = number of seeds to be sampled; t = the value of the normal deviate corresponding to the desired confidence level; s = the standard deviation of the factor to be tested; d = the level of precision or margin of error.

The size of sample required at different confidence probability and levels of precision was thus calculated using this formula for varying standard deviations. Since the seed lot taken for each population was finite and where the sampling fraction was more than 5% of the finite population, a correction to the sample size had to be applied. This was done using a correction formula which is as follows (Cochran 1953):

$$n' = \frac{n}{1 + \frac{n}{N}}$$

where n' = corrected sample size; n = uncorrected sample size; N = population of the seed lot.

Using the two formulae, a table showing the sample sizes necessary for moisture determination at desired confidence probability and precision was constructed. Once the actual standard deviation of the variability of moisture content among the three populations of the *H. odorata* seeds was known, the sample size could be determined.

Comparison of oven drying temperatures

Five drying temperatures, that is 70, 80, 90, 103 and 130°*C* were compared. For the first four temperatures, recordings were made at four hourly intervals for a period of 40 *h*. For 130°*C*, hourly recordings for a period of ten hours were taken. Three treatments of seeds, that is whole seeds, seeds that had been cut into two (halved) and into four (quartered) were also tested in this study. From each of the three populations, five replicates of 25 seeds were taken for each drying temperature and seed treatment. A regression analysis was carried out on the data obtained and comparisons were made to obtain the best seed treatment and the most suitable temperature for moisture determination.

Results

Seed size and weight

Measurements taken of individual seeds from the three populations to evaluate the variability in size and weight of seeds are shown in Table 1. Intact seed weight

Table 1. Average weight and size of individual seeds of *H. odorata* taken from three different populations

Population	Weight (*g*)			Size (*cm*)			
	Seed	Wings	Seed+ Wings	Seed		Wings	
				Length	Width	Length	Width
1	0.18±0.03	0.08±0.03	0.25±0.05	1.05±0.08	0.67±0.08	4.65±0.04	0.98±0.01
2	0.21±0.03	0.06±0.01	0.27±0.04	1.06±0.08	0.69±0.06	4.59±0.04	0.96±0.02
3	0.21±0.03	0.09±0.02	0.30±0.07	1.07±0.06	0.67±0.08	4.71±0.02	0.98±0.01
Mean	0.20±0.03	0.08±0.01	0.27±0.02	1.06±0.01	0.68±0.01	4.65±0.05	0 97±0.01

from the three populations varied between 0.25 to 0.30 *g* with a mean weight of 0.27 ± 0.02 *g*. The seeds that were dewinged weighed between 0.18 to 0.21 *g* with a mean of 0.20 ± 0.01 *g*. The average seed size was found to be 1.06 ± 0.01 *cm* long and with a width of 0.68 ± 0.01 *cm* at its widest circumference.

Estimation of sample size

Table 2 gives the determination of the size of sample required for testing the moisture content of *H. odorata* seeds at various confidence probability and levels of precision.

Table 2. A table for determining the size of sample required for testing the moisture content of *H. odorata* seeds at various levels of confidence and precision (based on a seed lot of 1500 seeds)

Desired precision (%)	Confidence probability	Standard deviation of seed moisture content								
		1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75	3.00
		Sample size (number of seeds)								
0.5	0.90	11	17	24	32	42	53	65	78	92
	0.95	16	25	35	47	61	77	94	112	132
	0.99	42	64	90	121	154	189	227	267	307
1.0	0.90	3	4	6	8	11	14	17	20	24
	0.95	4	6	9	12	16	20	25	30	35
	0.99	11	17	24	32	42	52	64	77	90
1.5	0.90	1	2	3	4	5	6	7	9	11
	0.95	2	3	4	5	7	9	11	13	16
	0.99	5	7	11	14	19	24	29	35	42
2.0	0.90	1	1	2	2	3	3	4	5	6
	0.95	1	2	2	3	4	5	6	8	9
	0.99	3	4	6	8	11	13	17	20	24

The variability of moisture content expressed as standard deviation for the three populations over four replicates is presented in Table 3.

Table 3. The variation of moisture content in relation to the three different populations of *H. odorata*

Population	Moisture content (%) with standard deviation				
	R1	R2	R3	R4	Mean
1	44.1127	41.4224	41.8862	42.2412	41.8383
	(2.939)	(2.397)	(2.327)	(2.461)	(2.240)
2	42.0062	41.8852	42.2519	42.4268	42.1425
	(2.431)	(1.967)	(2.665)	(2.441)	(2.356)
3	42.5011	42.5454	42.6037	42.5309	42.5453
	(2.164)	(2.477)	(2.068)	(2.630)	(2.306)
Mean	42.8733	41.9510	42.2473	42.3996	42.1754
	(2.511)	(2.280)	(2.353)	(2.511)	(2.301)

Note: a. R1 - R4 = replicates; b. moisture content is expressed as a percentage on wet weight basis; c. values in parenthesis are the variability in moisture; content expressed as standard deviation

From Table 3, it was observed that the variability in moisture content expressed as standard deviation ranged between 2.25 and 2.50. From Table 2, for this amount of variation, at a precision level of 0.5% and at confidence probability of 0.95, between 74 to 94 seeds would be required for moisture test while at the 1.0% precision level and at the same confidence probability, only about 20 to 25 seeds would be required.

Suitable oven drying temperature

Five temperatures and three seed treatments were tested over a period of 1 to 40 *h*. The results were analysed using a regression analysis and these are shown in Figures 1, 2, 3, 4 and 5. The results indicated that drying at 103°*C* for 20 *h* or at 90°*C* for 24 *h* gave consistent results. Seeds that had been halved or quartered lost moisture more uniformly when compared to whole seeds.

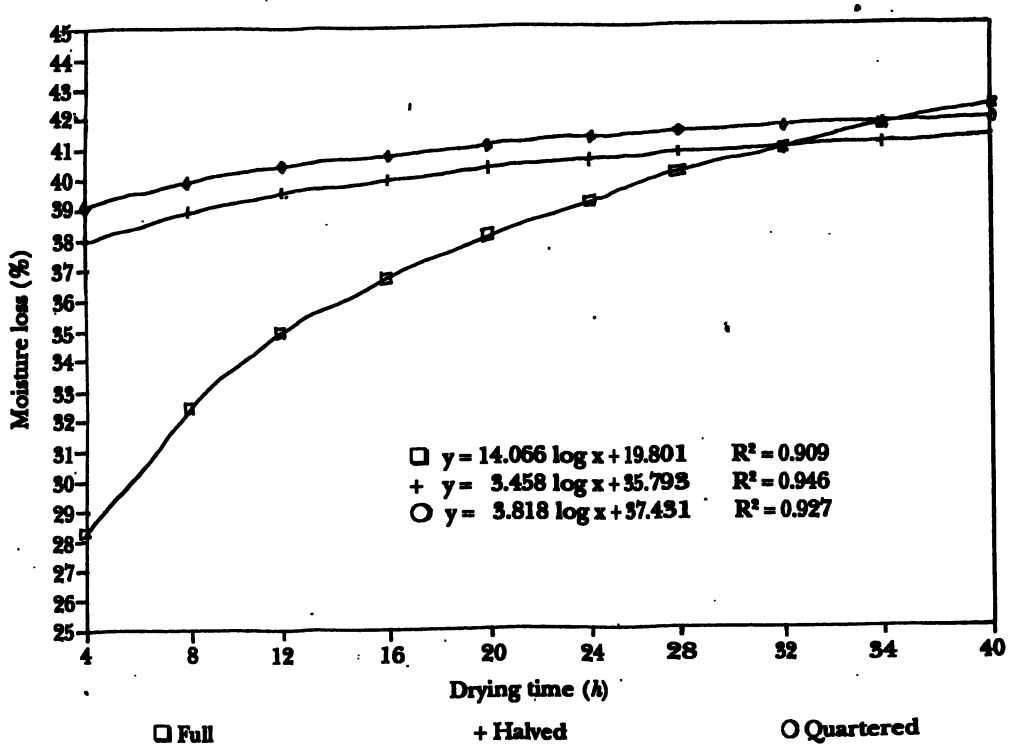

Figure 1. Regression curves for fully halved and quartered seeds of *H. odorata*, oven dried at 70°*C*

Discussion

Seed moisture is one of the most important factors affecting viability and storability of seeds. It is essential therefore that proper and sufficiently precise methods be devised for seed testing. To date, very little work has been done on establishing standards for large seeds of tropical species. The available information and guidelines are those that have been developed for orthodox seeds (ISTA 1985).

The present study carried out for *H. odorata* showed that the mature seeds though collected from three different locations were fairly uniform in terms of size (measuring 1.06±0.01 x 0.68±0.01 *cm*) and weight (0.20±0.01 *g*). The variation in moisture content expressed as standard deviation over the three populations showed a range of 2.28 to 2.51 (Table 3). Over this range, for a confidence

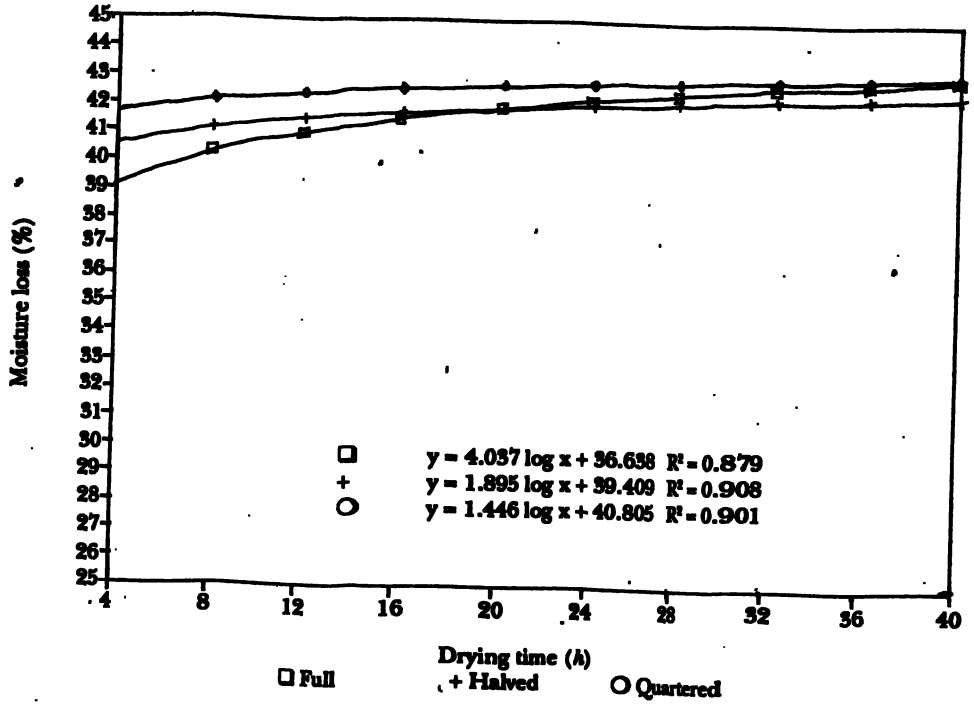

Figure 2. Regression curves for full, halved and quartered seeds of *H. odorata*, oven dried at 80°C

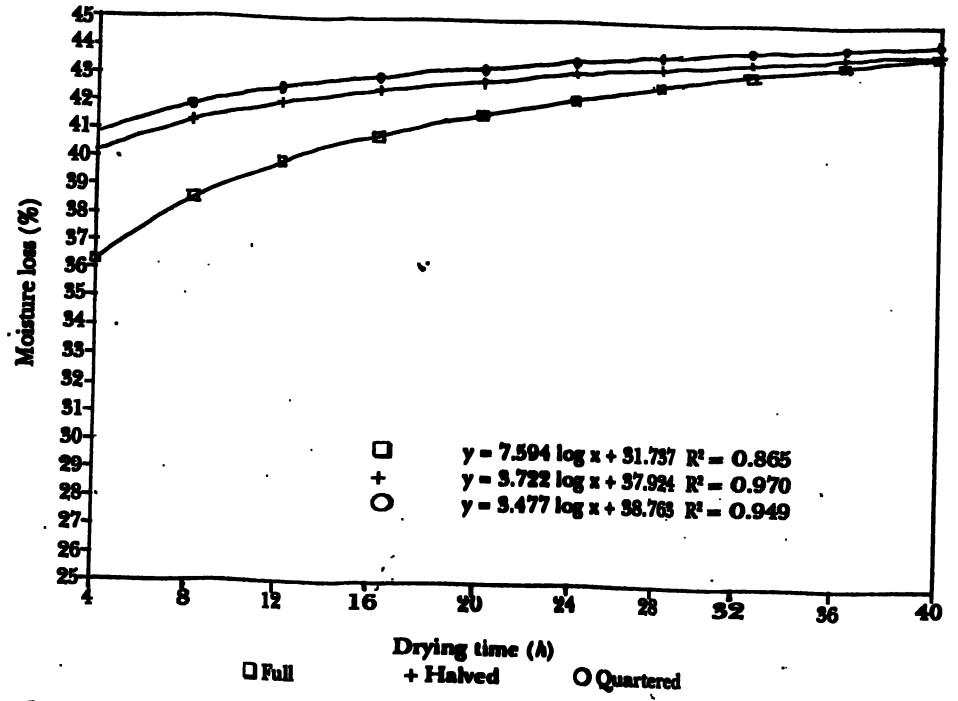

Figure 3. Regression curves from full, halved and quartered seeds of *H. odorata*, oven dried at 90°C

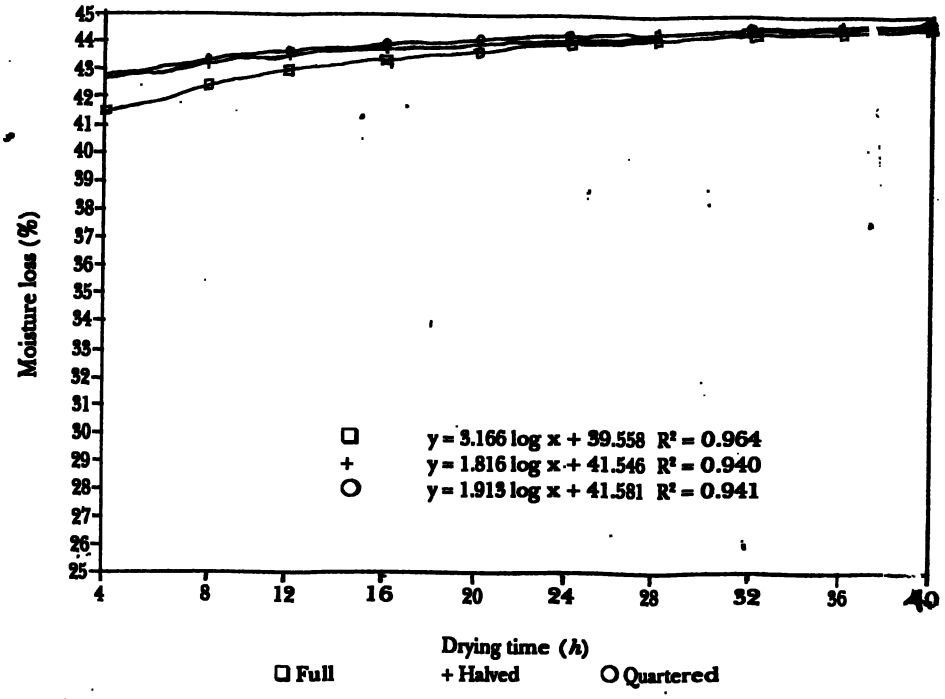

Figure 4. Regression curves for full, halved and quartered seeds of *H. odorata*, oven dried at 103°C

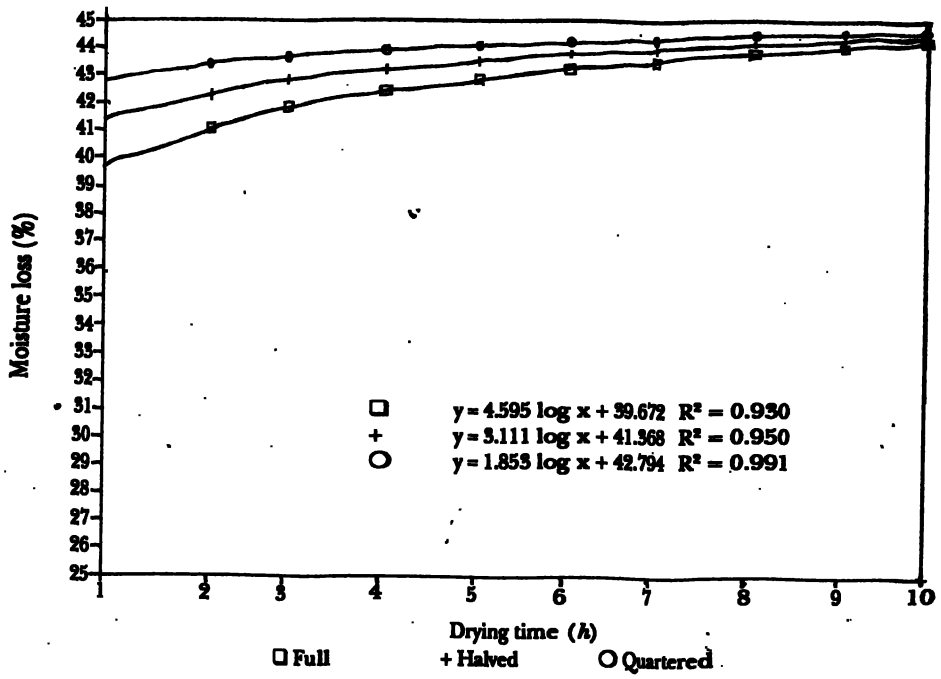

Figure 5. Regression curves for full, halved and quartered seeds of *H. odorata*, oven dried at 130°C

probability of 0.95 and at a precision level of 1.0%, a sample size of 20 to 25 seeds was found to be sufficient to accurately determine the moisture content in these seeds (Table 2). A 1.0% level of precision is selected because the moisture test is a destructive process and only 20 to 25 seeds will be used for this test. If a 0.5% precision level is used, this would involve destroying 74 to 94 seeds which may be undesirable in tropical species where the scarcity of seeds and the small quantity available are major constraints. Furthermore, Bonner (1982) in working with recalcitrant seeds of temperate species has suggested that a precision to tolerance of variability of up to 2.5% is acceptable for large seeds with moisture content of more than 12%. Hence, in this study, a 1.0% level of precision taken was sufficiently accurate for the sample size of *H. odorata*. Mok (1972), working with oil palm seeds, Manokaran (1983) and Tan (1985) working with horticultural recalcitrant seeds were able to establish the minimum sample size for the respective species using a similar statistical approach attempted in this study.

The guidelines for oven drying of recalcitrant tropical seeds are not prescribed in the ISTA rules (1985). At the 21st International Seed Testing Association Congress held in Brisbane, Australia (1986), there was active discussion on developing methods for accurate assessment of moisture content. Many researchers continue to use the temperature regime recommended by ISTA for orthodox seeds (103°*C* for 16 *h*) for drying large recalcitrant seeds without knowing if this temperature may be too high for such seeds. Of recent, it has been debated that for many of the recalcitrant seeds that have high oil content, oven drying at 103°*C* for 16 *h* may not be desirable. It has therefore been suggested (Berjak 1989) that for each species, a range of temperature and corresponding time, when constant weight is achieved, be evaluated.

In this present study where five temperatures were evaluated, results showed that for *H. odorata* seeds, drying them at 103°*C* for 20 *h* or 90°*C* for 24 *h* seemed suitable (Figures 3 & 4) for moisture content determination. At 70 and 80°*C* (Figures 1 & 2), there continued to be a loss in moisture content up to 40 *h* showing that these temperatures may be too low for drying. At 130°*C* (Figure 5), moisture loss was rapid and weight loss was recorded up to ten hours indicating probably that after complete removal of water, other volatiles were also lost and the seeds began to char.

Seeds that were cut into halves or quarters were found to lose moisture more uniformly as compared to whole seeds (Figures 3 & 4). According to Bonner (1982), any seed with an average length or diameter of 10 *mm* or more should be broken up to get a good assessment of the moisture content. In this study, similar results were also seen where seeds that were halved or quartered gave a more uniform drying pattern.

Drying the seeds at 90°*C* for 24 *h* or 103°*C* for 20 *h* is desirable because it fits an overnight schedule very well. Samples put into the oven at the beginning of a work day will be ready to cool and weigh the following mid-morning.

Conclusion

From this study, a sample size of 20 to 25 seeds was found to be sufficient for accurate moisture content determination for seeds of *H. odorata*. The suitable drying temperature for moisture content determination was found to be either 103°C for 20 *h* or 90°*C* for 24 *h*.

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