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Appendix 20

Technical note on teak germination

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Introduction

Teak (*Tectona grandis*) is a high value timber species that may grow well in Sabah (Malaysia). However, the research on genetic improvement and silviculture is at a very early stage in the State, as well as the collection of genetic material, that is still poor.

With the idea of expanding the genetic resources of Teak in Sabah and to allow subsequent selection and commercial production of good genetic materials, the PISP started in 1996 a programme to establish new Teak genetic collections and testing. Two provenance/progeny trials have been planned to be planted within the forest concession of ICSB, one in Luasong (Tawau) and one in Taliwas (Lahad Datu).

The first step was the importation of a number of seedlots from different geographic origins, obtained both from the commercial market or through CIRAD-Foret. The next step was the germination of the seeds. To avoid to waste the precious material, a research on seed germination has been implemented.

It is well known that the germination of Teak is not easy: in general, the germination percentage is low (5-20%), and the germination is scattered over long time (3 months to one year or more). In order to explain the bad germination results, some authors made reference to the existence of a physical or chemical dormancy (Gupta *et al.* 1975, Fairlamb & Davidson 1976).

Different methods have been tried in the past to overcome the problem: alternation of soaking in water and drying, scarification of the fruits, high temperature, treatment with sulfuric acid, with gibberellic acid, partial fermentation, etc. (see review in Vallauri 1994). However the results were not really satisfying, first of all because even in the best cases the germination rate was improved only to a small extent, secondly because the repeatability of the experiments was very low, i.e. two teams working on same or different seedlots came to very different conclusions (compare for example Behagel & Kadio 1995, with Vallauri 1994).

For this reason we decided to proceed with our own experimentation on Teak seed germination. Several methods have been compared: extraction of the seeds from the fruit by carefully cracking this latter (Dabral 1976), and subsequent germination in Petri dishes; water soaking and drying of the fruits (Vallauri 1994); soaking the fruits in sulfuric acid (Behagel 1993 and 1996, Behagel & Kadio 1995); *in vitro* germination on the extracted seeds.

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Material and Methods

In order to establish new genetic material in provenance / progeny trials, we germinated 72 different Teak seedlots. Most of the seedlots were collected in a clonal seed orchard in Ivory Coast (La Sangoué, CIRAD-Foret: seedlot numbers with 94... as first two digits). The rest of the seedlots were true provenance from India (numbers with 83... and 88.... as first two digits), Thailand (numbers with 86.... at the beginning), Papua New Guinea (PNG), Solomon Islands (SI...), Indonesia (PARE) and Sabah (SEGAMA). See Table 1.

The reception of the different seedlots has been scattered over several months. So we sequentially implemented the experiments according to the quantity of seeds received and to the results observed with the previous tests. Some of the comparison below are not orthodox because the treatments were done either on the same seedlots but at different times, or on seedlots more or less differing for their composition.

The first batch of fruits from the clonal seed orchard La Sangoué arrived in late August 1996; we received approximately 700 grams of fruits per seedlots and we split all the lots into 2 parts. The first half was germinated in the PISP nursery (Luasong, Sabah) by extracting the seeds from the cracked fruits, followed by germination in Petri dishes (method described below and referred hereafter as cracking method). This method was chosen because, according to Dabral (1976), it is the one giving the fastest germination. According to the germination rate of the first half of the seedlots, the second half was again subdivided into 2. The best seedlots were germinated in the nursery using the same cracking method and the worse ones (germination below 5 %) were sent to the Plant Biotechnology Laboratory (PBL, Tawau, Sabah) for *in vitro* germination (described below).

Later on, we were able to find in the market some seedlots (PNG, SEGAMA, PARE) on which to test other germination methods: water-soaking and drying, soaking in sulfuric acid (described below), cracking method.

Late December 1996, we received a second batch of fruits to complete the seedlots from the clonal seed orchard La Sangoué (approximately 300 grams per seedlot). Half of the seedlots (the best germinating ones according to the previous test) were germinated in the nursery after a soaking/desiccation treatment. The remaining seedlots were germinated *in vitro* in the PBL.

Description of the germination methods

1) Extraction of the seeds, germination in Petri dishes (cracking method)

This technique was derived with modifications from Dabral (1976). The fruits were soaked in water for few hours and cracked with a hammer in order to extract the seeds. This technique is time consuming: it takes 3 hours to extract the seeds from 300 grams of fruits.

The seeds were counted and placed into Petri dishes (diameter 9 cm) on a thick filter paper (50 seeds per box). We moistened the seeds with a mixture of water and fungicide (Thiram, 2 grams/liter) and we removed the excess of water by reversing the boxes. The Petri dishes were placed under a mist system in order to keep the temperature low. The moisture was nearly 100% and the light was about 5% compared to open conditions.

True Provenances

seedlot n.	Provenance
8304167	India Chandrapur Maharastra
8606568	Thailand Mae Huat Lampang (wild)
8606569	Thailand Mae Huat Lampang (plant)
8807822	India Sakrebail Karnataka
8807823	India Sakrebail Karnataka
8807824	India Virnoli Vir. Karnataka
8807831	India Karadibetta Karnataka
8807832	India Gilalegundi Karnataka
8807833	India Virnoli Vir. Karnataka
8807835	India Maukal Karnataka
8807836	India Maukal Karnataka
8807838	India Maukal Karnataka
8807839	India Maukal Karnataka
8807841	India Maukal Karnataka
8807842	India Maukal Karnataka
8807844	India Maukal Karnataka
PARE	Indonesia Pare Pare
PNG	Papua New Guinea ex Brown River
SI 4314	Solomon Island Arara
SI 5212	Solomon Island Viru
SEGAMA	Malaysia Sabah

Progenies of the Ivory Coast Clonal Seed Orchard

seedlot n.	provenance	origin of the clone
9410109	Ivory Coast	India Nellicutha
9410110	Ivory Coast	India Nellicutha
9410122	Ivory Coast	India Nellicutha
9410123	Ivory Coast	India Nellicutha
9410128	Ivory Coast	India Nellicutha
9410129	Ivory Coast	India Nellicutha
9410134	Ivory Coast	India Nellicutha
9410135	Ivory Coast	India Nellicutha
9410136	Ivory Coast	India Nellicutha
9410140	Ivory Coast	India Nellicutha
9410145	Ivory Coast	India Nellicutha
9410147	Ivory Coast	India Nellicutha
9410148	Ivory Coast	India Nellicutha
9410111	Ivory Coast	India Nilambur
9410113	Ivory Coast	India Nilambur
9410117	Ivory Coast	India Nilambur
9410118	Ivory Coast	India Nilambur
9410119	Ivory Coast	India Nilambur
9410120	Ivory Coast	India Nilambur
9410121	Ivory Coast	India Nilambur
9410137	Ivory Coast	India Nilambur
9410141	Ivory Coast	India Nilambur
9410142	Ivory Coast	India Nilambur
9410156	Ivory Coast	India Purunakote
9410157	Ivory Coast	India Purunakote
9410162	Ivory Coast	India Purunakote
9410152	Ivory Coast	India Masale Valley
9410159	Ivory Coast	India Masale Valley
9410143	Ivory Coast	India Vernolirge
9410146	Ivory Coast	India Vernolirge
9410150	Ivory Coast	India Vernolirge
9410114	Ivory Coast	Ivory Coast Bamoro
9410163	Ivory Coast	Ivory Coast Bamoro
9410116	Ivory Coast	Ivory Coast Kokondekro
9410154	Ivory Coast	Laos Paklay
9410161	Ivory Coast	Laos Paklay
9410115	Ivory Coast	Senegal Djibelor
9410151	Ivory Coast	Tanzania Bigwa
9410153	Ivory Coast	Tanzania Bigwa
9410160	Ivory Coast	Tanzania Bigwa
9410112	Ivory Coast	Tanzania Kihuhwi
9410131	Ivory Coast	Tanzania Kihuhwi
9410133	Ivory Coast	Tanzania Kihuhwi
9410124	Ivory Coast	Tanzania Mtibwa
9410126	Ivory Coast	Tanzania Mtibwa
9410138	Ivory Coast	Tanzania Mtibwa
9410155	Ivory Coast	Thailand Ban Cham Pui
9410158	Ivory Coast	Thailand Ban Pha Lay
9410164	Ivory Coast	Thailand Ban Pha Lay
9410125	Ivory Coast	Thailand Huoi-Nam-Oon
9410127	Ivory Coast	Thailand Huoi-Nam-Oon
9410139	Ivory Coast	Thailand Huoi-Nam-Oon
9410130	Ivory Coast	Thailand Mae Huat
9410144	Ivory Coast	Thailand Mae Huat
9410132	Ivory Coast	Thailand Pong Salee
9410149	Ivory Coast	Thailand Pong Salee

Table 1:
List of the seedlots, with their geographic origin, for the two types of material: true provenances (bulks), and families from a clonal seed orchard (Ivory Coast), established with plus trees selected in a multiprovenance trial (Ivory Coast).

Every day, we moistened the filter papers in the Petri dishes and removed the excess of water. This operation was aimed to rinse the seeds and the filter paper and also to apply a daily fungicide treatment. The rotted seeds were discarded. Once the seeds started to germinate (radicle = 2 to 3 millimeters), they were transplanted into polybags. The transplanted seedlings remained under the mist system until the emergence of the first leaves (2 cotyledons + new leaves).

2) Soaking and drying of the fruits (soaking/drying method)

This technique is well known, for Teak, to be an efficient method to increase the germination rate and speed (Vallauri 1994); however, it may give very variable results according to the seedlot.

The fruits were soaked in the water during the night and dried under the sun, on a plastic sheet, during the day. This operation was repeated 7 days before sowing the fruits. The fruits were then sown very superficially in a sand seedbed in the open. We covered the seedbed with a wire netting to prevent animal attacks. The germinated seedlings were counted and transplanted on a week basis at the 2 leaves stage.

3) In vitro germination (in vitro method)

In vitro seed germination was carried out according to a protocol developed in the Plant Biotechnology Laboratory (Tawau, Sabah). The tetracarpic fruits were washed several times and then cracked with a small hammer. Healthy seeds (usually 1 to 2 per pod) were extracted from within each chamber of the pod and immersed in distilled water until further manipulation. The disinfecting treatment called for soaking the seeds in 70% ethanol for 5 min followed by treatment with 0.1% mercuric chloride for 5 min. The seeds were then rinsed in sterile water three times and inoculated onto a basal culture medium in test tubes for germination. The test tubes were kept in total darkness at 26°C until germination, usually observed 5 to 7 days later.

It has to be noted that, in order to save space and material in the PBL, an intensive selection on the seed quality has been carried out. Approximately 40% of the seeds were thus discarded based on their appearance and color.

4) Soaking the fruits in sulfuric acid (sulfuric acid method)

The method was derived with modifications by Behagel (1996). The fruits were soaked for 6 hours in sulfuric acid at 96% diluted 10 times with water, and then rinsed overnight in running water. They were then sown in a sand seed bed under shade net giving a light percent of 15% compared to open conditions. This method has been tested and compared to the other treatments based only on a subsample of the seedlots.

Results and discussion

A single Teak fruit may contain one, two or (rarely) more seeds. For this reason, the germination percentage has been calculated here based on the number of seeds and not of fruits. With the cracking and *in vitro* methods, we had direct access to the count of the number of seeds

per fruit. Over all the seedlots, the mean number of seeds per fruit was of 1.44. For the soaking/drying method, the number of seeds per fruit has been extrapolated, seedlot by seedlot, on the basis of the counts obtained with the two other methods.

It has to be noted that not all the seedlots were germinated with all the three techniques. So the comparison among the three methods could be carried out both based on the total germination, and on the germination of those seedlots tested with all methods. In the same way, the dates of the different treatments did not exactly coincide. In particular, the cracking and *in vitro* methods have been tested upon the two batches of fruits from CIRAD-Foret, received one in September 1996 and one in December 1996; the soaking/drying has been tested only on the batch of December 1996. Further biases were generated by: 1) the fact that the seedlots given to the PBL for *in vitro* germination were selected, according to the cracking method, among the worst germinating ones, and 2) the fact that in the PBL the selection on the seeds was much stronger than in the PISP nursery (approximately 40% and 5% respectively).

In the Petri dishes, the germination started 3 days after sowing and ended 15 days after. Using the soaking/drying method, the germination in the seedbeds started two weeks after sowing (January 2, 1997), had a major peak at about 4 weeks (January 30, 1997) but continued for more than four months after sowing (Figure 1). A second, less important, germination peak was observed at about 3 and a half months (April 12, 1997). For the practical purpose of planting the provenance / progeny trials with homogeneous material, the germination was considered close after this last peak, even if the germination was pursuing at a low rate. In *in vitro* conditions, the first germinations were observed three weeks after inoculation and two months later the germination was considered finished.

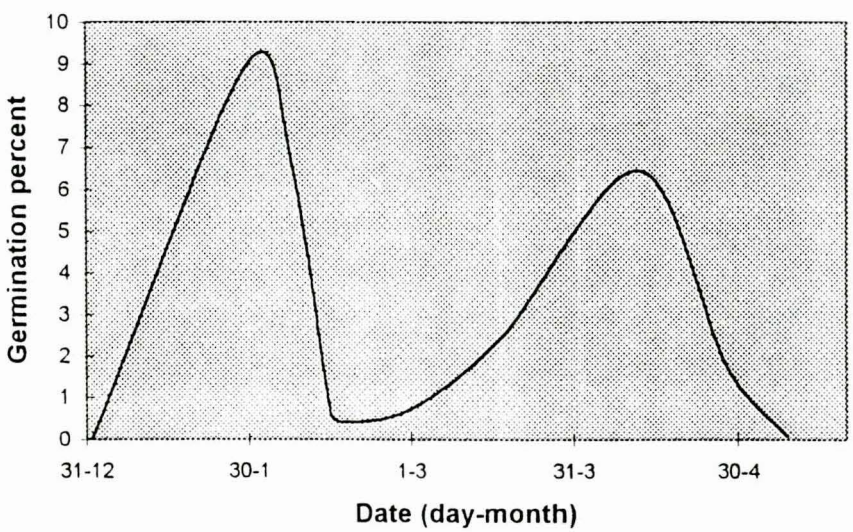

Figure 1. Germination of Teak over time (soaking / drying method, 1997).

Germination rates by seedlot are reported in Appendix 1. In Table 2, the first column refers to the total germination and the second to the germination of the seedlots tested for the three methods (common lots).

Table 2: Comparison of the 3 germination techniques.

	Average germination (*number of seeds tested*)	Av. germination common lots (*number of seeds tested*)	Minimum to Maximum	Standard deviation common lots
Cracking method	4% (*44808*)	**1%** (*11760*)	0% to 31%	6%
Soaking/drying method	27% (*14294*)	**20%** (*10484*)	0% to 86%	28%
***In vitro* method**	21% (*40337*)	**21%** (*11561*)	0% to 64%	16%

Overall, the best germination result was obtained by the soaking/drying technique. However, taking in account only the seedlots common to the three treatments (that means, as explained above, the worst germinating ones), the best result was comparably obtained by both the *in vitro* method and the soaking/drying method. The cracking method gave unexpected bad results, probably because of the physical damage to the germinants during transplantation and fungal attacks. The hypothesis of a chemical dormancy, broken by using the other two methods (that both require abundant soaking) but not with the cracking method, can not be discarded.

In Table 3, the results were shown subdividing the seedlots into 3 categories, according to the germination rate obtained with the soaking method. The soaking/drying method is better for the best germinating lots and the *in vitro* germination is more suitable for the lots with low germination (germination rate multiplied by 2 compare to soaking method).

After germinating the first half of the seedlots by the cracking technique, we tried several other fruits treatments; in particular, we compared the sulfuric acid method with the cracking and *in vitro* methods. The germination results of a test including about 1,000 seeds are summarized in Table 4.

Table 3:

Comparison of the 3 germination methods. Only the seedlots germinated by the 3 techniques are compared. The seedlots are classified into 3 categories according to the germination percentage.

lot	seeds/g	% craking	% soaking	% in vitro
8807823	1.27	2%	75%	46%
9410152	1.43	0%	67%	45%
9410150	1.76	0%	66%	64%
9410112	1.05	0%	49%	30%
9410159	1.56	1%	31%	27%
9410154	1.33	0%	24%	38%
9410144	1.75	0%	24%	7%
8807833	1.24	0%	23%	24%
9410111	1.25	1%	18%	34%
	Average	**1%**	**42%**	**35%**
9410146	2.04	1%	14%	31%
9410136	1.51	1%	14%	39%
9410151	1.25	1%	13%	6%
9410129	1.01	5%	9%	32%
9410109	1.21	0%	6%	5%
9410135	1.81	3%	6%	26%
9410115	1.87	0%	5%	6%
9410155	1.96	0%	3%	3%
9410125	1.04	0%	3%	5%
9410161	1.64	0%	0%	4%
	Average	**1%**	**7%**	**16%**
9410113	1.91	0%	0%	0%
9410122	1.54	0%	0%	1%
9410138	0.52	0%	0%	0%
9410153	0.87	0%	0%	0%
General	**Average**	**1%**	**20%**	**21%**

Table 4: Comparison of the sulfuric acid method with the cracking and *in vitro* germination methods.

Germination technique	Germination % (nb seedlings / nb seeds)
Cracking method	9%
In vitro method	26%
Sulfuric acid method	0%

The fruits treated by the acid did not germinated at all. The other treatment ranked comparably to the previous test. This result was probably due to the damages inflicted to the embryos by the acid. In general, not all the seeds are at the same stage of development, they may be more or less mature, with a more or less thick mesocarp and endocarp. It seems then very difficult to regulate the acid concentration and duration of the treatment: for the thickest envelop the given treatment may be not effective, for the thinnest ones it may corrode the fruit and damage the embryo.

Finally, other small experiments have been carried on a small scale: heating the fruits in a oven, drying the fruits under the sun for different periods... None of these treatments gave satisfactory results.

However, by visual observation, we noticed a difference between the germination tests conducted under the shade nets, and those conducted in the open: these last often gave better results. The hypothesis to explain this result is that light and sun heating can help to break the dormancy of the seeds. This observation was partially supported by the two separate germination peaks obtained after the soaking/drying treatment (Figure 1). These seeds were sown in the open, and the two peaks corresponded to two very dry and sunny periods. The intermediate phase with low germination corresponded to the winter monsoon that brought heavy and continuous rain over all February 1997. Of course, this hypothesis needs to be confirmed by further experiments.

Table 5: Advantages and drawbacks of the different germination methods

	Advantages	Drawback
Cracking method	-Speed up the germination so that all the seedlings are very homogeneous in stage	- Work intensive because of the cracking operation with a hammer - Physical damage of the seeds by the cracking operation - Contamination problems in the Petri dishes - High mortality after transplanting due to the early stage of the seedlings (fragility of the radicle, pest and diseases)
Soaking/drying method	-Rapidity of the soaking/drying operation -Good germination results -Low mortality after transplanting	- Germination length: 3 months or more between the first and the last germination. -Risk of mixing the seedlots during the soaking/drying operation
***In vitro* method**	-Limitation of the contamination problems -Good germination results -Poor mortality after transplanting	- Work intensive because of the cracking operation with a hammer - Physical damage of the seeds by the cracking operation - Cost of the operation due to the material required (test tubes, media, chamber...)
Sulfuric acid method		-Bad germination results -Even if in the literature some case of success have been reported, the risk to loose the seedlots is too important, and this method should be discarded

Conclusion

The advantages and drawbacks of the different methods were summarized in Table 5. The soaking/drying method gave satisfactory results for Teak seed germination, and was the less expensive technique both in terms of material and manpower. Some evidences were found that the exposition of the seeds to direct sunlight during the drying treatment and germination may enhance the germination. For seedlots that do not germinate well with the other techniques, the *in vitro* germination is a safe solution, giving good results in most cases.

The most important parameters that need to be studied further are:

- the number of cycles of the soaking/drying alternation
- the influence of the sun light during the drying treatment and the germination.
- the influence of the fruit conditions (maturation stage, age, hardiness of the endocarp, etc.) on the response to the different techniques.

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Appendix 1: Complete list of the seedlots including the weight of the fruits and the germination percentage for the 3 germination techniques.

seedlot	number of seeds per gram of fruits	Cracking method		Soaking/drying meth.		In vitro method	
		Weight(g)	Germ %	Weight(g)	Germ %	Weight(g)	Germ %
8304167	1.34	334	14%			330	64%
8606568	0.88	656	14%			1200	30%
8606569	1.06	292	1%			1491	24%
8807822	1.13	661	11%	600	24%		
8807823	1.27	336	2%	700	75%	335	46%
8807824	0.97	678	12%	600	74%		
8807831	1.18	669	12%	600	60%		
8807832	1.55	666	16%				
8807833	1.24	335	0%	700	23%	335	24%
8807835	1.45	674	31%				
8807836	1.20	333	5%			332	23%
8807838	0.79	442	12%			441	26%
8807839	1.79	667	19%				
8807841	1.50	333	1%			332	48%
8807842	0.81	757	17%				
8807844	1.39	657	14%	600	15%		
9410109	1.21	378	0%	300	6%	378	5%
9410110	1.79	354	0%			644	9%
9410111	1.25	354	1%	300	18%	353	34%
9410112	1.05	354	0%	301	49%	354	30%
9410113	1.91	352	0%	277	0%	351	0%
9410114	1.46	703	4%				
9410115	1.87	406	0%	281	5%	306	6%
9410116	1.26	711	9%			297	12%
9410117	1.42	405	1%			605	20%
9410118	1.35	407	0%			306	55%
9410119	2.44	406	0%			594	12%
9410120	0.64	409	0%			609	24%
9410121	1.45	352	0%			351	0%
9410122	1.54	355	0%	255	0%	354	1%
9410123	1.37	358	0%			658	7%
9410124	0.67	356	0%			616	25%
9410125	1.04	357	0%	299	3%	356	5%
9410126	1.83	356	1%			656	15%
9410127	1.04	359	0%			358	13%
9410128	1.61	355	0%			655	6%
9410129	1.01	348	5%	278	9%	347	32%
9410130	0.91	719	13%	279	62%		
9410131	2.67	359	1%			626	16%
9410132	1.47	355	1%			605	20%
9410133	1.62	359	2%			644	13%
9410134	1.78	358	0%			358	24%
9410135	1.81	357	3%	300	6%	357	26%
9410136	1.51	358	1%	265	14%	358	39%
9410137	2.25	351	1%			350	42%
9410138	0.52	357	0%	300	0%	357	0%
9410139	1.90	713	6%				
9410140	1.26	713	8%	600	86%		
9410141	1.84	357	0%			667	9%
9410142	1.44	358	2%			657	14%
9410143	3.65	721	9%				
9410144	1.75	357	0%	288	24%	357	7%
9410145	1.72	359	0%			658	11%
9410146	2.04	358	1%	300	14%	358	31%
9410147	1.07	358	2%			657	17%
9410148	1.28	359	0%			659	21%
9410149	1.52	355	0%			655	19%
9410150	1.76	358	0%	279	66%	358	64%
9410151	1.25	357	1%	300	13%	356	6%
9410152	1.43	360	0%	292	67%	360	45%
9410153	0.87	358	0%	300	0%	358	0%
9410154	1.33	359	0%	302	24%	358	38%
9410155	1.96	358	0%	251	3%	357	3%
9410156	1.32	358	0%			658	27%
9410157	1.29	356	2%			643	33%
9410158	1.36	357	0%			605	13%
9410159	1.56	357	1%	300	31%	357	27%
9410160	2.01	356	0%			662	17%
9410161	1.64	359	0%	317	0%	359	4%
9410162	0.85	353	0%			353	0%
9410163	1.12	711	14%				
9410164	0.96	354	0%			354	7%
General Average			**4%**		**27%**		**21%**
Standard deviation			*6%*		*28%*		*16%*