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Pollen and Stigma Viability in Teak (Tectona grandis L. f.)

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Abstract

Methods and results of tests on the viability of pollen and stigmas in Tectona grandis L.f. are described. In vivo and in vitro techniques revealed the extent of viability of pollen and stigma. Pollen grains from the day of anthesis to three days after were used in controlled pollinations. Pollen grains were also germinated in mixtures of boric acid, gibberellic acid and sucrose. Some pollen grains were stored in a deep freeze, a cooled incubator and a vacuum desiccator and later germinated. Sucrose at 14% gave the best germination; at 1% to 3% sucrose there was rupture and at 35% to 40% there was plasmolysis. Pollen grains appeared viable two days after anthesis although no seeds were obtained when these were used in controlled pollination.

Pollen grains had the highest percentage of viability between 12.00 and 14.00 hours, while viability decreased betwen 07.30 and 10.00 hours.

The uce of stored pollen for tree breeding appears possible.

Key words: **Pollen viability, in vivo, in vitro, pollen storage, pollen tube measurement, controlled pollination.**

Zusammenfassung

Unbehandelter Pollen von Tectona grandis L.F. war bis zu 2 Tagen nach der Anthesis keimfähig. Nach Aufbewahrung des Pollens in einem Exsikkator bei Minustemperaturen unterschied sich die Keimung nach 35 Tagen nur dadurch, daß die Pollenschläuche kürzer waren. In einigen Fällen konnte mit aufbewahrtem Pollen Befruchtung erzielt werden.

Introduction

Establishment of forest plantations is currently of great interest in Nigeria because of rapid depletion of the natural forest. However, there is little or no information on the reproductive biology and genetic variation of the species used in plantations. Information on the reproductive biology of such species is essential in designing effective breeding Programmes. BAWA and STETTLER (1969) stated that although breeding mechanisms of temperate forest-tree species are fairly well known they are poorly understood in tropical tree species.

The teak flower is actinomorphic and hermaphroditic and normally has six petals in a single whorl. The pistil is composed of an ovary with four ovules and a style with a forked stigma. Style and stamens are about 6 mm long and the diameter of the corolla is from 6-8 mm (BRYNDUM and HEDEGART, 1969). The flowers appear in large panicles in the outer, unshaded part of the tree crown. The flowering period for an individual inflorescence may last from 2 to 6 weeks.

The first study of some aspects of the reproductive biology of teak was begun in Thailand and was mainly on flowering and fruiting (BRYNDUM and HEDEGART, 1969). This work showed that the major pollinators of teak are insects; in Nigeria these are Euphaedra *janatta* B., Belenois calypso DR., *Acraea* bonasia F., belonging to the family *Nymphalidae* and the order Lepidoptera; Nomia tridenta SM. of the family *Apidae* and the order Hymenoptera; Melonospilus sternalis DALL. of the family Lygaeidae and the order *Hemiptera* (EGENTI, 1974). Another study by HEDECART (1973) showed that hand-pollination increased the percentage of fruit formation in comparison to pollination by natural agents. This finding led to the present study and the objectives were as follows:

(1) to determine the extent of pollen and stigma viability through in vivo and in vitro examination;
(2) to investigate the use of stored pollen for pollination.

Materials and Methods

Three studies were made (1) viability of pollen and stigma in vivo; (2) the viability of pollen in vitro (3) pollen storage in (a) a vacuum desiccator, (b) a cooled incubator and (c) a deep freeze.

Material for these three studies was obtained from 10 to 15 year old teak trees growing in plantations at Onigambari near Ibadan in Nigeria. Thirty trees were randomly selected from the plantations.

(I) For the in vivo studies, flowers were taken one day before anthesis (A — 1), on the day of anthesis (A), one day after anthesis (A + 1), two days after anthesis (A + 2) and three days after anthesis (A + 3).

After emasculation, fresh stigmata of the day of anthesis were pollinated with pollen of A — 1, A, A + 1, A + 2, A + 3 and bagged. In addition A and A + 1 stigmata were pollinated with fresh pollen of the day of anthesis (A) and bagged.

In practice it was not possible to use A — 1 and A + 1 stigmata as they could not be freed from the remaining parts of the flower without damage in the case of A — 1 because the flowers had not opened and the stigma had become dry in the case of A t 2. Each bag was numbered and dated together with the time of day when the pollina-

Table 1. — Viability of pollen and stigma as shown by *in vivo* studies

Bag number	Tree number	Number of flowers	Date of pollination	Stage of pistil	Stage of pollen	Time of day (GMT)	Percentage set
01	1	150	19/6/75	A	A	14.00	27
07	7	340	25/6/75	A	A	12.00	7
08	8	220	25/6/75	A	A	15.00	14
020	20	189	29/6/75	A	A	13.00	26
021	21	201	29/6/75	A	A — 1	15.00	0
016	16	280	28/6/75	A	A — 1	12.00	0
04	4	180	22/6/75	A	A + 1	11.00	9
011	11	215	26/6/75	A	A + 1	17.00	2.3
012	12	320	27/6/75	A	A + 1	09.00	4.6
05	5	270	24/6/75	A	A + 2	09.00	1
013	13	280	27/6/75	A	A + 2	14.00	0.7
015	15	258	27/6/75	A	A + 2	09.00	4
022	22	270	29/6/75	A	A + 2	17.00	1.8
06	6	210	24/6/75	A	A + 3	13.00	0
014	14	178	27/6/75	A	A + 3	17.00	0
02	2	305	19/6/75	A + 1	A	09.00	0.6
03	3	120	22/6/75	A + 1	A	13.00	0
09	9	230	26/6/75	A + 1	A	09.00	1.3
010	10	175	26/6/75	A + 1	A	13.00	0
017	17	301	28/6/75	A + 1	A + 1	15.00	0
018	18	315	29/6/75	A + 1	A + 2	09.00	0
019	19	95	29/6/75	A + 1	A + 3	12.00	0

tion was done. Pollination began at 09.00 hours and ended at 17.00 hours and there were on an average 250 flowers in each bag. All the flowers in each inflorescence were at the same stage of development and received the same treatment. Daily observations were made and the bags were removed from the inflorescences fourteen days after pollination.

(II) Pollen for the *in vitro* germination studies was germinated on slides in the following mixtures

(1) boric acid (BA) 1000 ppm, gibberellic acid (GA) 200 ppm and 10% sucrose,
(2) boric acid 1000 ppm, GA 500 ppm and 10% sucrose,
(3) boric acid 2500 ppm, GA 500 ppm and 10% sucrose,
(4) sucrose alone in the range 1% to 40%.

Slides were examined under a microscope every 2 hours to assess the germination. On each slide the number of pollen grains germinated for each exposure on the four replicates was averaged and recorded as a percentage of the total number observed. Pollen tube length was measured when the length of the pollen tube was twice the diameter of the pollen grain (Stairs and Troendle, 1969).

(III) For the work on pollen storage, fresh pollen from the day of anthesis was used. The anthers were excised before dehiscence and gently brushed into 5 cm long glass vials plugged with non-absorbent cotton. Two vials were stored in a vacuum desiccator over anhydrous calcium chloride in the laboratory at a day temperature of 21 to 27° C and a night temperature of 17 to 23° C. Another two vials were stored in a cooled incubator at +5° C and the remaining two in a deep freeze at —10 to —15° C.

All the vials were stored for 35 days and at the end of this period the pollen was germinated in the media described in Study 2, and measured for the length of pollen tube.

Results and Discussion

The viability of the pollen and stigma *in vivo* appears in *Table 1*. Some pollen usually was viable until the second day after anthesis (see bags 05, 013, 015, 018 and 022) and some fertilization was effected if the stigma was fresh (see the same bag numbers). Viability of pollen was zero three days after anthesis (bags 06, 014, 019) and no fertilization was obtained; the same applied to the pollen collected one day before anthesis (bags 016, 021). The stigma was still viable on the day after anthesis (see bags 02, 09) but was not longer viable after A + 1. Best results were obtained by using *A* pollen on *A* stigma (bags 01, 08, 020).

These results must remain preliminary because of the difficulties in their interpretation. Because pollinations were made on different days and at different times of the day, it was not possible to isolate the effects of these variables from those of the stage of either the pollen or of the stigma.

Further observations on the onset and duration of flowering included the maturation period of flower buds and the chances of abortion after fertilization in plantations of teak outside the experimental area. Observations showed that the rate of abortion could be as high as 90% of the flowers on a panicle.

In Nigeria, flowering of teak starts early in June and continues until early September. The flower buds mature 9 days after their appearance. In 10 days unfertilized ovaries fall off with the calyx, while 14 days from the day

of pollination, ovaries which fail to fall off usually grow into fruits. This is the reason why pollination bags were moved from the panicles 14 days from pollination. The growth of fruit is rapid and reaches maximum size in 62 days; mature fruits can be picked 5 months after pollination.

The different mixtures used for the *in vitro* studies of pollen germination were intended to reveal the best media for germinating teak pollen grains. Only those media that gave early and uniform germination and maximum growth of pollen tubes were considered. *Table 2* shows the percentage germination of pollen grains after 8 hours on the various media. The best medium was 14% sucrose. When pollen grains were germinated in 1% to 3% sucrose, there was rupture and when germinated in 35% to 40% sucrose, plasmolysis set in. Germination began at 5% sucrose but was not uniform. Germination of pollen grains collected from 12.00 to 14.00 hours was speedier and more profuse than those collected from 07.30 to 10.00 and at 18.00 hours when germinated in 14% sucrose. *Table 2* shows that some pollen was still viable on sucrose alone three days after and one day before anthesis.

Table 2. — Pollen grain germination in four media

Mixtures	Average Percentage of Pollen grains Germinating after 8 hours — Stage of Pollen				
	A — 1	A	A + 1	A + 2	A + 3
BA 1000 ppm GA 200 ppm 10% sucrose	0	5	0	0	0
BA 1000 ppm GA 500 ppm 10% sucrose	0	10	0	0	0
BA 2500 ppm GA 500 ppm 10% sucrose	0	12	1	0	0
14% sucrose	10	100	60	40	10

The failure of A — 1 pollen and A + 3 pollen to fertilize the ovary in the *in vivo* studies was probably due to immaturity and weakness of the pollen tube in the case of A — 1 and weakness of the latter in the case of A + 3. For pollen germinated in 14% sucrose, pollen tube length was measured every 2 hours for the first 8 hours, and again at 24 hours using an ocular micrometer attached to the microscope. Pollen tubes reached a maximum of 9.3 mm in 24 hours with an overall mean of 5.53 mm.

The pollen stored in the vacuum desiccator, cooled incubator, and deep freeze was germinated on the different media and 14% sucrose gave the best germination. All the media gave profuse germination of pollen grains but the pollen tubes were weaker and shorter when compared with those from fresh unstored pollen.

The mean length of the tubes produced by pollen stored in the vacuum desiccator was 3.66 mm while the mean length of those stored in the cooled incubator and deep freeze were 3.02 and 3.23 mm respectively. All pollen gave profuse and uniform germination.

Tables 3 and *4* show the combined analysis of variance and the means for the time intervals and the treatments. Storage was the most differentiating factor. The pre-storage showed very highly significant difference from the post-storage treatments. Significant differences also existed between the post-storage treatments. The time intervals were highly significant and the trend was linear that is the longer the interval the greater the length of pollen tube.

The stored pollen was used for polination and *Table 5* summarises the fruit set using this source. The stored pollen from all three sources was mixed before being used so it was not possible to ascertain which source gave the highest fertilization. Repeated germination tests of the

Table 4. — Table of means measurements of pollen tubes in mm.

Time	Mean	Treatment	Mean
2 hrs.	2.80	Pre-storage	5.53
4 hrs.	3.50	Storage (35 days)	3.31
6 hrs.	4.02	Vacuum Desiccator	3.66
8 hrs.	4.36	Cooled Incubator	3.02
24 hrs.	4.63	Deep freeze	3.23
S.E.	0.186	S.E.	0.166[1]
L.S.D.	0.53		0.096[2]
		L.S.D.	0.47[3]
			0.32[4]

[1] = S.E. for each of 4 treatment mean.
[2] = S.E. for combined mean of the 3 storage treatments.
[3] = L.S.D. for comparisons between individual treatment means.
[4] = L.S.D. for comparison between pre-storage mean and mean of all other treatments combined.

Table 3. — Pollen tube length: Combined analysis of variance

Source	D.F.	M.S.	F
Treatment	3	65.376	47.10**
Pre-storage versus Post-storage	1	185.927	133.95**
Between Post-storage	2	5.343	3.85*
Time	4	21.139	15.23**
Linear Effect	1	72.092	51.94**
Other Effects	3	4.154	2.99*
Interactions	12	0.308	0.22 n.s.
Pooled Error	144	1.388	

* Significant at 5%.
** Significant at 1%.
n.s. not significant.

Table 5. — Result of fruit development after pollinating with stored pollen.

Bag number	Tree number	Number of flowers	Date of pollina-	Stage of pistil	Time of day	Number set
023	23	80	19/7/75	A	10.00	4
024	24	120	19/7/75	A	11.00	6
025	25	95	19/7/75	A	13.00	8
026	26	50	19/7/75	A	14.00	2
027	27	58	19/7/75	A	15.30	3
028	28	45	19/7/75	A	16.30	0
029	29	60	19/7/75	A	17.30	0
030	30	48	19/7/75	A	17.30	0

stored pollen showed that longer pollen tubes were produced by pollen stored in the vacuum desiccator than in the other two media. It is, therefore possible that increased fertilization followed but further investigations are needed for confirmation.

No attempt was made to study either the variation in viability and pollen tube length or the variation in pollen production in the provenances of teak planted in Nigeria. Such studies are planned for the future.

Conclusion

Knowledge of the viability of both the pollen and stigma of the teak is necessary for the effective application of controlled pollination. The *in vivo* study showed that pollen was viable until the second day after anthesis and not viable on the third day. The stigma was viable for one day after anthesis and it was possible to obtain fertilization from pollinations made early in the morning. As expected the best result was obtained by using *A* pollen on *A* stigma. Pollen was no longer viable if collected three days after or one day before anthesis. The *in vitro* study confirmed that sucrose at 14% concentration was a suitable medium for germinating teak pollen because it gave early and profuse germination. When pollen was stored for 35 days in a vacuum desiccator, or in a cooled incubator or a deep freeze and germinated, there was no significant difference in viability but there was significant difference between the mean lengths of pollen tubes. Some fruit was set after pollinating with stored pollen suggesting that it can be uesd for controlled pollination.

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Variabilité géographique du poids de la graine de Pinus contorta

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Résumé

La variabilité géographique du poids de la graine chez *Pinus contorta* a été étudiée sur la majeure partie de l'aire naturelle de l'espèce grâce aux récoltes de graines effectuées par l'I.U.F.R.O.; 140 provenances ont été observées.

La sous-espèce *murrayana* des Cascades de l'Oregon et de la Sierra Nevada (Californie) présente une graine nettement plus lourde que les autres sous-espèces *(bolanderi, contorta, latifolia)*.

La variabilité interprovenance du poids de la graine «s'explique» de façon assez satisfaisante en fonction des variables géographiques (latitude, altitude, longitude); cependant les lois de variabilité (clines) ne sont pas très différentes entre sous-espèces, excepté pour la sous-espèce *bolandera* (qui occupe une aire restreinte en Californie) dont la graine est très légère compte-tenu de sa latitude méridionale.