DORMANCY MECHANISM AND EFFECTS OF TREATMENTS ON THE GERMINATION OF *GARCINIA GUMMI-GUTTA* (CLUSIACEAE) SEEDS

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ANILKUMAR, C., BABU, K. P. & KRISHNAN, P. N. 2002. Dormancy mechanism and effects of treatments on the germination of *Garcinia gummi-gutta* (Clusiaceae) seeds. *Garcinia gummi-gutta* (Syn. *Garcinia cambogia*) is normally propagated by seeds. Germination is uneven and occurs over six to nine months period which results in differentially-aged seedlings. Experiments were conducted to overcome dormancy both by chemical and mechanical methods. There was no response of seeds pretreated with petroleum ether, methanol or hydrogen peroxide. When seeds were decoated, 100% germination occurred in three months. Uniform germination of decoated seeds in different concentrations of pulp (barring the incidence of 40% seed mortality due to fungal attack) and water and methanol extracts of seed coat indicated the absence of chemical inhibitors. Positive results of decoating and acid scarification confirmed the role of seed coat in governing seed dormancy. Nicking of seeds dried in shade for 15 days was an effective method for raising even-aged, agronomically-sound seedlings in village nurseries.

Key words: *Garcinia gummi-gutta* - dormancy - seed coat - chemical and mechanical scarification

ANILKUMAR, C., BABU, K. P. & KRISHNAN, P. N. 2002. Mekanisme kedormanan dan kesan rawatan terhadap percambahan biji benih *Garcinia gummi-gutta* (Clusiaceae). *Garcinia gummi-gutta* (Syn. *Garcinia cambogia*) biasanya dibiak dengan biji benih. Percambahan adalah tidak sekata dan berlaku dalam tempoh enam hingga sembilan bulan. Ini menghasilkan anak benih yang berbeza-beza umurnya. Ujian yang menggunakan kaedah kimia dan mekanik ini adalah untuk mengatasi masalah kedormanan. Tidak ada tindak balas bagi biji benih yang diprarawat dengan eter petroleum, metanol atau hidrogen peroksida. Apabila kulit luar biji benih dibuang, 100% percambahan berlaku dalam masa tiga bulan. Percambahan seragam biji benih yang dibuang kulit luar di dalam pelbagai kepekatan pulpa dan juga pati kulit luar biji benih yang diekstrak menggunakan air dan metanol menandakan tiadanya perencat kimia. Keputusan positif dalam pembuangan kulit luar dan pelelasan asid mengesahkan peranan kulit luar biji benih dalam mengawal kedormanan biji benih. Menakik biji benih yang dikeringkan di bawah naungan selama 15 hari merupakan kaedah paling berkesan untuk membesarkan anak benih yang sama umurnya dan sesuai dari segi agronomi di tapak semaian di kampung.

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Introduction

Dormancy is regarded as the failure of an intact viable seed to complete germination under favourable conditions (Bewley 1997). In most cases, a considerable period may be required for effective action of natural agents to break dormancy under nursery conditions. Seed coat or pericarp can inhibit seed germination by various mechanisms such as preventing gas exchange, water uptake, light penetration or escape of inhibitors from the embryo (Taylorson & Hendricks 1977). High temperatures, hot water treatments and a combination of alternating temperatures have a great influence in overcoming seed dormancy in *Dichrostachys cinerea* (Fabaceae) (van Staden *et al.* 1994).

Garcinia gummi-gutta, an endemic species to peninsular India and Sri Lanka belongs to the family Clusiaceae and is an economically important spice tree grown for its dried rind. Decoction of fruit has medicinal properties in curing rheumatism and bowel complaints (Anonymous 1980). Normal propagation is through seed, which is represented by a hypocotyl enclosing a linear embryo devoid of cotyledons (Jansen 1992). Germination is sporadic and occurs over six to nine months which results in differentially-aged seedlings. This protracted and uneven germination may be due to dormancy. The objective of the present study is to determine the cause of dormancy as well as to standardise suitable treatments for achieving optimum germination responses in *G. gummi-gutta.*

Materials and methods

Ripened fruits of *G. gummi-gutta* were collected from Shola vegetation of southern Western Ghats of Kerala. Golden yellow berries usually have 6 to 12 seeds, each enclosed in juicy arillode. Average length and breadth of seeds are 2.4 ± 0.13 and 1.15 ± 0.11 cm respectively.

All seed treatments consisted of five replicates of 10 seeds each. Germination tests were carried out on Whatman No. 1 filter paper in Petri dishes kept in a closed chamber under laboratory conditions (25 ± 5 °C /80% RH, dark). In this paper, seeds are referred to pulped seeds while decoated seeds are those with their coats removed and hypocotyl exposed. All values were expressed as the mean percentage of germination ± standard error. The data were analysed statistically following analysis of variance (ANOVA), the means were tested as per the Duncan's multiple range test (DMRT) and least significant difference (LSD) was calculated at 0.05%.

Experiments were conducted to increase the permeability of seed coat both by mechanical and chemical methods. Mechanical scarification included nicking the seed at broader region with an aseptic needle. To study the effect of water and heat, seed samples were soaked separately in distilled water maintained for 24 hours at 30, 40, 50 and 60 °C. Chemical scarification consisted of soaking separate samples of seeds in petroleum ether (90%) or methanol (90%) for either 30 min

or one hour. Effect of chemical scarification was also investigated using 0.125, 0.25 and 0.5 m hydrogen peroxide (H_2O_2), each by pretreating the seeds for 30 min. Effect of acid scarification was tested by soaking seeds in 98% H_2SO_4 at 2 min intervals up to 16 min.

The content of the pulp makes it suitable for the presence of microbes, which attack seeds in the field and laboratory. To study the influence of any chemical inhibitors present in the pulp, decoated seeds were allowed to germinate in 0 (distilled water), 5, 10, 25, 50, 75 and 100% pulp. Presence of water-soluble and organic solvent-soluble inhibitors in the seed coat was tested separately by allowing decoated seeds to germinate in stock solutions of seed coat extracts. Seed coat extracts were prepared by grinding 50 mg seed coat either in distilled water or methanol. The mixture was then centrifuged at 6000 rpm. Supernatant was made up to 100 ml of stock solution by adding distilled water. However, in the case of organic solvent extraction, water was added only after complete removal of methanol by evaporation. The concentrations of stock solutions in this study were the same as the pulp. The germination tests were carried on for 60 days.

Detailed investigation of seed coat revealed its three layered nature; the outer layer dark brown and fibrous, the middle brown and the innermost yellow brown. These three layers are naturally appressed as if a single layer. To investigate the role of individual layers, germination studies were conducted after removing the coat layer by layer. Concomitantly another set of seeds were allowed to germinate after nicking individual coat layers for confirming their own specific role in controlling speed of germination. In an another experiment depulped seeds were dried in the shade for a period of 15 days by which time the seed coat became brittle enough to be separated from the yellow kernel. At this stage, a gentle twist broke the dark brown coat, and such seeds were allowed to germinate.

Results

Depulped seeds as control remained dormant for up to three months, after which they germinated to 78% six months after sowing (Table 1). Pre-sowing scarification of seeds enhanced germination from 16 to 82% by the third and sixth month respectively. Wet heat pre-treatments (30, 40, 50 and 60 °C) were less effective and temperatures above 50 °C were lethal. Soaking of seeds in petroleum ether, methanol and H_2O_2 enhanced neither speed nor percentage of germination. Seeds pretreated up to 6 min in 98% H_2SO_4 showed only negligible germination (below 30%) within three months, though germination percentage increased approximately to 84% by the sixth month. Seeds soaked in acid for 8 min germinated as much as 96% in six months, of which 70% germinated within three months. Seeds treated for 10 min in acid germinated to an average 72% in three months, but no additional germination was observed thereafter.

As pulp concentration of the germination medium increased, infection rate and mortality for decoated seeds increased up to 40% (Table 2). When the decoated seeds were allowed to germinate in different concentrations (0–100%) of extracts

of the seed coat in water and methanol they did not show any deleterious effect on germination percentage. In this case, 92–95% seeds germinated after 60 days, irrespective of the concentrations tested.

Table 1 Effect of various pretreatments on the germination of *Garcinia gummi-gutta* seeds

Treatment	Percentage of germination ± SE	
	3 months	6 months
Control	0	78 ± 8.4
Mechanical scarification	16 ± 5.5	82 ± 8.3
Wet heat / 24 hours		
30°C	14 ± 5.5	84 ± 8.9
40°C	14 ± 5.5	80 ± 10
50°C	18 ± 8.4	28 ± 8.4
60°C	0	0
LSD at 0.05%	7.72	9.83
Chemical scarification		
Petroleum ether / 30 min	10 ± 7.1	80 ± 10
Petroleum ether / 1 hour	8 ± 8.4	82 ± 8.3
Methanol / 30 min	10 ± 7.1	76 ± 8.9
Methanol / 1 hour	12 ± 8.4	78 ± 8.4
0.125 M H_2O_2 / 30 min	14 ± 5.5	80 ± 10
0.25 M H_2O_2 / 30 min	16 ± 5.5	84 ± 8.9
0.5 M H_2O_2 / 30 min	16 ± 5.3	82 ± 8.3
LSD at 0.05%	8.49	41.58
Acid scarification (98% H_2SO_4)		
2 min	26 ± 8.9	82 ± 8.4
4 min	28 ± 10.9	84 ± 5.5
6 min	34 ± 13.4	84 ± 8.9
8 min	70 ± 10.0	96 ± 5.5
10 min	72 ± 10.9	72 ± 10.9
12 min	74 ± 8.9	74 ± 8.9
14 min	53 ± 20.8	53 ± 20.8
16 min	37 ± 5.8	37 ± 5.8
LSD at 0.05%	14.31	17.53

Table 2 Effects of treatments on the germination of decoated *Garcinia gummi-gutta* seeds

Treatment	Percentage of germination on 60th day							LSD (0.05%)
	0% (Control)	5%	10%	25%	50%	75%	100%	
Pulp	96 ± 2.8	79 ± 4.2	75 ± 8.3	71 ± 4.2	67 ± 8.7	62 ± 4.2	60 ± 3.7	4.45
Water extract of seed coat	96 ± 2.8	96 ± 4.2	88 ± 8.3	89 ± 4.2	89 ± 4.2	96 ± 4.2	92 ± 8.3	49.23
Methanol extract of seed coat	96 ± 2.8	90 ± 7.1	95 ± 5.0	92 ± 2.5	95 ± 5.0	95 ± 5.0	95 ± 5.0	7.94

Seeds with all three coat layers started to germinate from the fourth month onwards and 78% germinated by the sixth month (Table 3). When the outer most layer was nicked, 94% seeds germinated six months after sowing. Germination began at the third month when the outer layer was completely removed. Nick scarification of the middle layer further accelerated germination during the first month and 78% seeds germinated by the fourth month. Upon complete removal of the middle layer, seeds with innermost layer germinated to 90% in four months. Nicking of the innermost layer hastened germination and all the seeds germinated by the fourth month. Decoated seeds germinated to 98% in two months. When depulped seeds were nicked after 15 days being dried in the shade, by the time of which the brown seed coat had started to separate from the yellow hypocotyl and moisture content was 30.48%, more than 70% germinated seeds were observed within three months of sowing.

Table 3 Effect of seed coat layers on the germination of *Garcinia gummi-gutta* seeds

Treatments	Percentage of germination					
	Month					
	1	2	3	4	5	6
3rd layer (Control)	0	0	0	6 ± 8.9	28 ± 8.3	78 ± 13.03
3rd layer, nicked	0	0	0	12 ± 8.4	74 ± 11.4	94 ± 5.5
2nd layer	0	0	36 ± 8.94	56 ± 8.94	96 ± 5.5	
2nd layer, nicked	6 ± 5.5	12 ± 4.5	36 ± 5.5	78 ± 4.5	96 ± 5.5	
1st layer	12 ± 4.5	16 ± 5.5	74 ± 8.9	90 ± 7.1	96 ± 5.5	
1st layer, nicked	28 ± 8.37	66 ± 5.5	84 ± 5.5	100		
Decoated	74 ± 8.9	98 ± 4.5	100			
Chipped, semi-dried seeds	0	26 ± 8.9	78 ± 8.4			

Discussion

Our observations indicated that 78% of the intact seeds germinated within six months whereas decoated seeds took only three months for complete germination. These values are higher that the 50 and 90% reported respectively by Chacko and Chandrasekhara (1977).

Wet heat pretreatments of *Grewia optiva* seeds soaked for 10, 20 and 30 min gave about 29% germination, higher compared with the control (Lata & Verma 1993). Further, the authors reported that germination of the seeds soaked in hot water for 24 hours started from nine days of sowing, while the control seeds took 19 days for germination to start. Similarly, hot water treatments promoted germination of rice seeds (Hayashi 1977, Lago *et al.* 1977). In our experiment, wet heat pretreatments did not overcome seed dormancy in *Garcinia.*

Taylorson and Hendricks (1979) used methanol and ethyl ether to overcome dormancy of *Panicum capillare* seeds. In the present investigation, methanol and petroleum ether had no effect on germination of *Garcinia* seeds. Coumans (1974) used H_2O_2 to overcome dormancy in sugar beet seeds. In our study however, H_2O_2 treatment was not effective with *Garcinia* seeds. Sugawara (1973) found that dormancy in *Oryza glaberrima,* which lasts about six months after harvest, could be overcome with H_2SO_4 treatments. When *Garcinia* seeds in our study were treated with 98% H_2SO_4 for eight minutes, 70% germinated in three months period.

In our study, as the pulp concentration increased in the germination medium, the rate of fungal infection also increased, which resulted in seed mortality (Table 2). *Garcinia gummi-gutta* seeds germinated equally in different concentrations of seed coat extracts in water and methanol, confirming the absence of any chemical inhibitors. Removal of the pulp prevented fungal infection and thus enhanced the germination percentage as reported in *Hancornia speciosa* seeds by Oliveira and Valio (1992). Similarly the role of seed coat and methanol-extractable phenolic substances in regulating seed dormancy of *Cynoglossum officinale* was studied by Qi *et al.* (1993). They found that seed coat did not retard water uptake, nor did it contain water soluble germination inhibitors. However, seed coat control oxygen availability to the embryo. The critical hydration level is species specific and corresponds to the critical water potential of the seed, below which germination cannot take place (Hadas & Stibbe 1973). The critical water potential of *Garcinia* seeds with initial moisture content of 41% was found to be low, since only 1% fresh weight enhancement was observed even with sprouted seeds. Our observations on the seed coat of *Garcinia gummi-gutta* showed increasing rate of germination and germination percentage when the seed coats were removed layer by layer. Thus each seed coat layer has its own role in controlling dormancy by checking water uptake, perhaps oxygen availability too, to the embryonic axis. Removal of all the three seed coat layers resulted in about 98% germination in two months.

In studying the differential degree of coat and seed desiccation, it was found that the coat dried first and became brittle enough to be easily separated from the seed. Even though seed dormancy is an innate mechanism favouring species perpetuation, it posses a problem in raising uniform seedlings in village nurseries. In this context, decoating of shade-dried seeds is an easy and cost effective method for overcoming *Garcinia* seed dormancy.

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