Danida Forest Seed Centre

IPGRI

THE PROJECT ON HANDLING AND STORAGE OF RECALCITRANT AND INTERMEDIATE TROPICAL FOREST TREE SEEDS

Newsletter - March 2001 8

Contents

Collection of *Anadenanthera colubrina.* Photo: BASFOR

This newsletter is a product of the “Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds” coordinated by the International Plant Genetic Resources Institute (IPGRI) and financed by Danida.

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The newsletter is edited by Danida Forest Seed Centre. Articles of relevance to the project, comments, discussions, etc. are welcome from everybody. Please contact Kirsten Thomsen, Danida Forest Seed Centre, Krogerupvej 21, DK-3050 Humlebaek, Denmark.

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The newsletter can be ordered free of charge at the same address.

Danida Forest Seed Centre (DFSC) is a Danish non-profit institute which has been working with development and transfer of know-how in management of tree genetic resources since 1969. The development objective of DFSC is to contribute to improve the benefits of growing trees for the well-being of people in developing countries. DFSC’s programme is financed by Danish International Development Assistance (Danida).

International Plant Genetic Resources Institute (IPGRI) is a member of the Consultative Group on International Agricultural Research (CGIAR). IPGRI’s mandate is fulfilled by encouraging, supporting and undertaking activities to improve the management of genetic resources worldwide so as to help eradicate poverty, increase food security and protect the environment. IPGRI focuses on the conservation and use of genetic resources important to developing countries and has an explicit commitment to specific crops.

More results!

The past six months have been quiet with regard to workshops and technical backstopping visits, but as you will see in this newsletter, the partners have been busy with their trials, and results are accumulating. Meanwhile we have been busy reading progress reports, making contracts, reporting to Danida, planning the Asian workshop and editing this newsletter.

Co-ordination of this project is very stimulating and rewarding, since we have the privilege of being in contact with so many good colleagues and are getting first hand knowledge of new interesting results. However, the format for the submission of the project progress reports is often a source of confusion. The project has established the progress report format which all of you are using correctly (see format for preliminary results on the homepage). This report should be preceded by the IPGRI summary sheet, which is included in appendix A of the letters of agreement. Please remember to fill this out when submitting your progress reports. Also, we would be very happy to hear from you if there are procedures we could improve in order to make things easier. Please do not hesitate to contact us if you have any suggestions or ideas.

Since we work with many species spread over the world, many of us will not be familiar with some (or many) of these species. DFSC is aiming to produce seed leaflets (see back page) on all the species included in the project, while IPGRI is busy setting up a database on tropical tree species of economic and conservation value. These are sources of information for many of the tropical tree species of concern to us on, for example, natural distribution, use and seed biology. Please submit any information about tropical forest species which you think we should include in our database. Photos are also very useful in getting to know species, so, during your trials, we would like to encourage all of you to take photographs of the stand, the adult tree, fruits and seeds, the staff in action etc. and submit them to Kirsten for use in the newsletter and on the homepage. Drawings are also very welcome.

Month after month, we receive more and more requests from seed scientists and seed research institutions, wanting to join the project, proof that the project is successful and that it receives a wide coverage. While the project has not sufficient resources to take new partners on board, interested parties are very welcome to join the network of seed scientists which the project is generating. The number of network members has now reached 474. Please submit your names and contact details to Kirsten and you will be added to the network and receive more information about the project. As you will notice, the newsletter often contains articles submitted by scientists outside this project working on recalcitrant seed. This is one way of sharing your experiences with other members of the network. Any inputs relating to recalcitrant/intermediate seed are very welcome, and those that are interested in submitting an article can contact Kirsten for directions.

We are busy organising the regional workshop on recalcitrant seeds for Asia which will be held in Thailand, hosted by ASEAN Forest Tree Seed Centre, in the beginning of April. Seed scientists from Australia, China, India, Indonesia, Laos, Malaysia, Vietnam and Thailand will be participating in this workshop. We look forward to bringing a full report in the next issue of the newsletter.

Ehsan Dulloo, IPGRI
and
Kirsten Thomsen, DFSC

Preliminary Results

Anadenanthera colubrina

Collecting partner	Edilberto Rojas Espinoza Centro de Semillas Forestales BASFOR Avenida Atahuallpa Final 5453, Cochabamba Bolivia
Replicating partner	Jaime Magne Carrera Ingenieria Forestal Universidad Gabriel Rene Moreno Santa Cruz Bolivia
Collection date	July 4th 2000
Seed source	Tin Tin, Mizque, Cochabamba

Initial trials (BASFOR)

Fruit weight ± SD	7.92 ± 2.29 g
Fruit size (length/width) ± SD	11.99 ± 2.68 cm / 2.06 ± 0.27 cm
Seed weight ± SD	0.32 ± 0.08 g
Seed diameter ± SD	1.72 ± 1.09 cm
Mc before processing %	41
Mc after processing %	37
Initial germination %	97

Initial trials (Universidad Gabriel)

Arrival date	July 7th 2000
Initial mc %	31
Initial germination %	89

Desiccation trial (BASFOR)

Mc after desiccation (%)	36.2	32.4	27.5	23.3	13.8	6.9
Germination (%)	**97**	**94**	**97**	**97**	**95**	**98**

Desiccation trial (Universidad Gabriel)

Mc after desiccation (%)	31	26	22	17	7
Germination (%)	**89**	**76**	**80**	**74**	**65**

Storage trial (BASFOR)

Mc before storage (%)	37 (fresh)		13.5	10.3	7.9	4.3
Germination before storage (%)	**97**		**97.3**	**97.3**	**97.7**	**98.7**
Mc after 3 months storage at 18°C (%)	40.3		16.3	14.2	9.2	8.1
Germination (%)	**0**		**3**	**5**	**84**	**94**
Mc after 3 months storage at 4°C (%)	40.1		16.4	13.1	10.9	7.8
Germination (%)	**0**		**60**	**63**	**92**	**93**
Mc after 3 months storage at -20°C (%)	40.0		16.0	13.7	10.1	8.0
Germination (%)	**0**		**92**	**92**	**93**	**90**

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Comments and conclusions

The seeds were fully desiccation tolerant and exhibited orthodox storage behaviour after three months of storage. The increase in mc during storage experienced at BASFOR is probably due to a sodium hypochloride treatment after desiccation, before storage. Lower germination percentages were obtained in the desiccation trial carried out at Universidad Gabriel, probably due to a three-week delay of the desiccation trial because silica gel was not available until then.

Anadenanthera colubrina
Photo: BASFOR

Buchanania lanzan

Collecting partner

S.C. Naithani.
Seed Biology Lab. School of Life Sciences
Pt. Ravishankar Shukla University
Raipur – 492 010 (M.P.)
India

Collection date April 24th 2000

Seed source Village Sambalpuri, District Raigarh

Initial trials

Fruit weight 0.64 ± 0.05 g
Seed weight 0.24 ± 0.02 g

	Fruit	Embryonic axes	Cotyledons
Mc (%)	46.3 ± 4.3	35.7 ± 0.9	15.4 ± 2.5

Mc before processing (%) 19.4 ± 1.2
Mc after processing (%) 18.2 ± 1.8
Initial germination (%) 93

Desiccation trial

Mc after desiccation (%)	12.4	6.3	3.9	3.3	2.7
Germination (%)	**91**	**81**	**80**	**73**	**60**

Storage trial

Storage for 10, 15, 30, 75 and 120 days. Results after 30 days presented below.

Mc before storage (%)	18.2	12.5	6.3	3.9	3.4	2.7
Germination (%) after storage at -20°C	**0**	**0**	**50**	**52**	**53**	**40**
Germination (%) after storage at 0°C	**0**	**0**	**50**	**60**	**57**	**60**
Germination (%) after storage at 15°C	**40**	**51**	**50**	**75**	**76**	**59**
Germination (%) after storage at 25°C	**60**	**48**	**60**	**60**	**35**	**30**

Comments and conclusions

The critical moisture content is somewhere between 6.3 and 12.4%. Seeds with the two highest moisture contents are sensitive to 0 and -20°C, the viability was 0% already after 10 days of storage.

***Calophyllum brasiliensis* (2000 collection)**

Collecting partner	William Vasquez CATIE 7170 Turrialba Costa Rica
Replicating partner	Danida Forest Seed Centre Krogerupvej 21, 3050 Humlebaek Denmark
Collection date	12 May 2000
Seed source	Volcan Buenos Aires, Puntarenas (BL096)
Initial trials (Costa Rica)	
Mc before processing %	56.0 ± 1.3
Mc after processing %	35.0 ± 1.5
Initial germination %	86.0 ± 7.6
Initial trials (Denmark)	
Arrival date	22 May 2000 (hand carried)
Mc at arrival %	33.78
Germination %	95

Desiccation trial (Costa Rica)			
Mc after desiccation (%)	31.8 ± 1.2	29.8 ± 0.8	28.4 ± 0.9
Germination (%)	**91.0 ± 8.9**	**91.0 ± 3.8**	**94.0 ± 5.2**
Germination (%) of control			94.0 ± 5.2
Desiccation trial (Denmark)			
Mc after desiccation (%)	33.6 ± 1.1	30.9 ± 1.6	27.6 ± 0.7
Germination (%)	**95.0 ± 3.8**	**91.0 ± 5.0**	**92.0 ± 3.3**
Germination (%) of control		98.0 ± 2.3	93.6 ± 0.8

Storage trial (Costa Rica)						
Mc before storage (%)	31.8	29.8	31.8	29.8	31.8	29.8
Temperature	Ambient		15°C		5°C	
Mc after 1 month of storage (%)	35.9	29.6	32.6	29.1	33.4	30.0
Germination after 1 month of storage (%)	**60**	**81**	**91**	**75**	**26**	**56**
Mc after 3 months of storage (%)	47.1	33.3	34.4	27.1	28.7	28.5
Germination after 3 months of storage (%)*	16**	55**	68	46	0	0

Storage trial (Denmark) The seeds were germinated after storage for 3 months at 5 and 15°C, but the germination test was stopped after 5 weeks due to massive fungal attack. 15-32% of the seeds appeared fresh when cut. After 6 months of storage the seeds will be desinfected before germination is initiated.

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* Germination after 7 weeks, test not completed
** Fungi and caterpillars were a problem during germination at ambient temperature

Calophyllum brasiliensis cont../

Comments and conclusions	The germination period was very long, the first seeds started to germinate after 3-4 weeks, peak germination was between 5-10 weeks and the total period was 5 months. No reductions in viability was found for the tested moisture contents in the desiccation trial, so 28 to 34% mc seems to be a safe interval. The preliminary results of the second trial confirm that the seeds are sensitive to 5°C. After 3 months of storage, best results are achieved for seeds with a moisture content around 30% at ambient temperature and 15°C. The difference in viability between the two moisture contents stored at 15°C indicates that moisture contents below 30% affect storability negatively.

Harpephyllum caffrum

Collecting partner	Sthandiwe Shange, Deon Erdey and Patricia Berjak Plant Cell Biology Research Unit School of Life and Environmental Sciences University of Natal, Durban 4041 South Africa
Collection date	3 July 2000
Seed source	University of Natal, Durban, KwaZula-Natal

Initial trials

Fruit weight	365.24 g
Seed weight	121.03 g
Initial mc (embryo axis) %	55.78
Initial mc (embryo) %	12.09
Initial germination %	10

Desiccation trial

Mc of embryo after desiccation (%)	16.10	7.10	4.10
Germination after desiccation (%)	**20**	**10**	**0**
Mc of embryo of controls (%)	10.01	5.09	7.20
Germination of controls (%)	**10**	**0**	**0**

Comments and conclusions

The seeds are characterised by axes with a high moisture content (55.78 %) and cotyledons with a low moisture content (12.09 %). However, it is difficult to establish conclusively the effects of desiccation on H. caffrum survival in these experiments. Control seeds exhibited poor germination (10%), as did seeds that were dried for 46 h to a moisture content of 7.10 %. The survival of some of these seeds to such low water contents seems to suggest that these seeds are not recalcitrant.

It is possible that the inability of these seeds to germinate is due to the increase in mechanical resistance of the stone during desiccation, rather than damage per se to the seed itself. A similar response has been observed for *Sclerocarya birrea* (a related species).

Fruits and seeds of
Harpephyllum caffrum (Bernh)
Photo: Patricia Berjak

Madhuca indica

Collecting partner	S.C. Naithani. Seed Biology Lab. School of Life Sciences Pt. Ravishankar Shukla University Raipur – 492 010 (M.P.), India
Collection date	June 10th (desiccation trial) and June 26th (storage trial) 2000
Seed source	Village Attari, District Raipur 7 km from Pt. Ravishankar Shukla University

Initial trials

Fruit weight	15.8 ± 2.8 g
Seed weight	3.3 ± 0.9 g
Seed size (length/breadth)	3.7 ± 1.2 / 1.8 ± 0.4 cm

	Fruit	Seed coat	Embryo	Embryonic axis	Cotyledons
Mc (%)	68.0 ± 3.1	63.8 ± 1.0	57.2 ± 3.4	65.2 ± 4.2	46.9 ± 3.1

Mc before processing (%)	54.8 ± 3.2
Initial germination (%)	100

Desiccation trial

Mc after desiccation (%)	53.0±2.6	48.0±0.4	37.7±3.6	28.4±4.0	14.8±2.1	9.4±2.3	4.8±0.2
Germination (%)	**100**	**100**	**89**	**40**	**10**	**0**	**0**

Storage trial

Mc before storage (%)		53.0 ± 2.6	48.0 ± 0.4	37.7 ± 3.6	28.4 ± 4.0
Storage period	3 days				
Germination (%) after storage at -20°C		**0**	**0**	**0**	**0**
Mc (%) after storage		55.2 ± 2.3	50.1 ± 3.1	35.2 ± 1.4	28.7 ± 3.1
Germination (%) after storage at 0°C		**0**	**0**	**0**	**0**
Mc (%) after storage		53.1 ± 2.1	47.8 ± 4.8	35.2 ± 2.3	28.3 ± 0.4
Germination (%) after storage at 15°C		**100**	**40**	**40**	**40**
Mc (%) after storage		54.4 ± 2.7	48.3 ± 4.8	34.7 ± 0.6	28.5 ± 0.7
Germination (%) after storage at 25°C		**100**	**100**	**14**	**20**
Mc (%) after storage		45.5 ± 1.8	41.6 ± 3.0	31.7 ± 1.1	28.0 ± 3.4
Storage period	30 days				
Germination (%) after storage at -20°C		**-**	**0**	**0**	**0**
Mc (%) after storage		-	51.8 ± 0.9	32.7 ± 1.9	30.1 ± 2.2
Germination (%) after storage at 0°C		**-**	**0**	**0**	**0**
Mc (%) after storage		-	48.6 ± 1.8	31.9 ± 3.4	25.8 ± 1.6
Germination (%) after storage at 15°C		**35**	**20**	**5**	**0**
Mc (%) after storage		49.4 ± 2.3	40.8 ± 4.8	21.8 ± 3.6	15.2 ± 3.1
Germination (%) after storage at 25°C		**10**	**5**	**0**	**0**
Mc (%) after storage		17.2 ± 1.6	15.2 ± 4.2	12.2 ± 2.7	9.9 ± 2.3
Storage period	60 days – all dead				

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Madhuca indica cont../

Comments and conclusions

The seeds were sensitive to drying below approx. 40% moisture content, and did not tolerate even short storage at -20°C and 0°C. Viability was lost fast, after 30 days of storage at 15°C, only 35% of the seeds stored at the highest moisture content germinated. After 60 days, all seeds were dead, and moisture contents had decreased significantly.

Psychotria capensis

Collecting partner

Sthandiwe Shange, Deon Erdey and Patricia Berjak
Plant Cell Biology Research Unit
School of Life and Environmental Sciences
University of Natal, Durban 4041
South Africa

Collection date 14 June 2000

Seed source University of Natal, Durban, KwaZula-Natal

Initial trials

Fruit weight	32.9 g
Seed weight	6.095 g
Initial mc %	42.27
Initial germination %	100

Desiccation trial

Mc after desiccation) (%)	35.20	27.85	17.58	10.85	13.51
Germination after desiccation (%)	**100**	**100**	**100**	**100**	**100**
Mc of controls (%)	36.55	33.95	19.64	12.65	12.72
Germination of controls (%)	**100**	**100**	**100**	**100**	**100**

Comments and conclusions

The seeds were harvested at a relatively high moisture content (36.51 - 42.47%) and 100% of the seeds germinated. The seeds showed quick germination, as radicle extension occurred within 7 days for most of the seeds. This could be due to the fact that all the seeds tested were fully mature and extracted from mature fruits. Moisture loss during drying in silica gel was rapid, reaching 35.20% and 27.85% within 4.5 and 21 hours respectively. Drying to seed moisture levels lower than 27.85% was not as rapid, however, reaching 17.58% and 13.51% within 44 and 86 hours. However, even though the rate of water loss decreased, seed viability remained unaffected (i.e. 100%). This was also the case for seeds maintained in vermiculite for equivalent periods. The survival of these seeds at a moisture content of 13.51% seems to suggest that this species is not recalcitrant. Further investigations will be undertaken to establish the survival of these seeds at lower moisture contents.

Shorea assamica

Collecting partner — Jayanthi Nadarajan and Daniel Baskaran Krishnapillay
Forest Research Institute Malaysia (FRIM)
Kepong 52109
Kuala Lumpur
Malaysia

Collection date — 12 April 2000

Seed source — Ulu Tranum Forest Reserve Bentong, Pahang (one tree)

Initial trials

Initial germination %	100
Initial mc (before extraction) %	47.87
Initial mc (after extraction) %	47

Component	Fruit	Whole seed	Seed coat	Embryo	Storage tissue/ cotyledons
Mc (%)	63.00	57.00	31.22	76.10	45.16

Desiccation trial

Mc (%)	45.11	43.60	31.34	28.76	19.59	11.27
Germination (%)	**92**	**85**	**82**	**80**	**47**	**0**
Mc of control (%)	47.07	46.21	45.24	44.89	46.20	45.27
Germination of control (%)	**95**	**95**	**96**	**97**	**85**	**65**

Storage trial (Malaysia)

Storage duration (weeks)	2	4	6	8	10	12	14	16	18	20
Mc (%) after storage at 25°C, initial mc = 42%	43.72	37.25	35.07	33.85	33.29	31.47	23.26	17.02	10.36	8.25
Germination (%)	**92**	**84**	**80**	**78**	**76**	**66**	**58**	**28**	**0**	**0**
Mc (%) after storage at 25°C, initial mc = 47%	47.3	44.9	43.2	40.7	38.6	32.8	25.1	19.1	15.7	12.5
Germination (%)	**94**	**90**	**92**	**82**	**78**	**62**	**54**	**28**	**0**	**0**
Mc (%) after storage at 16°C, initial mc = 42%	42.8	44.9	40.9	38.5	36.8	38.3	27.1	18.4	11.2	10.1
Germination (%)	**96**	**90**	**88**	**86**	**76**	**68**	**60**	**34**	**0**	**0**
Mc (%) after storage at 16°C, initial mc = 47%	46.8	44.6	40.4	39.7	36.4	31.6	27.4	23.0	17.9	11.1
Germination (%)	**96**	**92**	**86**	**82**	**70**	**62**	**58**	**44**	**32**	**0**

Comments and conclusions

Germination declined significantly when the seeds were dried down to 19% moisture content and below, the critical moisture content seems to be between 20 and 29%. Germination percentages stay high, above 60%, for 3 months. Thereafter, viability drops. Moisture contents decrease throughout the storage period, and after 16 to 18 weeks it has dropped below the critical level identified at the desiccation trial. The number of presprouted and dead seeds increase during storage, after 20 weeks approximately 40% of the seeds had presprouted.

Shorea roxburghii

Collecting partner	Suomal Saelim ASEAN Forest Tree Seed Centre Muak Lek District 18180 Saraburi Thailand
Replicating partner	Jayanthi Nadarajan and Daniel Baskaran Krishnapillay Forest Research Institute Malaysia (FRIM) Kepong 52109 Kuala Lumpur Malaysia
Collection date	31 March-1 April 2000
Seed source	Sakaerat environment research station

Initial trials (Thailand)

Fruit weight	0.91 g
Seed weight	0.73 g
Initial mc (before extraction) %	47.23
Initial mc (after extraction) %	45.17

Initial trials (Malaysia)

Date of arrival	24 April 2000				
Pre-sprouted seeds %	4				
Germination %	90				
Component	Fruit	Whole seed	Seed coat	Embryo	Storage tissue/ cotyledons
Mc (%)	38.9	38.0	21.7	52.3	46.6

Desiccation trial (Thailand)

Mc after desiccation (%)	36.03	27.3	25.04	21.23	12.07
Germination after desiccation (%)	**96**	**91**	**65**	**50**	**2**

Desiccation trial (Malaysia)

Mc (%)	39.0	31.0	25.4	21.2	9.0	7.3
Germination (%)	**88**	**80**	**60**	**27**	**10**	**0**
Mc of control (%)	36.7	38.7	37.8	34.5	36.3	35.7
Germination of control (%)	**90**	**84**	**82**	**78**	**74**	**66**

Storage trial (Thailand)

Storage moisture content	As in desiccation trial
Storage temperatures	5°C, 15°C and ambient
Storage containers	Cloth, plastic and foil bag

Most storage conditions resulted in no germination, therefore results below only include treatments with viable seeds after storage

Mc before storage (%)	36.03	27.3	25.04
Mc after storage for 3 months at 5°C in plastic bags (%)	25.33	28.67	23.97
Germination after storage for 3 months at 5°C in plastic bags (%)	**14**	**24**	**16**
Mc after storage for 3 months at 5°C in foil bags (%)	26.85	27.9	24.57
Germination after storage for 3 months at 5°C in foil bags (%)	**9**	**14**	**6**

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Shorea roxburghii cont.../

Germination after 3 months storage at ambient temperature, initial mc = 27.3, mc after storage = 34.19	**2 %**

Storage trial (Malaysia)

Storage duration (weeks)	2	4	6	8	10	12	14	16	18	20
Mc (%) after storage at 25°C, initial mc = 33%	31.5	30.8	28.9	29.8	27.9	24.6	18.7	14.2	9.4	6.2
Germination (%)	***88***	***82***	***76***	***62***	***64***	***48***	***25***	***18***	***5***	***0***
Mc (%) after storage at 25°C, initial mc = 28%	28.1	27.6	25.9	23.7	19.4	17.8	13.8	10.5	8.4	7.6
Germination (%)	**86**	**84**	**80**	**82**	**78**	**62**	**58**	**44**	**28**	**12**
Mc (%) after storage at 16°C, initial mc = 33%	32.4	31.2	29.1	27.0	26.8	22.9	19.2	16.1	11.4	9.7
Germination (%)	**90**	**86**	**82**	**74**	**64**	**58**	**42**	**34**	**15**	**0**
Mc (%) after storage at 16°C, initial mc = 28%	27.8	26.7	24.8	22.6	18.4	17.6	15.9	11.5	9.5	8.2
Germination (%)	**88**	**86**	**82**	**76**	**74**	**60**	**48**	**38**	**26**	**16**

Comments and conclusions

The seeds tolerate partial desiccation, but below 27% moisture content viability was reduced significantly. In the storage trial made in Thailand, 24% of the seeds with an initial moisture content of 27.3% survived storage at 5°C for 3 months. Whereas almost no seeds survived storage at ambient temperature. In the Malaysian storage trial germination around 50-60% was achieved after 12 weeks at 16°C and 25°C, even though the moisture contents had dropped to 18-25%.

All these results will be presented on our homepage as pdf files under this address:
http://www.dfsc.dk/Workplanupdate.htm

The results already available are as follows:

Acridocarpus natalitius
Cavacoa aurea
Cinnamamon cassia
Genipa americana
Hancornia speciosa
Illicium verum
Neobalanocarpus heimii
Strychnos cocculoides
Ximenia americana

DFSC Seed Leaflets

DFSC has initiated a new series of publications called Seed Leaflets with information on practical seed handling for a number of tree species. Each leaflet includes a paragraph on taxonomy, distribution, uses, botanical description, phenology, seed collection, processing, storage, pretreatment and nursery techniques, but emphasis is on seed handling and storage.

The leaflets are only two pages long and will be distributed as single sheets. The reason for this is that the single sheet format is easy to update. As new information is generated, e.g. on storage methods for recalcitrant species, the latest results will be added to the leaflet in question. It is possible that the leaflets will be posted in pdf format on DFSCs homepage in 2001.

The target group is seed technicians, extension workers and farmers, and focus is on simple, low technology methods that are possible in small scale seed production and in local nurseries.

The species are selected from priority lists from the countries that collaborate in making the leaflets. They are mainly species that are grown in a geographically limited area and are important to local communities as a source of income or as a supplement to fuel, fodder etc.

The collaborating partners are seed programmes in various countries. It is important that the information on practical seed handling methods comes from practitioners that are familiar with the species and not just from literature, which is often insufficient or out of date.

We hope our readers will give us feedback on the leaflets; any additional information, criticism or ideas are most welcome!

Dorthe Jøker
DFSC

List of Seed Leaflets available 1. January 2001. The leaflets can be ordered from DFSC and are distributed without charge. Species marked with * are included in the project on recalcitrant seed.

1. *Alnus acuminata*
2. *Acacia auriculiformis*
3. *Acacia mangium*
4. *Acacia mearnsii*
5. *Acacia senegal*
6. *Afzelia xylocarpa*
7. *Albizia lebbeck*
8. *Alnus nepalensis*
9. *Alstonia scholaris*
10. *Anacardium excelsum*
11. *Anacardium occidentale*
12. *Azadirachta indica* *
13. *Azadirachta excelsa*
14. *Casuarina equisetifolia*
15. *Grevillea robusta*
16. *Guazuma ulmifolia*
17. *Neolamachia cadamba*
18. *Swietenia mahagoni*
19. *Acacia tortilis*
20. *Cedrela odorata*
21. *Balanites aegyptica*
22. *Ceiba pentandra*
23. *Cinnamomum camphora*
24. *Quercus humboldtii* *
25. *Cordia alliodora*
26. *Dalbergia cochinchinensis*
27. *Dovyalis caffra* *
28. *Faidherbia albida*
29. *Senna siamea*
30. *Swietenia macrophylla*
31. *Afzelia quanzensis*
32. *Astronium graveolens* *
33. *Swietenia humilis*
34. *Acacia seyal*
35. *Pentaclethra macroloba*
36. *Pterocarpus angolensis*
37. *Pterocarpus indicus*
38. *Vitex keniensis*
39. *Vochysia guatemalensis* *
40. *Pinus caribaea*
41. *Pterocarpus macrocarpus*
42. *Chukrassia tabularis*
43. *Cunninghamia lanceolata*
44. *Styrax tonkinensis*
45. *Tamarindus indica*
46. *Calophyllum brasiliensis* *
47. *Hieronyma alchorneoides* *
48. *Maesopsis eminii*
49. *Hopea odorata* *
50. *Vitellaria paradoxa* *

Screening of *Dovyalis caffra* seeds

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Species information

Dovyalis caffra is a member of the family Flacourtiaceae. It is a shrub or small tree, up to 5 m in height, with many sharp 50 mm spines. It is found in bush and wooded grassland in southern Africa, but its seeds and seedlings are widely available for sale as the plant produces an almost impenetrable hedge. It grows well over a wide geographical range, and from 200-2000 m altitude (W. Omondi & K. Thomsen, pers. comm.). It is hardy and drought resistant. It produces fruit, which when ripe is yellow, containing up to 20 small seeds and an edible pulp. The fruit has a high vitamin C content (17 mg / 100 g), and is most often made into jam. If the fruits are fermented, an acidic herbicide is produced. The plant is also useful for fodder and bee forage (Mbuya *et al.*, 1994). It has been described as orthodox; mature and properly dried seeds can be stored at 3°C in airtight containers for several years (Albrecht, 1993). The seeds contain an oily endosperm and the embryo has thin, flat cotyledons (Corner, 1976).

Results

Initial determinations on the seed lot
The seeds were collected on 13 October 1999, partially cleaned, and despatched to Wakehurst Place by the Kenya Forestry Research Institute. Approximately 1.5 kg material was received on 21 October 1999. The bulk of the remaining fruit tissue was removed under running water, and the seeds blotted dry with laboratory tissue paper. Finally, individual seeds were separated from the flesh. The total weight of seed extracted was 180 g.

Initial measurements were carried out on 25 seeds. The length and width were 7.1 ± 0.6 mm and 4.2 ± 0.5 mm respectively. The average seed weight was 0.037 ± 0.09 g. Thus, the total number of seeds received was c. 4900.

Following hand picking to remove sprouting seeds, the initial moisture content analysis was carried out on 25 seeds. The seeds contained a layer of endosperm that adhered closely to the coat (testa). Consequently, moisture content determinations were made for the embryo (42 ± 7 %) and the rest of the seed tissue (41 ± 7 %).

The initial level of germination was 97 ± 2 %.

Drying experiment
Seeds were placed in bags with an equal weight of silica gel, and controls set up with bags of moist vermiculite. The experiment was run at 26°C. Seeds were dried for 7 h, 18 h, 1, 2, 4, and 12 d, to moisture contents of 26.6, 21.3, 13.9, 15.4, 6.0 and 3.9 % respectively, and then sown for germination on agar plates at 26 °C (12 h photoperiod). Germination (radicle emergence) was monitored for 21 d. A proportion of the seeds from the final drying stage was sealed into foil laminate bags and stored at both -20 and -70 °C for 7 days, after which germination capability was checked.

Drying seeds did not sprout during the experiment, and those that sprouted before the start of the trial were discounted. Between moisture contents of 42 to 15 %, germination was c. 95 %. As the moisture level was reduced to 3.9 %, germination fell to 71 %. The application of 7 days' storage at -20 and -70°C had no effect on germination - it remained at 70 % after both treatments.

Estimates of the mean time for seed germination at 26°C following drying were also made based on the equation:

$$\text{Mean Time to Germinate (MTG)} = \frac{\sum (T \times G)}{\sum \nu}$$

where T = the day of the germination test, G = the number of seeds germinating on that day, and $\sum \nu$

= the total number of seeds to germinate. MTG was 1.96 days for the initial germination and this value rose steadily during drying, to 6.4 d and 7.9 d for 6 % and 4 % moisture content seeds respectively. After storage at –20 and -70°C, the mean germination time was 7.3 and 7.7 days respectively.

Some of the control seeds sprouted during hydrated storage, and so viability was calculated as:

$$P + [(100 - P) / 100]G$$

where P is the percentage of seeds that presprouted during the experiment, and G is the percentage germination of non-presprouted seeds sown on agar. Hence [(100 – P) / 100]G is the percentage of all the seeds that did not presprout, but germinated on agar. Adding this value to the percentage of seeds that did presprout gives the overall viability. During control storage, viability fell from 96 to 56 % over 12 days, due to fungal infection of the moist seeds.

The equilibrium relative humidity of the seeds (a sample of 5) was measured after cleaning (93% RH), and after 4 d (57 % RH) and 12 days (32 % RH) of drying. Additionally, 5 seeds were equilibrated to constant weight over saturated salt solutions (≥ 17 % RH) or silica gel (c. 5 % RH). Equilibration periods of 4 weeks were used for the 33 to 83 % RH treatments and 8 weeks for the 17 and c. 5 % RH treatments. There was reasonable agreement between mc/RH relationship during drying with silica gel and after equilibrium with salt solutions. The general relationship between moisture content and RH (the isotherm) is similar to that of groundnut, which has an oil content of 45 % dry mass basis (Cromarty *et al*, 1982)

Germination temperature experiment
Fresh seeds were sown at temperatures from 6 to 35 °C and germination recorded over 21 days. Germination was highest (92-98 %) between 16 and 35 °C, but even at 6 °C germination reached 22 %. Germination was fastest at 26 °C, where the mean time to germinate was only 1.9 days. The mean germination time rose steadily above and below this temperature.

Conclusions and recommendations

The slight decrease (25 %) in germination when seeds were dried from 15 % to 4 % moisture content was probably due to desiccation sensitivity in a few seeds that were just about to sprout / germinate as the treatment was started. However, a germination level of over 70 % after desiccation to 4 % moisture content indicates that the seeds of this species are not recalcitrant. The desiccation response appears orthodox in nature and dry seeds do not display sensitivity to short-term (7 d) cooling at -20°C, unlike the seeds of some dryland palm species (Davies and Pritchard, 1998).
Although the optimum temperature for seed germination is around 26°C, high levels of germination occur from 11 to 35 °C. Such a response is consistent with the wide geographical range of this species.

Acknowledgements

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Desiccation stress in neem seeds : Physiological and biochemical considerations

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Abstract
Neem seed tolerated desiccation to 10.9% moisture content while further dehydration lead to absolute loss of viability. Pronounced decline in seed vigour was evident before the loss of germinability. Free radical mechanisms seemed to be involved in dehydration-related loss of viability. Overproduction of superoxide during loss of viability in neem seed may be responsible for accelerated rates of lipid peroxidation ultimately resulting in leaky membranes during imbibition. Accumulation of free radicals when the moisture content was below 10.9% was closely associated with simultaneous failure of antioxidant enzymes. Successful cryopreservation of the seeds was possible after fast drying of the seeds to 7.5% moisture content with 60% survival after one year of storage. However, decline in survival during storage, even in liquid nitrogen (LN2), warrants the necessity of studies on long-term storage in LN2 together with assessment of various vigour parameters.

Introduction
Neem (*Azadirachta indica* A. Juss; synonymous: *Melia indica* Brandis, *Melia azadirachta* Linn.), a native of the Indian subcontinent, is a very important tree not only for the people of this region, but also in other tropical parts of the world. The species belongs to the family *Meliaceae* and has been well known since ancient times as nim, Indian lilac, margosa tree etc. and exploited since the Vedic period in India over the past 5000 years. Its wood and derivatives have been used for multiple purposes; toiletries, cosmetics, livestock production, insecticide, timber, shade, fodder, public health, contraceptives, oil etc (Randhawa and Parmar, 1996).

However, as for other tropical tree species, the short span of viability of the neem seeds has become a major problem in its conservation and use (Gamene *et al.*, 1996). This paper examines the desiccation-induced oxidative stress and related changes in dehydrating neem seeds. Attempts were also made to evaluate the survival of neem seeds at LN2 temperatures for long term conservation after drying it to intermediate moisture content.

The seeds used for the trials were collected from Gariabandh forest, nearly 91 km from Raipur, Central India.

Storage behaviour
Like most recalcitrant and intermediate seeds, the mature neem seeds were shed from the tree at high moisture content (40-42%, fresh weight basis). The seeds were desiccation-sensitive and exhibited absolute loss of viability within 140-160 days after harvest when stored at ambient conditions (26-28°C, 35-40% RH), although varying viability periods were evident in neem seeds harvested in five years from 1995-1999. The lowest safe moisture content (LSMC) for the seeds was 10.9% (see figure 1). This is a relatively low moisture content compared to recalcitrant seeds where LSMC is 28-40%. A rapid loss of viability was experienced when the seeds were dried below 10.9% moisture content. Vigour tests, e.g. germination index (mean daily germination x peak value) showed that a decline in vigour preceded the loss of germination (Varghese and Naithani, 2000).

These results suggest that neem seeds exhibit intermediate storage behaviour. The desiccation sensitivity below 10.9% moisture content may be due to loss of "*structured or bound water*", which in turn, can result in metabolic imbalances and loss of stability of sub-cellular structures and membranes.

Solute leakage
Loss of viability below 10.9% seemed to be closely related to irreparable membrane perturbations, particularly during germination, since enhanced efflux of vital cellular constituents into seed leachates: sugars, proteins and inorganic constituents like K^+, Zn^{++} and Ca^{++}, was seen immediately after dehydration below LSMC.

[1] This article contains a summary of the most important results of Boby Varghese's Ph.D. thesis (awarded August 2000) at Pt. Ravishankar Shukla University, Raipur, India.

Damage by free radicals

Desiccation induced increased rates of peroxidation of membrane lipids, mediated by increased liberation of superoxide radicals (figure 2). Increased production of superoxide radical in dehydrating seed is suggested to be responsible for enhanced lipid peroxidation (Chaitanya and Naithani, 1994). The free radical damage to cellular membrane phospholipids and the resultant fatty acids lead to increased membrane dysfunction. Formation of malondialdehyde (MDA), one of the sensitive markers of lipid peroxidation *vis-à-vis* membrane damage, exhibited a significant correlation with the moisture content of seeds. Thus, it is proposed that the loss of viability in neem seeds below 10.9% moisture content may be due to the cumulative toxic effect of peroxidized products of the stored polyunsaturated fatty acids and membrane lipids in the embryo.

Antioxidants

Desiccating neem seeds are protected against oxidative injury above the critical moisture content by enzymic antioxidants: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (AsPOD) and guaiacol peroxidase (GPOD). The combined action of the antioxidants converts the potentially dangerous superoxide radical and hydrogen peroxide into water and oxygen, thus averting cellular damage (Leprince *et al*., 1993). A differential expression of SOD and CAT/AsPOD/GPOD was observed in the seeds (figure 3). Increased SOD with simultaneous reduction in CAT, AsPOD and GPOD activities immediately following drying of the seed below the critical moisture content on the one hand favours dismutation of superoxide whereas it, on the other hand, may favour accumulation of other reactive oxygen species (ROS). The accumulation of ROS (including highly reactive hydroxyl radicals via the transition metal catalyzed Haber-Weiss cycle) due to impairment of antioxidant enzymes may account for loss of viability in neem seeds below critical moisture content. Desiccation tolerance in orthodox seeds is particularly linked with higher activities of SOD, CAT and POD (Zhang and Kirkham, 1994) and massive loss of these enzymes was closely linked to viability loss in desiccation-sensitive seeds (Chaitanya and Naithani, 1998). Therefore, it is suggested that collapse of CAT/AsPOD and GPOD and not of SOD activity *per se* in response to desiccation below the critical moisture content is critical for the '*intermediate storage physiology*' of neem seeds.

Cryopreservation

Cryopreservation is considered one of the most promising approaches for long term storage of recalcitrant and intermediate seeds (FAO, 1993). As the neem seeds could be dried down to intermediate moisture contents without significant loss in seed viability, the possibility of its cryopreservation was explored (Varghese and Naithani, 1999). In our experiments, the desiccated excised neem seeds with 7.5% moisture content survived in LN2 for 12 months with 60% germinability (figure 4), and can hence be categorized as moderately cryotolerant. The seeds also exhibited sufficiently high vigour although loss was evident along with increased membrane perturbations at later stages (after 12 months) of LN2 storage.

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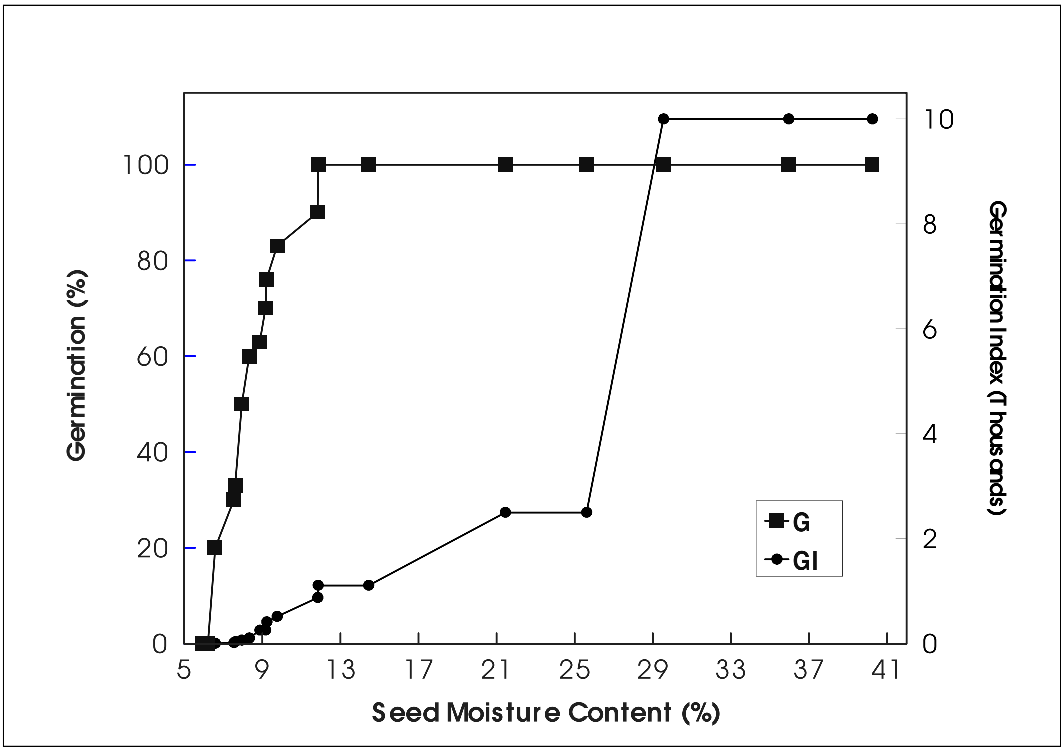

Figure 1: Loss in percentage germination (G) and germination index (GI) of neem seeds during desiccation.

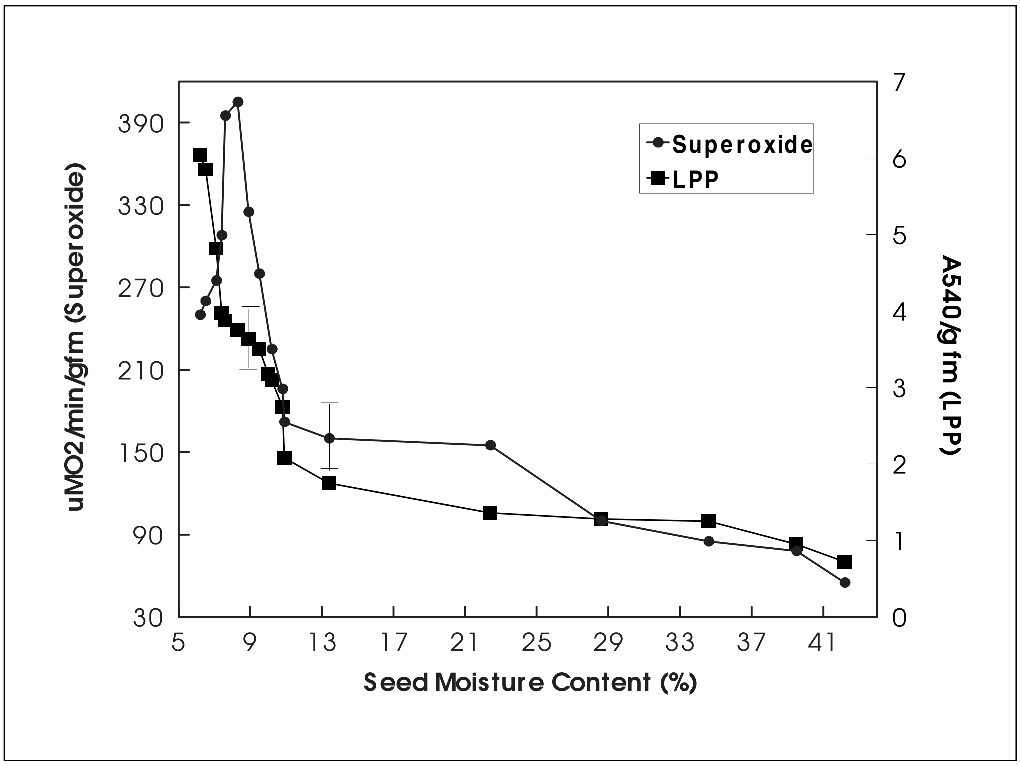

Figure 2: Changes in accumulation of superoxide and lipid peroxidation products (LPP) in the embryonic axes of neem seeds during desiccation. Vertical bars represent maximum ± SD.

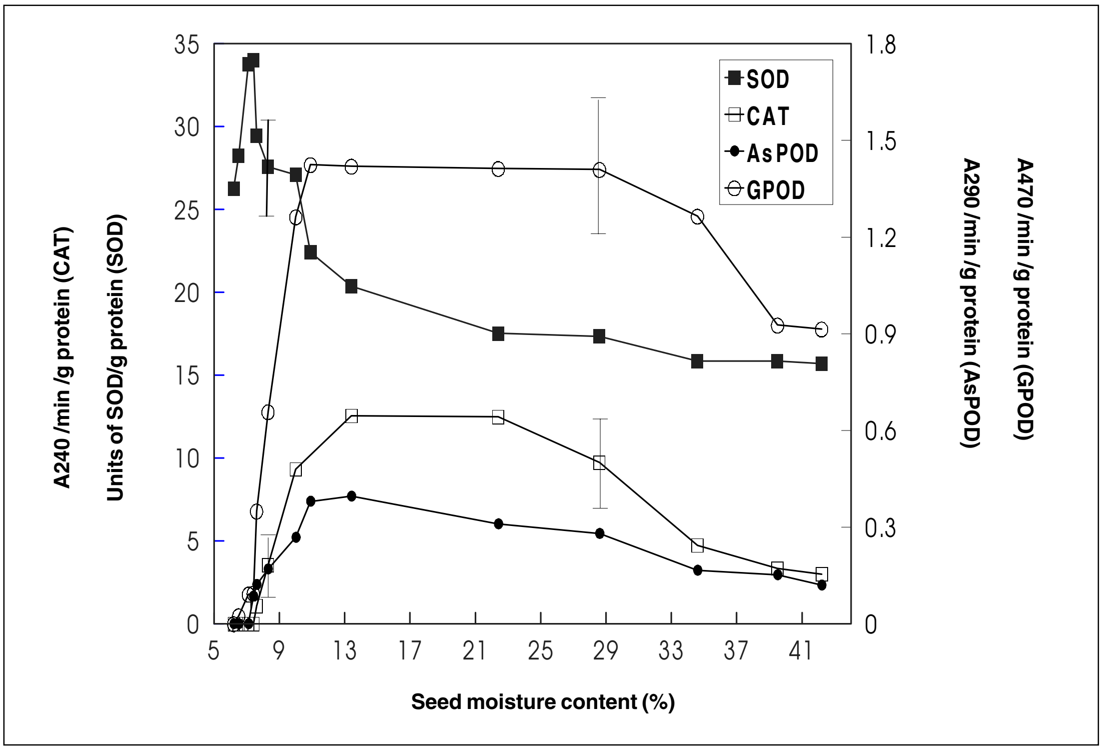

Figure 3 : Changes in antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (AsPOD) and guiaicol peroxidase (GPOD) in embryonic axis of neem seeds during desiccation. Values are mean of six replicates. Standard deviation has been plotted only for points with maximum value.

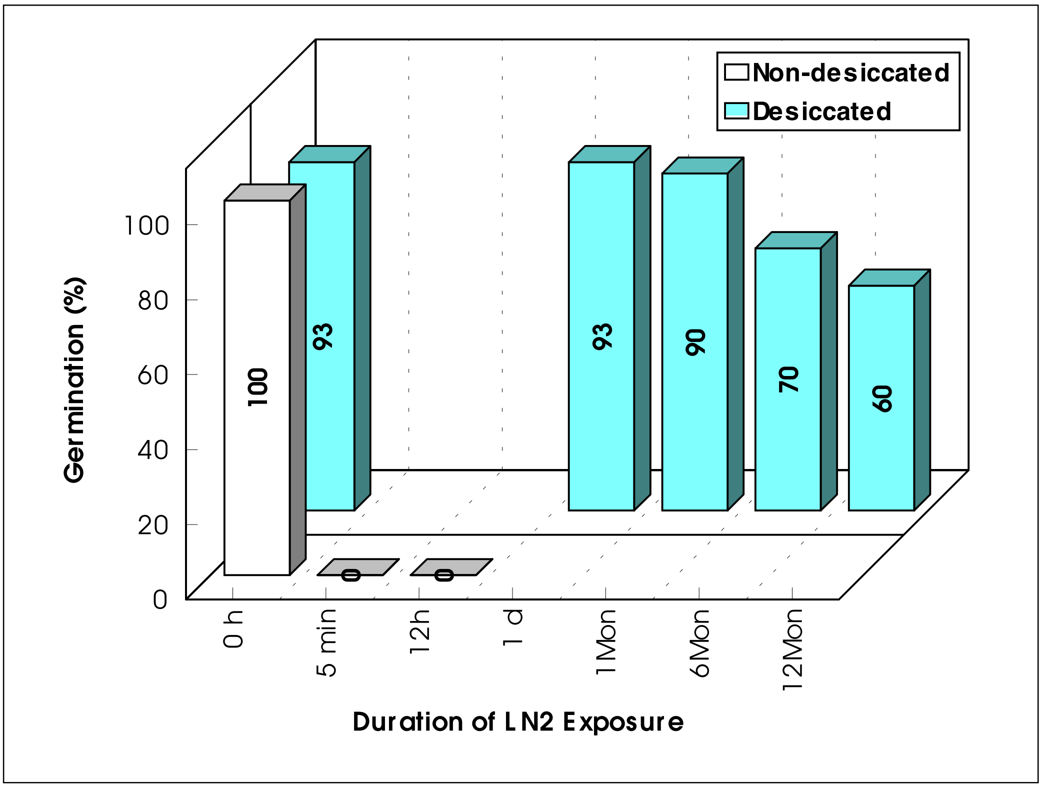

Figure 4 : Survival of non-desiccated and desiccated excised neem seeds after cryostorage for one year. Each value is a mean of 45 observations.

Desiccation and storage of *Dipterocarpus retusus* seed

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Dipterocarpus retusus Bl. (synonym: *Dipterocarpus macrocarpus* Vesque) is a tropical evergreen tree species belonging to the family Dipterocarpaceae. The height of the tree ranges between 45-48 m and the girth lies between 3.6-6.7 m with clean bole up to 40 m (Joshi,1980). It is distributed throughout the South and Southeast Asia extending from Assam in India to Burma, Thailand, Vietnam, Malay Peninsula, Java, Bali, Lombok and Sumbawa. In India, it grows in moist evergreen forests of the Brahmaputra valley of upper Assam, the foothills of Tirap, Changlang and Lohit districts of Arunachal Pradesh and Mokokchung and Tuensang district of Nagaland.

In eastern India the species is used for plywood timber, constructional timber, railway sleepers, poles etc. It also produces an oleoresin on blazing.

Flowering starts in July and continues to November. Fruits mature from January to March and the seeds are collected in February and March. The fruits have long wings, are 2.5-3.3 cm in diameter and 3-4 cm long, more or less contracted at the apex and the stalk is up to 0.2 cm long. The calyx lobes (wings) are removed manually to facilitate sowing. One kg of seed contains approximately 40-60 seeds. The seeds are short-lived under natural conditions, (Dent, 1948).

Methodology

Seed collection
Seeds were collected from the ground; those that fell first were rejected, as most of them were insect-infected. After selection of the trees, all vegetation, debris and old seeds were cleared from the ground and seeds were collected daily.

Determination of moisture content and germination percentage
For determination of moisture content, 3 replications of 5 seeds were cut into quarters and dried at 103°C for 17 hours. Moisture content was expressed as percentage of fresh weight. Viability was determined as germination percentage with 2 replicates of 50 seeds. The dewinged seeds were cut in halves and the pericarps were removed from the distal half, in which the embryonic axis is located, and this half was placed upside down on moist paper in trays. The trays were placed in a germinator at 30°C with a daily cycle of 8-hrs light and 16 hrs dark. Number of germinated seeds was recorded up to 30 days.

Table 1. Results of desiccation trial

Seed Moisture Content (%)				Germination (%)	Germination of control (%)
Embryonic axis	Peripcarp & Seed coat	Cotyledons	Whole seed		
76	46	58	55	80	80
76	27	56	50	80	72
72	20	54	47	75	78
65	18	50	36	67	90
47	15	36	30	29	77
36	13	34	23	0	76

Drying experiments
Seeds were mixed with dried silica gel and placed in a closed airtight box. Silica was changed every day and seeds were dried down to 50%, 47%, 36%, 30% and 23% moisture content. Seeds for control were stored in a ventilated box with moist perlite and 0.2% bavistin (fungicide).

Storage trial
The seeds were stored at shedding moisture content in perforated polybags with 0.2% bavistin at 0-5°C and 15°C for 3 months.

Results and conclusions

The initial moisture content of seed was 55% and the initial moisture content of pericarp, cotyledon and embryo were 46%, 58% and 76% respectively. The initial germination percentage was 82 ± 4%.

Table 1 shows the germination results of the various drying treatments with corresponding moisture contents of pericarp, cotyledons, embryonic axis and the whole seed. The results of the desiccation trial show that an appreciable decrease in germination percentage starts at 36% moisture level of the whole seed and 65% of the embryonic axes. At 23% moisture content of the seed and 36% of the embryonic axis, total loss of viability was observed. This trial shows that the seeds of *Dipterocarpus retusus* are desiccation sensitive. As reported earlier (Tompsett, 1992,1998) recalcitrant storage behaviour is common in dipterocarps.

Short-term storage at 5°C and 15°C showed a total loss of viability at 5°C, whereas at 15°C the seeds retained 61% of the initial germination after 3 months. The chilling sensitivity of the seeds also supports its recalcitrant nature.

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Desiccation sensitivity of avocado (*Persea americana* Mill.) seeds

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The recalcitrant behaviour of avocado seeds causes quick loss of viability under ambient conditions with gradual decrease in moisture content. In this study the lowest safe moisture content was found to be 49%, below which a rapid reduction in germination and seedling vigour was noticed along with increased electrical conductivity.

Avocado (*Persea americana* Mill.) is a important salad fruit distributed widely in the tropical and subtropical regions. The buttery pulp contains about 3 to 30 % oil, which is highly digestible and used in the cosmetic industry. The avocado plant is mainly seed propagated, but the seed loses its viability very soon under normal conditions after extraction from the fruit. The avocado seed has been classified as recalcitrant because it has a high critical moisture content. Several attempts have been made in a number of recalcitrant crop species to determine the critical moisture content but not yet in avocado. Hence a study was carried out to determine the critical moisture content for retaining the seed viability in avocado.

Material and method

Fully ripened avocado fruits were collected from the college orchard, TNAU, Coimbatore, and the seeds were extracted by cutting the fruits into two halves. Healthy and uniformly sized seeds were placed in single layers in trays kept at room temperature for desiccation. Seed samples were taken at two days intervals for assessing the viability as well as moisture content.

For determining the moisture content of the seeds, they were cut into small pieces. Then they were dried at a constant temperature of 105°C for 16 h and cooled in a desiccator before weighing (ISTA, 1999).

Three replicates of ten seeds were germinated in sand and kept in a germination room maintained at 25 ± 2°C and 90 ± 3% RH (ISTA, 1999). Fifty days after sowing, germination percentage, shoot length, shoot diameter and root length were assessed. The vigour index values were derived as per Abdul-Baki and Anderson (1973). Speed of germination was calculated by taking the germination count at weekly intervals up to 50 days and computed as per the method proposed by Maguire (1962).

Electrical conductivity of seed solute leakage was measured by placing three replicates of one seed in 50 ml of distilled water for 21 h at room temperature, where after the electrical conductivity of the leak water was measured (Presley, 1958). The data obtained were statistically analysed as per Panse and Sukhatme (1967).

Results and discussion

Recalcitrant seeds are shed from the parent plant with high moisture content ranging from 30 to 70% (Chin *et al*., 1984). In avocado, the initial moisture content of the seeds was 68%.

A significant decrease in viability was found for moisture contents below 49%. Only 33% of the seed germinated when the moisture content was reduced to 38%, and hardly any seeds germinated at 37% moisture content. At 34% moisture content, the seed had completely lost its viability (Table 1). Poulsen and Eriksen (1992) reported that recalcitrant seeds decline in viability below a certain critical moisture content. For example, the critical moisture content below which seeds are killed is 35% for *Hopea helferi* (Tamari, 1976), 15 to 20 % for *Hevea brasiliensis* (Chin *et al*., 1981) and 26% for *Theobroma cacao* (Hor *et al*., 1984). For avocado the lowest safe moisture content, in this case, was 49%.

Decline in germinability was associated with reduction in speed of germination, shoot length, root length, vigour index and shoot diameter (Table 1 and 2).

Table 1. Effect of desiccation on seed moisture content, germination, speed of germination, shoot length and root length in avocado seeds.

Treatments (number)	Seed moisture content (%)	Germination (%)	Speed of germination	Shoot length (cm)	Root length (cm)
Initial	68.10 (90.0)	100	0.072	66.40	13.42
2	58.84 (90.0)	100	0.058	50.40	6.57
4	49.08 (81.15)	93	0.058	30.17	8.19
6	45.08 (61.15)	77	0.059	22.04	7.50
8	40.82 (53.85)	65	0.044	21.58	5.47
10	39.49 (46.92)	53	0.036	12.82	5.07
12	37.88 (35.01)	33	0.035	12.80	3.33
14	37.20 (8.86)	7	0.026	10.67	3.37
16	34.20 (0.00)	0	0.000	0.00	0.00
18	31.85 (0.00)	0	0.000	0.00	0.00
SEd	0.78	6.41	0.003	3.61	1.50
CD(P=0.05)	1.64	13.37	0.007	7.53	3.12

Values in parenthesis indicate arc sine values.

Table 2. Effect of desiccation on vigour index, shoot diameter and electrical conductivity in avocado seeds.

Treatments (days)	Vigour Index (cm)	Shoot diameter (dSm-1)	Electrical conductivity
Initial	7985	0.767	0.354
2	5700	0.667	0.367
4	3553	0.633	0.425
6	2154	0.600	0.467
8	1790	0.567	0.475
10	960	0.533	0.508
12	552	0.467	0.539
14	80	0.400	0.547
16	0	0.000	0.680
18	0	0.000	0.819
SEd	362.73	0.06	0.02
CD(P=0.05)	756.64	0.12	0.03

Electrical conductivity of seed leak water increased during desiccation (Table 2). Weakening of cell membranes is believed to be the cause for leakage of metabolites like electrolytes and other soluble compounds into the imbibing medium (Heydecker, 1972). It is also possible that a reduction in moisture content in avocado seeds causes a loss of membrane integrity and nuclear disintegration which lead to the increased levels of electrolytes. Similar results were observed by Chin *et al.* (1981) in sun dried rubber seeds.

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Newsletter of IUFRO Research Group on Seed Physiology and Technology

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The International Union of Forestry Research Organizations (IUFRO) is divided into specialized areas of forestry research. One area is Seed Physiology and Technology identified as IUFRO Research Group (RG) 2.09.00. As the result of recent elections in IUFRO, Dr. Jack Vozzo has been elected Coordinator of 2.09.00. Deputy Coordinators will be Dr. Daniel Baskaran Krishnapillay, Forest Research Institute of Malaysia, Kuala Lumpur, Malaysia; and Dr. Tannis Beardmore, Canadian Forest Service, New Brunswick, Canada.

IUFRO is headquartered in Vienna, Austria and is composed of member countries structured by approved regulations. Individuals may become members of one of the IUFRO Research Groups provided that their individual institute is a recognized IUFRO member. If one's institute is not yet a IUFRO member, one may still be active in an RG by invitation from that RG. A limitation would be that only members of IUFRO may vote and/or hold office within IUFRO.

If you are currently not a participant in IUFRO RG 2.09.00, please accept this invitation to join us. There are no extra fees involved and there are no commitments to participating individuals or institutes.

The IUFRO RG also has a NEWSLETTER. It is not limited to tropical tree seeds and it is distributed only to each member of RG 2.09.00. This newsletter will be combined with The Tropical Tree Seed NEWSLETTER, begun by Dr. Frank Bonner in October 1990. The format will be a compromise of both. We intend to distribute the combined NEWSLETTER effort to both 2.09.00 members and to persons currently receiving the Tropical Tree Seed mailings. You do not have to become a member of RG 2.09.00 to continue receiving the NEWSLETTER if you formerly received the Tropical Tree Seed NEWSLETTER. Some of the persons are on both mailing lists already. The combined NEWSLETTER will be printed in English, Spanish, and French. You may select one language mailed to you, or you may access it in any of the languages using the web page at the 2.09.00 internet site. Details will come later.

The editor for the combined format will be Dr. Kris Connor, United States Forest Service, Starkville, Mississippi. We will continue to have NEWSLETTERS in February, June, and October annually.

RG 2.09.00 has organized international symposia where we meet to present our research, hold workshops, and publish the proceedings. These symposia are commonly held once a year and deliberately moved to different regions internationally to attract a wide participation. Our next meeting will be hosted by the College of Forestry and Natural Resources at the University of the Philippines, Los Banos. It is scheduled for April 30th to May 3rd 2001. This will be a combined meeting with IUFRO RG 1.07.00, Tropical Silviculture, Dr. Jerry Vanclay, Coordinator.

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SEED LEAFLET

No. 46 October 2000

Calophyllum brasiliense Cambess.

Taxonomy and nomenclature

Family: Clusiaceae

Synonyms: *Calophyllum chiapense* Standley, *C. rekoi* Standley, *C. antillanum* Brit. Standley.

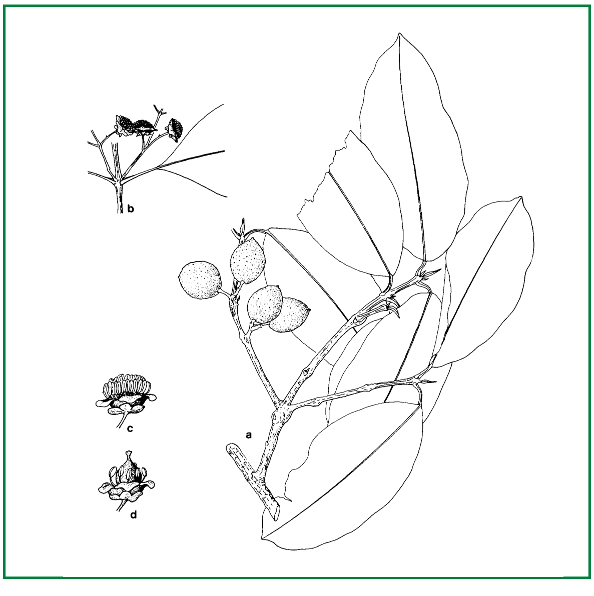
Vernacular/common names: Santa maría, maría, jaca, jacareúba, Brazil beauty-leaf.

Distribution and habitat

The natural range includes southern Mexico, Central America and northern South America. It is also found in the Antilles from Cuba to Jamaica and Trinidad-Tobago. It grows as a canopy tree in the humid tropical forests. It is found from sea level and up to 1500 m altitude in areas with annual rainfall of more than 3000 mm and temperatures of 24-28°C. It grows well on sloped areas with alluvial or clay soils even when very humid and acid (pH 4.5-6.0).

Uses

The species is mainly grown for the attractive wood. It is used for both outdoor and indoor constructions and is durable in contact with soil and water. Depending on origin, the wood is heavy or only moderately so, with specific weight of 0.45 – 0.69 g/cm^3. The fibres can be used for paper pulp and the latex has medicinal properties.



A, fruiting branch; b, part of inflorescence; c, staminate (male) flower; d, bisexual flower. From: Flores 1994.

Botanical description

Tree up to 45 m tall with straight bole without butresses and branchless for about 2/3 of the height. The bark is thick and contains a yellow-green latex. Leaves are simple and very variable in size depending on climatic conditions, the smallest leaves on trees growing in dry areas. The species is andromonoecious, i.e. each tree has both male and bisexual flowers. The flowers are about 1 cm in diameter with small, cream coloured petals. Male and bisexual flowers in separate, 3-9 cm long inflorescences, each with 2-10 flowers.

Fruit and seed description

Fruit: round berry, 2.5-3.0 cm long, green at first later with brown patches. The pericarp is leathery and dotted with numerous laticifers containing yellow latex. Each fruit contains one, large seed.

Seed: 1.8-2.3 cm long, with thick, brown testa and large, oily cotelydons. There are 415-440 seeds/kg.

Flowering and fruiting habit

In dry areas the trees defoliate at the end of the dry season but in other places the leaves remain. The flowers are visited by numerous insects but the pollinator is not known and it is also unknown whether both male and bisexual flowers produce viable pollen. There are large variations in flowering and fruiting times between zones, but in most of the geographical range flowering occurs in June-July. Most trees bloom only once every year but in Central America there can be a second period of flowering in November-December. The fruits mature in October-November but it is possible to find some fruits in December-January. In Central America a second fruiting often occurs in May-July. The trees begin to produce fruits when they are about 5 years old. They produce fruits every year but the annual harvest varies in size.

Harvest

The fruits are green at maturity but the colour becomes less bright as they ripen. When the pericarp is easy to remove, the seeds are mature and ready for collection. The fruits can be collected directly from the tree or from the ground. 3 kg of fruits yield about 1 kg seed.

Processing and handling

The mature seeds have high moisture content and the fruits must be transported to the processing site in gunny sacks or open bags allowing respiration. At the processing site the fruits are dried for one day in the shade. Extraction is done manually by placing the fruits in one layer in trays and crushing them gently to break the pericarp. Once the pericarp is loose it is easily removed.

Storage and viability

Fresh seeds have a moisture content of 60% and storage behaviour is probably recalcitrant but the results are not conclusive. A recent trial in Costa Rica showed that the seeds could be desiccated to 25% moisture content and still retain germination close to 100%. The preliminary results also indicate that the seeds are sensitive to 5°C.

Until more is known, it is recommended to store the seeds in loosely folded bags at no less than 25% moisture content and temperatures above 15°C.

It is also possible to store whole fruits and extract the seeds just before germination.

Dormancy and pretreatment

The seeds are not dormant but it is advisable to soak them for 24 hours prior to germination in order to soften the seedcoat. The soaking ensures a more uniform germination and minimises the number of seedlings with abnormal growth.

Sowing and germination

Germination is epigeal. Although the cotyledons remain inside the seedcoat, the petioles elongate and perforate the seedcoat to make an opening through which the shoot emerges. In 10-15% of the germinating seeds the petioles fail to make way for the shoot and the shoot is often damaged. In these cases axillary buds give rise to one or two shoots but these seedlings show slow or abnormal growth and have a lower survival rate.

The radicle begins to emerge after three weeks and in the nursery germination is normally complete after one month. If the seeds are germinated in a cabinet, germination may take longer. The seeds can be sown directly in containers or in germination boxes and then transferred to containers when the radicle has emerged. When the seedlings have been transferred they should be kept in the shade for 15 days. When the seedlings after 5-6 months are 25-30 cm tall they are ready for planting in the field.

Phytosanitary problems

The larvae of an unknown coleoptera can attack the seeds and the fruits are predated by monkeys and rodents.

Selected readings

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Flores, E. M., 1993. *Calophyllum brasiliense.* Trees and Seeds from the Neotropics Vol. 3, no 1. Museo National de Costa Rica.

IPGRI/DFSC. Preliminary results from the Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds, phase 2.

Fruits and seeds of *Calophyllum brasiliense.* Photo: Dorthe Jøker, DFSC.

THIS NOTE WAS PREPARED IN COLLABORATION WITH CENTRO AGRONÓMICO TROPICAL DE INVESTIGACIÓN Y ENSEÑANZA

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