

SEED CHARACTERISTICS, STORAGE BEHAVIOR and VIABILITY EQUATION of *Jatropha curcas* (L.)

Lucille Elna Parreño-de Guzman

University Researcher
Seed Science and Technology Laboratory
PSSD, Crop Science Cluster
College of Agriculture, UP Los Baños

OUTLINE

Introduction

- ▶ Seed characteristics, moisture content and drying
- ▶ Seed viability and vigor testing
- ▶ Seed storage and prediction of seed longevity

Seed Science & Technology Laboratory
PSSD, CSC-CA, UPLB
June 2007 – March 2010

INTRODUCTION

- seed is a basic crop production input
 - ▶ quality is determined through seed testing
 - ▶ methods must be based on:
 - scientific knowledge of seed
 - accumulated experience of the seed analyst
 - ▶ research is focused on ~ 6,000 cultivated species
 - ▶ little attention is given to underutilized and neglected crops like *Jatropha curcas*

J. curcas

- well-documented — medicinal properties
- minimal or no information on:
 - seed physical and chemical characteristics
 - processing and seed quality testing
 - storage behavior
 - prediction of its longevity in storage
- ▶ genetic conservation
- ▶ plant propagation
- ▶ utilization

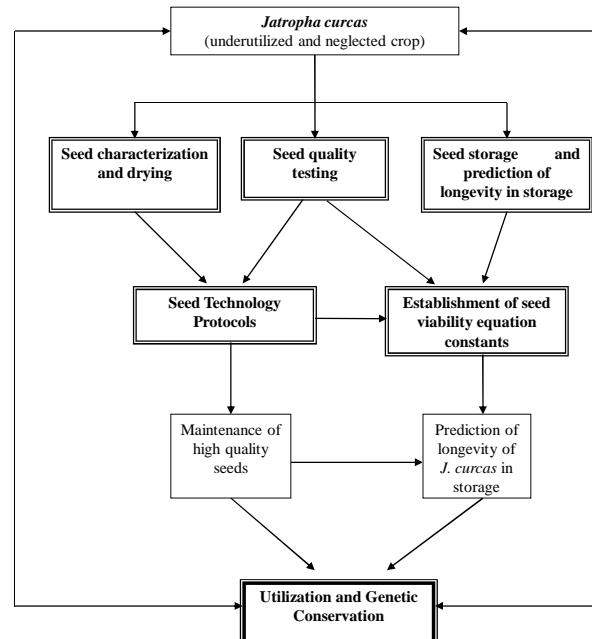
Objectives

General:

- To establish the seed technology of *J. curcas* and find out its viability constants

Specific:

1. To characterize and evaluate the morphological and chemical properties of *J. curcas* seeds.
2. To develop procedures for optimum seed drying, quality testing (viability and vigor tests and moisture content determination), and storage of *J. curcas*
3. To establish the species-specific temperature (C_H and C_Q) and moisture content (K_E and C_W) viability constants



Conceptual framework of the research

(1)

SEED CHARACTERISTICS, MOISTURE CONTENT and DRYING of *J. curcas*

Materials and Methods

Summarized passport data.

DETAILS	GENEBANK (GB) NUMBER			
	57,115	57,387	57,388	57,528 ¹
Collecting No.	LPG-07	LPG	LPG	MLHV/EEDR/ AGL-07-0321 TOMKASa
Trader/Source	Karen Tanquiamco	PNOC	D1 Oils Phil., Inc.	Tomas Chavez, Sr.
Collected from	Mahayahay, Digos, Davao del Sur	-	Cotabato	Edwards, T'boli, South Cotabato
Acquisition Date	January 18, 2007	July 5, 2007	August 24, 2007	September 12, 2007
Planting Material Collected / Remarks	seeds of mixed population	seeds	seeds	seeds from yellow fruits; sun-dried for 7-8 h after seed extraction

¹ Two seed lots were acquired and designated as 57,528(N) and 57,528(O) where N seeds were harvested 8 September 2007 and O seeds were harvested on 5 September 2007.

- ▶ Seed physical and chemical characterization
- ▶ Moisture (MC) content determination
 - 12 seed preparations - (7) whole seed, (1) seed coat & (4) kernels



- ▶ Seed drying
 - Moisture isotherm – 200 ml glycerol solution (6 RH)
 - Drying curve – 1:1 and 2:1 silica gel and seed



Moisture isotherm set-up

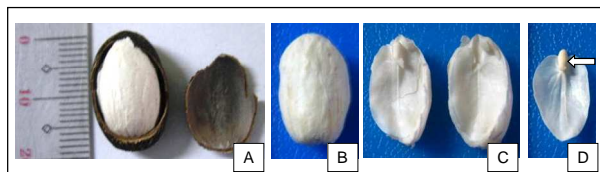


Drying curve set-up

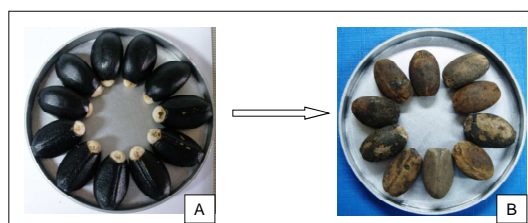
- ▶ Experimental design and data analysis:
 - CRD in 4 replications
 - SAS for Windows ver 6.12
 - LSD to compare treatment means

Results and Discussion

Physical characteristics



Internal morphology of *J. curcas* seed.



Physical transformation of *J. curcas* seed from newly harvested to aged or old seeds.

Seed dimensions, weight and number of seeds kg^{-1} of acquired bulk *J. curcas*.

PARAMETER	GENEBANK NUMBER (GB No)					MEAN
	57,115	58,387	58,388	57,528(N)	57,528(O)	
Dimensions ¹ (cm)						
Length	1.87 (0.10)	1.63 (0.17)	1.89 (0.10)	1.93 (0.07)	1.95 (0.08)	1.85
Width	1.17 (0.04)	1.08 (0.07)	1.17 (0.06)	1.13 (0.05)	1.15 (0.05)	1.14
Thickness	0.90 (0.05)	0.82 (0.07)	0.94 (0.07)	0.92 (0.04)	0.92 (0.04)	0.90
Weight ¹ (g)						
Whole seed	0.67 (0.13)	0.54 (0.15)	0.72 (0.12)	0.80 (0.10)	0.76 (0.10)	0.70
Seed coat only	0.27 (0.10)	0.20 (0.04)	0.28 (0.02)	0.29 (0.03)	0.29 (0.02)	0.27
Kernel only	0.43 (0.03)	0.35 (0.09)	0.45 (0.09)	0.50 (0.08)	0.47 (0.09)	0.44
1,000 seed weight (g)	681.4 (0.98)	525.1 (0.82)	721.8 (1.08)	807.0 (0.84)	765.3 (0.65)	700.1
No. of seeds kg ⁻¹	1,468	1,904	1,385	1,239	1,307	1,461

¹ Mean value of 100 individual seeds followed by standard deviation enclosed in parenthesis

Chemical characteristics

Proximate analysis¹ of whole seed, kernel, and seed coat *Jatropha* seeds.

SEED PART	GENEBANK NO.	PERCENTAGE (%)					
		Moisture	Fats	Protein	Ash	Fiber	NFE ²
Whole seed	57,115	3.4	31.13	16.59	4.65	32.20	12.07
	57,528	3.1	35.87	16.78	3.69	31.95	8.62
	58,387	1.4	33.83	16.45	4.45	34.70	9.15
	58,388	1.4	34.88	15.76	4.01	36.15	7.77
	Mean	2.3	33.93	16.40	4.20	33.75	9.40
	sd	(1.08)	(2.04)	(0.44)	(0.43)	(2.03)	(1.87)
Kernel	57,115	2.3	51.59	27.17	2.43	8.25	8.22
	57,528	2.0	51.82	24.72	3.21	9.00	9.25
	58,387	1.2	52.45	20.02	3.47	10.90	7.00
	58,388	0.9	54.59	23.28	3.71	10.30	7.21
	Mean	1.6	52.61	23.80	3.21	9.61	7.92
	sd	(0.66)	(1.37)	(2.99)	(0.56)	(1.21)	(1.03)
Seed Coat	57,115	5.7	4.25	10.09	4.44	54.85	20.72
	57,528	4.8	4.86	10.27	4.70	49.06	26.28
	58,387	3.1	4.89	10.18	4.70	50.70	26.44
	58,388	2.9	5.38	10.20	4.28	50.85	26.44
	Mean	4.1	4.85	10.19	4.53	51.37	24.97
	sd	(1.35)	(0.46)	(0.07)	(0.21)	(2.46)	(2.83)

Moisture content (MC) determination

Summary ANOVA on percentage MC of 5 seed lots of *J. curcas* using 12 seed preparations.

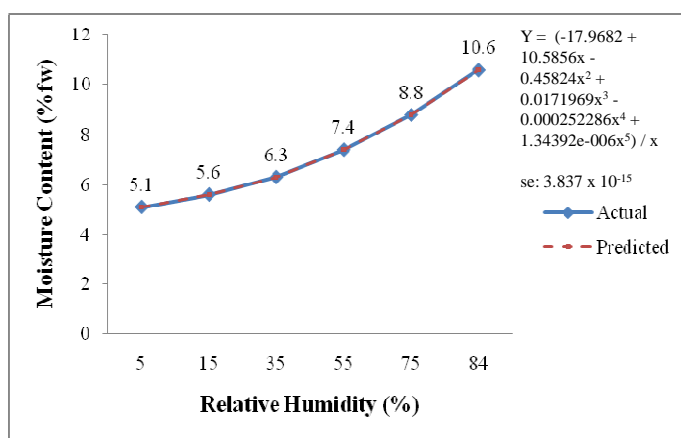
SOURCES OF VARIATION	DF	PR>F
Seed preparation (SP)	11	*
Seed lot (SL)	4	*
SP x SL	33	*
Error	117	
Total	155	
CV = 3.75%		LSD = 0.47

Moisture content (%) of 5 seed lots of *J. curcas* using 12 different seed preparations.

SEED PREPARATION	MOISTURE CONTENT (%) OF SEED LOTS (GB NO.)					MEAN
	57,115	58,387	58,388	57,528(N)	57,528(O)	
Whole seed (WS)	8.9 ef	9.6 d	8.8 ef	11.0 c	8.7 ef	9.4 w
WS ground	9.4 de	9.5 d	8.7 ef	10.9 c	8.4 fg	9.4 w
WS with SC cracked	9.5 d	9.6 d	-	-	-	9.6 w
WS (SC + K)	9.3 de	9.2 de	-	-	7.5 hi	8.7 x
WS cut by ½	8.9 ef	9.5 d	-	-	7.7 gh	8.7 x
WS cut by ¼	9.0 d	9.7 d	-	-	7.5 hi	8.7 x
WS cut by 1/8	8.8 ef	9.4 de	-	-	7.9 gh	8.7 x
Seed coat (SC) only	11.9 b	12.0 b	11.6 b	13.5 a	11.8 b	12.2 v
Kernel only	6.7 j	7.6 h	7.1 ij	8.7 ef	6.6 j	7.3 y
Kernel cut by 1/2	6.6 j	7.6 h	-	-	-	7.1 y
Kernel cut by 1/4	6.4 j	7.6 h	-	-	-	7.0 y
Kernel cut by 1/8	6.4 j	-	-	-	-	6.4 z
Mean	8.5 r	9.2 q	9.1 q	11.0 p	8.3 r	

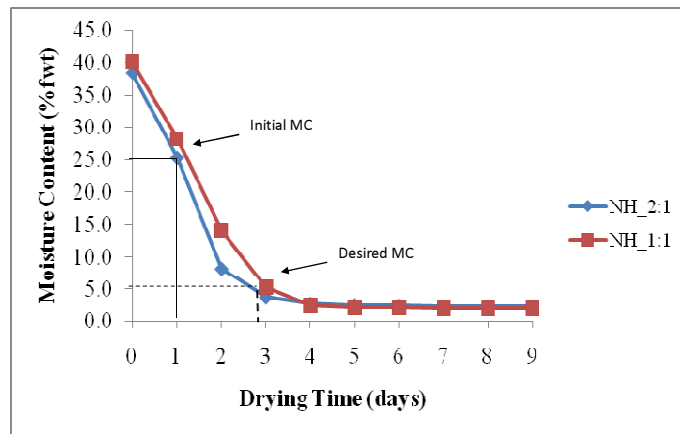
Treatment means with common letter are not significantly different at 5% LSD.
CV = 1.84% LSD = 0.47

Seed drying: Moisture isotherm

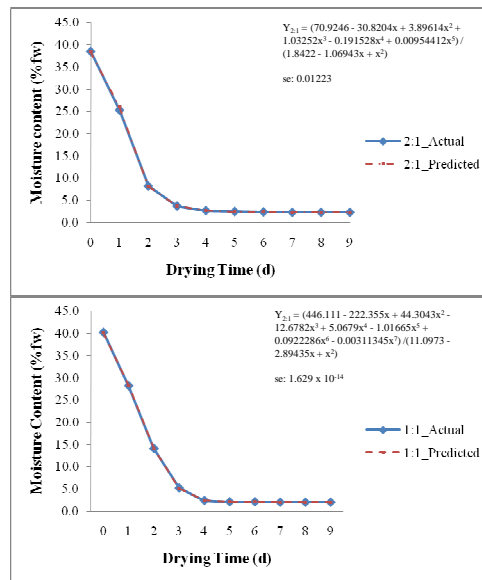


Actual and predicted moisture isotherm or EMC of *Jatropa curcas* seeds under 6 different RH levels. The polynomial equation and standard error were derived using FindGraph ver 2.181

Seed drying: Drying curve



Drying curve of newly-harvested *Jatropa curcas* seeds with initial MC of 38.5-40.2% using a 2:1 and 1:1 silica gel and seed ratio



Actual and predicted drying curves of *Jatropa curcas* seeds with initial MC of 38.5-40.2% using a 2:1 (top) and 1:1 (bottom) silica gel and seed ratio. The polynomial equations and standard errors were derived using FindGraph ver 2.181

Conclusion

- Baseline physical and chemical properties of local *J. curcas* seeds have been established
 - ▶ Black shiny seed coat and white-colored hilum can be used as indicators of high quality seeds
 - ▶ MC determination is best using ground whole seed, LCTOM of 103 ± 2 °C for 17 ± 1 h
 - ▶ Seed MC can be estimated without resorting to oven drying by using the moisture isotherm and drying curves that were developed

▶ Three protocols were developed specific for *J. curcas* seeds:

1. Moisture content (MC) determination
2. Establishment of Moisture isotherm
3. Establishment of Drying curves

Contents:

- Equipment and supplies
- Precautions/Notes
- Step by step procedure
- Calculation of results and Tolerances
- References

(2)

SEED VIABILITY and VIGOR TESTING of *J. curcas*

Materials and Methods

A. Viability tests

1. Germination test
2. Tetrazolium (TTZ) test
 - 3 quality levels (%G = 90, 50 & 30)
 - 3 pre-moistening treatments (soaked in water)
 - Whole seed (WS), 36 h
 - WS with cracked seed coat, 24 h
 - Kernel only, 6 h
 - 3 types of seed tissues immersed in 0.5% TTZ
 - Cotyledon + embryo
 - Embryo square
 - Embryo only
 - 3 soaking time (2, 3, & 4 h)



B. Vigor test through electrical conductivity (EC)

- seed quantity (10, 25, & 50 seeds)
- water level (50, 100, 150, & 200 ml)
- soaking time (EC measured every 2 h for 40 h)
- Final test
 - ▶ Simultaneous EC and viability test plus vigor test through Mean germination time (MGT)
 - ▶ 2 containers with distilled water only was used as control

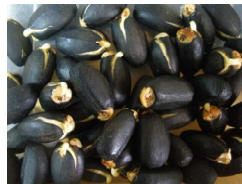
▶ Experimental design and data analysis:

- CRD in 4 replications
- SAS for Windows ver 6.12
- LSD to compare treatment means
- Correlation analysis of some parameters measured

Results and Discussion

Viability: Germination Test

- Initial results
 - 47 – 77%; 14 – 28 d



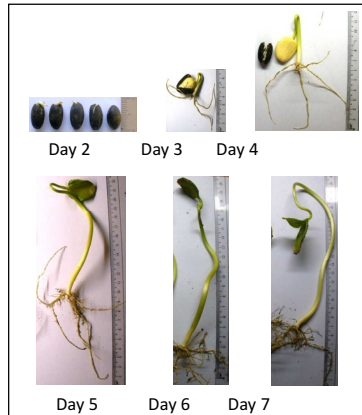
No dormancy in *J. curcas*

Newly extracted seeds that have already germinated while still inside yellow fruits.

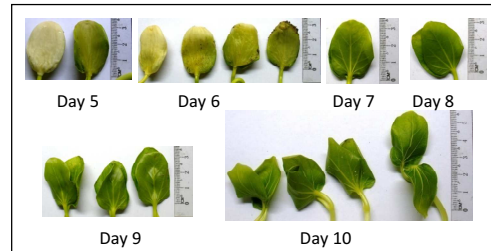


Stages of fruit maturity of *Jatropha curcas*. A – green; B – yellow green; C – yellow black; D – black (Aquino et al 2009).

Best maturity level: yellow green fruits
Germination = 96%



Transformation of *Jatropa curcas* seed during germination test, from radicle emergence at day 2 to a full-grown seedling at day 7.



Transformation of plumule into first true leaves during germination test of *Jatropa curcas*. The white colored-cotyledon in day 5 becomes transparent and jelly-like or papery in day 6 and eventually falls off until the first true leaves unfold from day 7 to 10.

- Germination test = 10 d with first count on the 7th d
- Media = sterilized moist, uncompressed sand

Viability: Tetrazolium (TTZ) Test

Summary ANOVA for the TTZ test *J. curcas*.

SOURCES OF VARIATION	df	GERMINATION Pr>F
Seed lot (SL)	2	*
Pre-moistening treatment (PMT)	2	*
Type of tissue exposed to TTZ (TT)	2	*
Soaking duration in TTZ (SD)	2	*
SL x PMT	4	*
SL x TT	4	ns
SL x SD	4	ns
PMT x TT	4	ns
PMT x SD	4	ns
TT x SD	4	ns
SL x PMT x TT x SD	48	ns
Error	81	
Total	161	
CV = 52.84%		LSD = 7.41

Viability (%) of *Jatropha curcas* seeds as affected by seed lot, pre-moistening treatment and their interaction.

PRE-MOISTENING (SOAKING TIME IN WATER) TREATMENT (PMT)	VIABILITY (%) OF SEED LOT (GENEBANK NO.)			MEAN
	57,528(O)	57,387	57,115	
Whole seed, 36 h	68 ab	23 c	24 c	38 m
Whole seed with seed coat cracked, 24 h	64 b	30 c	28 c	41 m
Kernel only, 6 h	72 a	10 d	11 d	31 n
Mean	68 o	21 p	21 p	

CV = 50.01%

LSD = 6.46

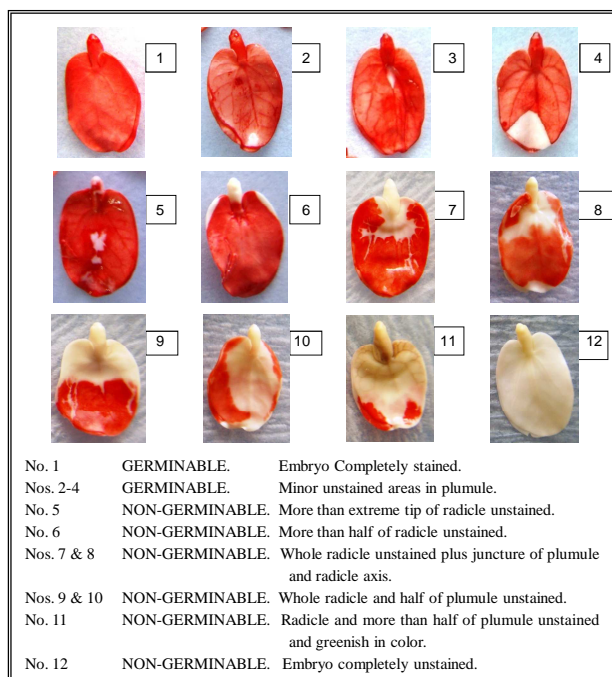
Treatment means with common letter within a column and within a row are not significantly different at 5% LSD.

Viability (%) of *Jatropha curcas* seeds as affected by type of tissue exposed to 0.5% TTZ and soaking duration in TTZ.

FACTORS AFFECTING TTZ TEST	VIABILITY (%)
Type of tissue exposed to TTZ (TT)	
Cotyledon plus embryo	32 b
Embryo square	37 ab
Embryo only	41 a
Mean	37
Soaking duration in TTZ (SD)	
2 h	42 a
3 h	36 ab
4 h	32 b
Mean	37

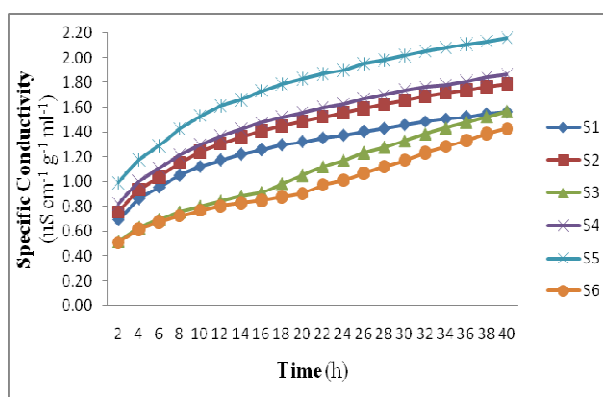
Treatment means with common letter among each factor are not significantly different at 5% LSD.

Tetrazolium staining patterns and their interpretation for *Jatropha curcas* seeds.



Vigor: Electrical Conductivity Test

Optimum soaking time



Specific conductivity curve for six *J. curcas* seed lots soaked in distilled for 40 h.

Specific conductivity of *J. curcas* using 10 and 25 seeds soaked in different water levels for 31 h.

WATER LEVEL (ml)	CONDUCTIVITY OF SEEDLOTS ($\mu\text{S cm}^{-1} \text{ g}^{-1} \text{ ml}^{-1}$)		
	10 Seeds	25 Seeds	MEAN
50	3.88	3.68	3.78 a
100	1.03	0.94	0.98 b
150	0.47	0.42	0.44 c
Mean	1.79 a	1.68 a	

CV = 12.32% $\text{LSD}_{\text{WL}} = 0.22$ $\text{LSD}_{\text{seed no.}} = 0.18$

Treatment means with common letter across columns and across rows are not significantly different at 5% LSD

Conclusion

- Germination of *J. curcas* seeds is best using sterilized sand as substrate with seeds sown with the hilum facing sideways; on a level layer of moist 20-25 mm uncompressed sand.
 - ▶ Uniform seed germination can be observed within 3 days after sowing (DAS) with initial and final counts done after 7 and 10 DAS, respectively.
 - ▶ The embryo is the best seed tissue to use for TTZ test and the optimum soaking duration in TTZ solution is 2 h.

- Through TTZ test, seed viability can be determined in **8h** for seeds with high viability using kernel soaked in water for 6h or in **26h** for seeds with low or unknown viability using WS with cracked seed coat soaked in water for 24h.
- ▶ the optimum conditions for conductivity test is 10 seeds soaked in 100 ml of distilled water for 31 h
- ▶ Protocols for the following tests has been developed:
 1. Germination
 2. Tetrazolium (TTZ)
 3. Electrical conductivity (EC)

(3)

**SEED STORAGE and PREDICTION OF
LONGEVITY of *J. curcas***

Materials and Methods

1. Sealed low temperature storage

- ▶ GB No 57,528(N)
- ▶ 4 MC levels: 4.0, 5.0, 6.5, and 9.5%
- ▶ 2 temperature conditions: 0 °C and 18-20 °C
- ▶ 2 y storage
- ▶ 8 samplings of MC and germination



2. Unsealed ambient temperature storage

- ▶ GB Nos 57,528(N) and 57,528(O)
- ▶ Placed in net bags
- ▶ Ambient temperature of 25-30 °C
- ▶ 13 mo storage
- ▶ monthly samplings of MC and germination

3. Prediction of seed longevity

- ▶ TCS seed lot
- ▶ 3 MC levels: 4.5, 6.8 and 9.4%
- ▶ 3 temperature conditions: 13, 30 and 40 °C
- ▶ germination test every 3 mo (50 seeds x 4 reps)

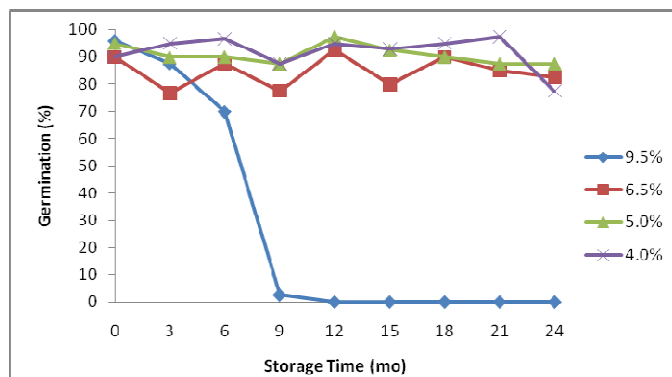
- ▶ Experimental design and data analysis:
 - CRD in 4 replications
 - SAS for Windows ver 6.12
 - LSD to compare treatment means
 - Correlation and regression analysis of some parameters measured

Results and Discussion

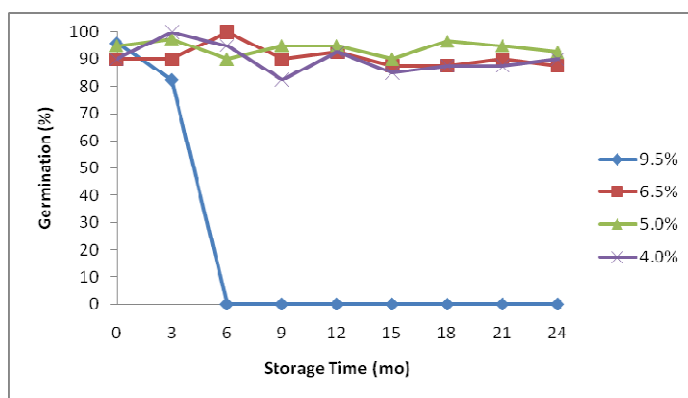
Seed Storage

Analysis of variance of germination and moisture content of *Jatropha curcas* seeds with four initial MC stored in airconditioned room (18-20 °C) and 0 °C temperature for 24 mos.

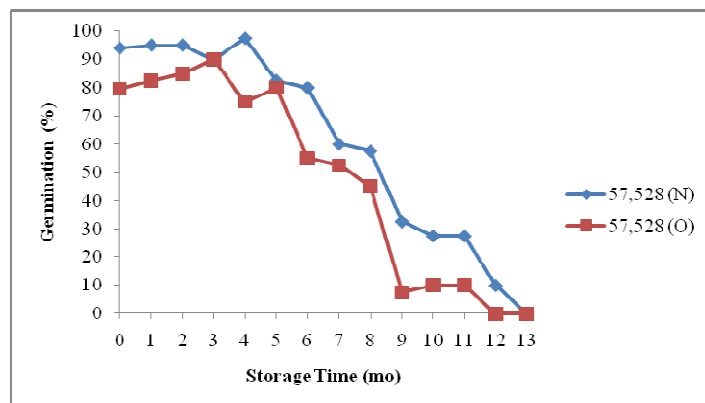
SOURCES OF VARIATION	df	Pr>F	
		GERMINATION (%)	MOISTURE CONTENT (%)
Sampling Time (ST)	8	*	*
MC	3	*	*
Temperature (T)	1	ns	*
Replication	3	ns	ns
MC x T	6	*	*
ST x MC x T	41	*	*
Error	174		
Total	236		
cv (%)		10.67	4.44
LSD		12.26	0.37



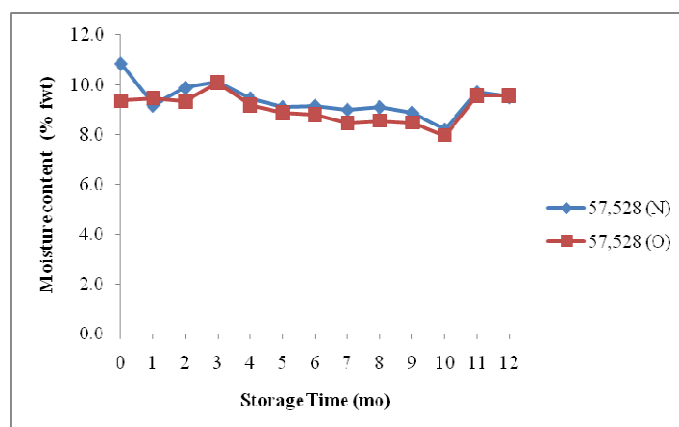
Germination percentage of *Jatropha curcas* seeds with different MC stored in sealed aluminum foil packs at 18-20 °C for 24 mos.



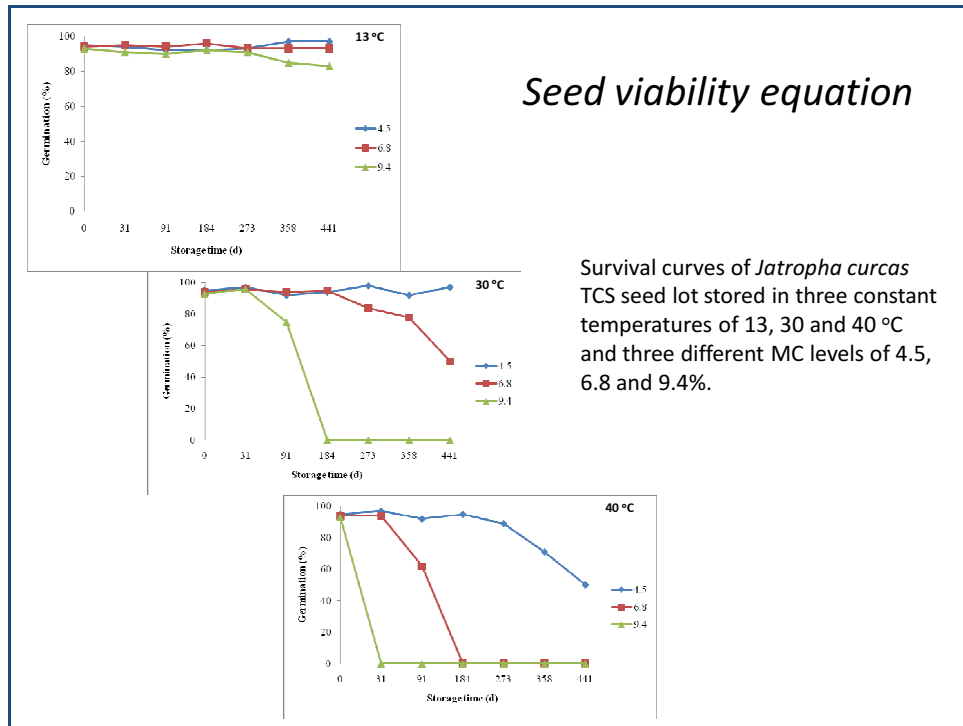
Germination percentage of *Jatropha curcas* seeds with different MC stored in sealed aluminum foil packs at 0 °C for 24 mos.



Germination percentage of *Jatropha curcas* GB No. 57,528 (N) and (O) seeds stored inside net bags under ambient conditions (25 – 30 °C) for 13 mos.



Moisture content of *Jatropha curcas* GB No. 57,528 (N) and (O) seeds stored inside net bags under ambient conditions (25 – 30 °C) for 12 mo.



- Results: Temperature of 13 °C can not be used

$$v = K_i - \frac{p}{10^{K_E - C_W \log m - C_H t - C_O t^2}}$$

where:

v = final viability (expressed as %, NEDs or probits) after p days storage.

P = storage time (days)

m = % moisture content (fresh weight basis)

t = temperature (°C)

K_i = initial viability of the seed lot at $p = 0$ days (seedlot constant)

C_H and C_O = species-specific temperature constants

K_E and C_W = species-specific moisture content constants

$$v = K_i - p/10^{K_L - C_1 m - C_2 t}$$

where:

v = final viability (expressed as %, NEDs or probits) after p days storage.

K_i = probit percentage viability at the beginning of storage

p = storage time (days)

m = % moisture content (fresh weight basis)

t = temperature (°C)

K_L = species-specific seed lot constant

C_1 = species-specific MC constant

C_2 = species-specific temperature constant

- seed death was defined as viability at 85%
 - ▶ Species-specific constants
 - $K_L = 2.81167$ (0.21024)
 - $C_1 = -0.02524$ (0.01316)
 - $C_2 = -0.00751$ (0.00527)
 - ▶ $R^2 = 0.0463$

Given:

$$T = 40\text{ }^{\circ}\text{C}$$

$$MC = 4.5\%$$

$$K_i = 95\%$$

$$v = 50\%$$

$$v = K_i - p/10^{K_L - C_1 m - C_2 t}$$

$$p = (K_i - v)10^{K_L - C_1 m - C_2 t}$$

$$= (0.95 - 0.50)10^{(2.81167) - (-0.02524 * 4.5) - (-0.00751 * 40)}$$

$$= (0.45)10^{3.22565}$$

$$= 757\text{ d}$$

50% viability was reached in 441 d which is 316 d earlier than that predicted using the derived constants

- seed viability equation experiment was not able to come up with reliable values for the MC and temperature constants
 - huge volume of seeds with high initial viability
 - manpower to monitor germination on a daily or even weekly basis
 - oven or cold storage facilities that could be maintained at a constant temperature for the whole duration

Conclusions

- *Jatropha curcas* seeds can be stored for 2 yr with little reduction in percent germination if seeds are dried to 4.0 - 5.0% MC before storage and are kept sealed in moisture-proof containers.
- ▶ storage under low temperature is not an absolute requirement for maintaining seed viability
- ▶ Under ambient storage MC must be lowered to 6.0 - 8.0% and seeds must be stored immediately after harvest in sealed containers to maintain high percentage germination.

- seed viability equation experiment was not able to come up with reliable values for the MC and temperature constants due to several limitations
- ▶ present results can be used as a basis for refining the methodology of similar studies in the future

Limitations

- Under local conditions, no distinct genotype of *J. curcas* has been identified
- Seeds used can only be described based on their passport data
- Results may be applicable only to the specific seed lots used

RECOMMENDATIONS

- Apply the protocols developed to other seed lots of *J. curcas* and find out if results would be comparable
 - ▶ Refinements of the protocols

► Options for future research include:

1. Sealed storage
 - ambient and low temperature
 - different MC levels
 - minimum length of storage of 4 – 5 y
2. Viability equation
 - temperature range of 25 – 45 °C at 5 °C interval
 - use the universal temperature constants but expand the MC to 10 levels
 - weekly or daily monitoring of germination
 - minimum length of storage of 4 – 5 y.

Thank you very much!