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Seed germination and storage studies in Jatropha (*Jatropha curcas* L.)

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Abstract
Germination and storage behaviour of six seed lots of *Jatropha curcas* L. revealed that among the three substrata tested, sand proved to be the best with maximum germination (82.3%) followed by Between Paper (77.2%). Temperature also affected the germination percentage with maximum germination (78.4%) observed at 30^{0}C. There was a gradual reduction in germination in all the seed lots with increase in storage period, irrespective of the containers used. Maximum reduction is germination was observed in muslin cloth bags in which germination was reduced from 93.8% to 48.0%. Polythene (700μ) bags maintained the germination up to 68.1% after 9 months of storage. Less reduction in oil content was observed in polythene (700μ) bags than muslin cloth bags and HDPE bags. Vigour index-I was also reduced as the storage period increased with maximum reduction in muslin cloth bags followed by HDPE. The results obtained during the study revealed that polythene (700μ) bag is best container for Jatropha seed storage and can maintain the viability up to 9 months with standard germination above 68%.

Keywords: Germination test, Jatropha seed, Oil content, Storage container, Seed storage

Introduction

Jatropha curcas L. is a multipurpose large shrub or small tree of Latin American origin. In recent past, the economic importance of Jatropha was increased due to its oil usage as biodiesel. It is, imperative to ascertain the quality of seed before taking up sowing and plantation. Neither seed and field standards nor germination protocol is available at national and international level for Jatropha. The information on above aspects is a prerequisite to understand the germination ecology. Even under low temperature conditions, seeds with high oil content may have their germination reduced during storage and may completely lose their germination capacity after one year (Nazreen *et al*., 2000). It is of common occurrence that the seed of one season of this crop cannot be used for sowing in next year due to quick fall of germinability and viability, if not stored properly. The present study was undertaken to identify germination method and suitable containers for storing Jatropha seeds under ambient conditions with different combinations of relative humidity (RH) and temperature.

Material and Methods

The present study was conducted at Department of Seed Science and Technology CCS Haryana Agricultural University (CCS HAU), Hisar, India. The study materials consisted of six seed lots of *Jatropha curcas* L. collected from different locations of Haryana *viz*. L_{1-} Medicinal and underutilized Section, Department of Plant Breeding, CCS HAU, Hisar, L_{2-} Department of Agro Forestry, CCS HAU, Hisar, L_{3-} Regional Research Station, Bawal, Distt. Rewari, L4 Ladwa (District Hisar), L_{5-}Umra (Distt. Hisar), L_{6-} Ratera (Distt. Hisar). The above seed lots were stored at ambient conditions in the Department of Seed Science and Technology, CCS HAU, Hisar.

***Germination study*:** Three replications with 100 seeds per replication for each seed lot were placed on sufficiently moistened substrata *viz*., sand, between paper (B.P.) and Top of the paper (T.P.) at four different temperatures 20, 25, 20-30 and 30^{0}C with 90-95% RH in seed germinators. At alternate temperature, seeds were kept at 20^{0}C for 16 hrs and 30^{0}C for 8 hrs. The final count of germination was recorded on 9th day (as maximum germination was observed on 9th day and further number of seedlings starts decreasing due to decay). Germination percentage was recorded on the basis of normal seedlings.

***Storage study*:** The seed samples of all the lots were stored in three different containers *i.e*., muslin cloth, polythene bags (700 μ) and HDPE bags under ambient conditions (14-30^{0}C and 45-81% RH). The initial germination, vigour, moisture and oil content were recorded and these observations were repeated in samples drawn at quarterly interval.

***Germination (%)*:** Three replications with 100 seeds per replication for each lot were tested in sand medium at 30^{0}C. The germinated seeds were evaluated into normal and abnormal seedlings, hard, fresh un-germinated and dead seeds. The germination percentage was calculated based on number of normal seedlings.

***Seed moisture content (%)*:** A working sample of 5 g seed from each lot was drawn and drying was carried out at 103^{0}C for 17 hr. The moisture content (percentage on wet weight basis) was calculated with the help of following formula:

$$\text{Moisture content (\%)} = \frac{(M_2-M_3)}{(M_2-M_1)} \times 100$$

Where,

M_1=Weight of container and its cover; M_2= Weight of container, cover and seed before drying; M_3=Weight of container, cover and seed after drying.

Seedling vigour index-I: The vigour index was calculated as: Vigour index-I=Standard germination (%) x Average seedling length (cm)

***Oil content*:** The oil content of each seed lot was calculated by using Soxhlet apparatus according to AOAC (1970). The seeds were dried at 50^{0}C for 16 hrs and allowed to cool in a dessicator. Fine powder (5 g) of dry seed kernels was transferred to an extraction thimble placed in a Soxhlet extractor to which sufficient quantity of petroleum ether (40-60^{0}C) was added and heated until seven siphonings were completed. The percentage of oil content was expressed as percentage of the sample weight.

Statistical analysis: The data was analyzed using Factorial Completely Randomized Design (CRD). Per cent data was transformed into angular values and subjected to the statistical analysis as described by Panse and Sukhatme (1967).

Result and Discussion

***Germination study*:** Highest germination was observed in sand medium (82.3%) followed by Between Papers (72.2%). This clearly indicates that sand is the best substrata for conducting germination tests which may be because seeds are covered fully thereby maintaining the required level of moisture necessary for germination and seedling development. Among different temperatures studied, 30^{0}C had maximum germination (78.4%) followed by 25^{0}C (72.5%). However, no germination took place at 20^{0}C and 20-30^{0}C on top of the paper method.

The interaction effect of germination media and temperature showed that maximum germination (94.4%) was observed in sand at 30^{0}C followed by between paper at 30^{0}C and sand at 25^{0}C (Table-1). At the alternative temperature of 20-30^{0}C lower germination was observed as compared to constant temperatures which could be attributed to the inability of the seeds to cope up the metabolic activities with a shift in the diurnal temperature (Kamaha and Maguire, 1992).

Storage study

***Standard germination (%)*:** With increase in storage period, a gradual reduction in germination was observed in all the seed lots, irrespective of the containers used. The reduction in germination in polythene (700μ) bags and HDPE bags was recorded from 93.8 to 77.7% and 93.8 to 73.0% respectively after 9 months of ambient storage. Minimum germination percentage was recorded in muslin cloth bags (48.0%) followed by HDPE bags (57.3%) after same period (Table 2). Fluctuation in seed moisture content during storage along with high respiration rates in muslin cloth bag stored seeds can possibly be the reasons for rapid loss of viability. Singh and Dadlani (2003) reported that seeds in polythene (700μ) bags maintained good germinability under ambient conditions of storage compared to seed packed in cloth bags particularly when the storage period coincides with the rainy seasons in soybean.

***Moisture content (%)*:** The seed moisture content remained constant from the start to germination except in muslin cloth bags in months of July-August coinciding with rains. Maximum fluctuations and increase in seed moisture content was found in muslin cloth bags due to the free flow of air from the surroundings. Moisture content increased from 7.37 to 7.43% across the lots. Minimum seed moisture content was observed in muslin cloth bags (7.16) after 9 months of storage (Table 3) due to decrease in the RH of the surrounding air. Similar results were observed by Shedage *et al*. (2012) in physic nut seeds.

Vigour index-I: Maximum reduction (from 3601.5 to 2143.1) in vigour index-I was found in muslin cloth bags and greater loss in vigour (from 3601.5 to 1596.9) was recorded after 9 months of storage (Table 4). Reduction in vigour index was observed as the storage period increased. This is due to reduction in germination as well as seedling length, the two components of vigour index-I. Similar results were recorded by Kaushik (2003).

Table 1. Effect of substrata and temperatures on germination (final count) of *Jatropha curcas* L. seeds

Lots	Germination (%) on different substrata at different temperature (^{0}C)												Mean
	Between Paper (B.P.)				Sand				Top of Paper (T.P.)				
	20^{0}C	25^{0}C	20-30^{0}C	30^{0}C	20^{0}C	25^{0}C	20-30^{0}C	30^{0}C	20^{0}C	25^{0}C	20-30^{0}C	30^{0}C	
L_1	48.7	81.3	78.0	89.3	52.3	89.3	87.7	93.7	ð -	41.0	ð -	48.0	59.1
	(44.2)	(64.4)	(62.0)	(70.9)	(46.3)	(70.9)	(69.4)	(75.4)		(39.8)		(43.8)	(48.9)
L_2	49.3	81.0	77.3	88.0	51.3	88.7	86.3	92.7	ð -	40.7	ð -	47.3	58.6
	(44.6)	(64.1)	(61.5)	(69.7)	(45.7)	(70.3)	(68.3)	(74.3)		(39.6)		(43.5)	(48.5)
L_3	53.0	86.3	85.0	92.0	56.0	90.7	89.0	94.3	ð -	44.0	ð -	51.3	61.8
	(46.7)	(68.3)	(67.2)	(73.5)	(48.4)	(72.2)	(70.6)	(76.2)		(41.5)		(45.7)	(50.9)
L_4	47.3	78.3	75.3	85.7	48.3	86.7	85.3	91.3	ð -	38.7	ð -	46.0	56.9
	(43.5)	(62.2)	(60.2)	(67.7)	(44.0)	(68.6)	(67.5)	(72.9)		(38.4)		(42.7)	(47.3)
L_5	58.0	91.7	89.0	95.7	63.0	95.0	92.7	98.7	ð -	46.7	ð -	56.0	65.5
	(49.6)	(73.2)	(70.6)	(78.1)	(52.5)	(77.1)	(74.3)	(83.4)		(43.1)		(48.4)	(54.2)
L_6	53.0	88.7	88.3	93.3	62.3	92.7	90.3	95.7	ð -	44.3	ð -	52.7	63.4
	(46.7)	(70.3)	(70.0)	(75.0)	(52.1)	(74.3)	(71.9)	(77.9)		(41.7)		(46.5)	(52.2)
Mean	51.6	84.6	82.2	90.7	55.5	90.5	88.6	94.4	ð -	42.6	ð -	50.2	
	(45.9)	(67.1)	(65.3)	(72.5)	(48.2)	(72.2)	(70.3)	(76.7)		(40.7)		(45.1)	

Figures in parenthesis are angular transformed values

CD at 1%: Substrata (A) : 0.22; Temperature (B) : 0.25; Lot (C) : 0.31; A x B : 0.44; B x C : 0.62; A x C : 0.54; A x B x C : 1.09

Mean of temperatures: Mean of 20^{0}C : 35.7; Mean of 25^{0}C : 72.5; Mean of 30^{0}C : 78.4; Mean of 20-30^{0}C : 56.9

Mean of substrata: Sand : 82.3; B.P. : 72.2; T.P. : 23.2

Table 2. Effect of packaging materials and storage periods on seed germination of *Jatropha curcas* L. seeds

Lots	Initial Germination (%)	Germination (%) in different storage containers									Mean
		Muslin cloths bags			HDPE bags			Polythene (700 μ) bags			
		Months									
	0	3	6	9	3	6	9	3	6	9	
L_1	92.7	85.7	71.0	48.0	87.0	74.3	59.3	87.7	79.0	68.0	73.3
	(74.3)	(67.7)	(57.4)	(43.8)	(68.8)	(59.5)	(50.4)	(69.4)	(62.7)	(55.5)	(59.5)
L_2	92.3	84.3	71.3	47.7	85.3	74.0	54.3	86.3	75.3	69.0	71.9
	(73.9)	(66.6)	(57.6)	(43.6)	(67.5)	(59.3)	(47.5)	(68.3)	(60.2)	(56.1)	(58.5)
L_3	93.3	85.0	72.0	51.0	85.7	77.7	59.0	86.3	79.3	69.7	73.9
	(75.1)	(67.2)	(58.0)	(45.6)	(67.7)	(61.8)	(50.2)	(68.3)	(62.9)	(56.6)	(59.8)
L_4	90.3	81.3	67.3	40.0	82.0	72.0	52.0	82.3	74.0	61.3	68.0
	(71.9)	(64.4)	(55.1)	(39.2)	(64.9)	(58.0)	(46.1)	(65.1)	(59.3)	(51.5)	(55.9)
L_5	98.3	88.7	72.0	51.0	90.3	77.7	60.7	91.7	80.0	72.3	76.0
	(82.8)	(70.3)	(58.0)	(45.6)	(71.9)	(61.8)	(51.1)	(73.2)	(63.4)	(58.2)	(61.5)
L_6	96.0	87.3	72.3	50.3	88.0	76.0	59.0	90.3	79.0	68.0	74.5
	(78.5)	(69.1)	(58.2)	(45.2)	(69.7)	(60.6)	(50.2)	(71.9)	(62.7)	(55.5)	(60.4)
Mean	93.8	85.4	70.9	48.0	86.4	75.3	57.3	87.4	77.8	68.1	
		(61.6)	(57.4)	(43.8)	(62.4)	(60.2)	(49.2)	(69.4)	(61.9)	(55.6)	

Figures in parenthesis are angular transformed values

CD at 1%: Storage period (A) : 0.27; Storage container (B) : 0.27; Lot (C) : 0.38; A x B : 0.47; B x C : 0.66; A x C : 0.66; A x B x C : 1.15

Mean of containers: Muslin cloth bags : 68.1; HDPE bags : 73.0; Polythene bags : 77.7

Mean of time: 3 months: 86.4; 6 months: 74.7; 9 months: 57.8

***Oil content (%)*:** Effect of storage period, packaging material and lots on oil content of *Jatropha curcas* seeds showed significant reduction in oil content with increased storage period (Table 5). Maximum reduction in oil content (from 30.8 to 29.7) was recorded after 9 months of storage and the reduction was maximum in muslin cloth bags (from 30.8 to 30.2) compared to other containers. Maintenance of proper moisture content of seed during ambient storage in polythene (700μ) bags, due to its impervious nature, might have resulted in slow rate of lipid peroxidation, thereby release of minimum free radicals, leads to maintenance of membrane integrity. This results in slow leakage of intracellular substances (electrolytes and other solutes), which is responsible for maintenance of seed germination during storage. Similar findings were reported by Shedage *et al*. (2012) in Jatropha.

Table 3: Effect of packaging materials and storage periods on seed moisture content (%) of *Jatropha curcas* L. seeds

Lots	Initial moisture content (%)	Moisture content (%) in different containers at different periods (months)									Mean
		Muslin cloths bags			HDPE bags			Polythene (700 μ) bags			
		Months									
	0	3	6	9	3	6	9	3	6	9	
L_1	7.16	7.01	7.75	6.93	7.11	7.13	7.08	7.14	7.13	7.11	7.15
	(15.52)	(15.35)	(16.16)	(15.26)	(15.45)	(15.47)	(15.48)	(15.50)	(15.48)	(15.46)	(15.51)
L_2	7.29	7.20	7.85	7.18	7.21	7.23	7.18	7.27	7.26	7.23	7.29
	(15.66)	(15.56)	(16.26)	(15.53)	(15.57)	(15.58)	(15.59)	(15.64)	(15.63)	(15.59)	(15.66)
L_3	7.88	7.78	8.42	7.74	7.81	7.82	7.77	7.83	7.82	7.76	7.86
	(16.30)	(16.18)	(16.86)	(16.15)	(16.22)	(16.23)	(16.23)	(16.24)	(16.23)	(16.17)	(16.27)
L_4	6.94	6.81	7.52	6.76	6.81	6.82	6.79	6.90	6.89	7.17	6.94
	(15.27)	(15.12)	(15.91)	(15.06)	(15.13)	(15.14)	(15.14)	(15.23)	(15.22)	(15.52)	(15.27)
L_5	7.49	7.19	8.05	7.16	7.36	7.35	7.32	7.47	7.46	7.33	7.41
	(15.87)	(15.54)	(16.48)	(15.52)	(15.74)	(15.72)	(15.72)	(15.85)	(15.84)	(15.70)	(15.79)
L_6	7.46	7.18	7.99	7.16	7.23	7.24	7.19	7.42	7.41	7.33	7.35
	(15.84)	(15.53)	(16.42)	(15.51)	(15.60)	(15.60)	(15.60)	(15.80)	(15.79)	(15.70)	(15.72)
Mean	7.37	7.19	7.93	7.16	7.25	7.27	7.22	7.34	7.33	7.32	
		(15.55)	(16.35)	(15.51)	(15.62)	(15.63)	(15.58)	(15.71)	(15.70)	(15.69)	

Figures in parenthesis are angular transformed values
CD at Storage period (A) : 0.03
1% Storage containers (B) : 0.03
Lot (C) : 0.04

A x B : 0.06
B x C : N.S
A x C : 0.08
A x B x C: N.S

Mean of containers:
Muslin cloth bags : 7.43
HDPE bags : 7.25
Polythene bags : 7.33

Mean of time:
3 months: 7.26
6 months: 7.51
9 months: 7.24

Table 4. Effect of packaging materials and storage periods on vigour index-I of *Jatropha curcas* L. seeds

Lots	Initial vigour index-I	Vigour index-I in different containers at different period (months)									Mean
		Muslin cloths bags			HDPE bags			Polythene (700 μ) bags			
		Months									
	0	3	6	9	3	6	9	3	6	9	
L_1	3547.0	2978.3	2177.4	1235.2	3123.4	2363.6	1587.9	3231.9	2551.9	1858.8	2345.3
L_2	3508.7	2974.5	2149.3	1244.0	3134.7	2318.7	1461.5	3251.9	2415.9	1869.9	2313.4
L_3	3796.0	3219.3	2419.1	1428.1	3346.6	2697.6	1713.0	3433.1	2781.9	2066.7	2567.2
L_4	2950.7	2395.9	1813.6	965.3	2553.3	2037.6	1298.4	2645.6	2148.4	1574.1	1936.9
L_5	3966.3	3242.1	2291.7	1431.5	3459.8	2573.3	1750.9	3605.6	2701.3	2160.4	2579.6
L_6	3840.3	3042.2	2162.8	1405.9	3176.6	2366.0	1691.3	3333.3	2533.3	2001.3	2412.5
Mean	3601.5	2975.3	2168.9	1285.0	3132.3	2392.7	1583.8	3250.2	2522.1	1921.8	

CD at 1%
Storage period (A) : 16.58
Storage container (B) : 16.58
Lot (C) : 23.44

A x B : 28.71
B x C : 40.61
A x C : 40.61
A x B x C : N.S.

Mean of containers:
Muslin cloth bags : 2143.1
HDPE bags : 2369.6
Polythene bags : 2564.7

Mean of time:
3 months: 2564.7
6 months: 2369.6
9 months: 2143.1

Table 5: Effect of packaging materials and storage period on oil content of *Jatropha curcas* L. seeds

Lots	Initial oil content (%)	Oil content (%) in different containers at different period (months)									Mean
		Muslin cloths bags			HDPE bags			Polythene (700 μ) bags			
		Months									
	0	3	6	9	3	6	9	3	6	9	
L_1	29.47	29.4	29.2	27.9	29.4	29.4	28.0	29.5	29.4	29.1	29.0
	(32.86)	(32.8)	(32.7)	(31.9)	(32.8)	(32.8)	(31.9)	(32.9)	(32.8)	(32.7)	(32.6)
L_2	33.07	33.2	33.2	31.9	33.4	33.3	32.1	33.5	33.4	32.6	32.9
	(35.33)	(35.1)	(35.2)	(34.4)	(35.3)	(32.2)	(34.5)	(35.3)	(35.2)	(34.8)	(35.0)
L_3	32.50	32.4	32.2	30.8	32.5	32.4	31.0	32.5	32.5	32.1	32.1
	(34.82)	(34.7)	(34.6)	(33.7)	(34.8)	(34.7)	(33.8)	(34.8)	(34.7)	(34.5)	(34.5)
L_4	29.33	29.2	29.2	27.7	29.2	29.2	28.1	29.3	29.2	28.9	28.9
	(32.78)	(32.7)	(32.7)	(31.7)	(32.7)	(32.7)	(31.9)	(32.7)	(32.7)	(32.5)	(32.5)
L_5	30.27	30.2	30.1	28.5	30.3	30.2	28.8	30.3	30.3	29.5	29.8
	(33.45)	(33.3)	(33.2)	(32.2)	(33.4)	(33.3)	(32.5)	(33.4)	(33.4)	(32.9)	(33.0)
L_6	30.43	30.2	30.2	28.5	30.3	30.2	29.0	30.3	30.3	29.8	29.9
	(33.47)	(33.3)	(33.3)	(32.2)	(33.4)	(33.3)	(32.6)	(33.4)	(33.4)	(33.1)	(33.1)
Mean	301.8	30.8	30.7	29.2	30.9	30.8	30.8	29.5	30.9	30.3	
		(33.6)	(33.6)	(32.6)	(33.7)	(33.7)	(33.7)	(32.8)	(33.7)	(33.4)	

Figures in parenthesis are angular transformed values

CD at 1%: Storage period (A) : 0.07; Storage container (B) : 0.07; Lot (C) : 0.38; A x B : 0.13; B x C : N.S.; A x C : N.S.; A x B x C : N.S

Mean of containers: Muslin cloth bags : 30.2; HDPE bags : 30.3; Polythene bags : 30.7

Mean of time: 3 months: 30.9; 6 months: 30.8; 9 months: 29.7

The study revealed that for germination studies the suitable media is sand and at 30 0C maximum germination is achieved up to 9 days. Among the storage containers the polythene bags () are best suited for Jatropha seed storage under ambient conditions retaining germination above 68%.

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