Establishment of *Jatropha curcas* L. Seedlings Based on Variation in Seed Size and Cuttings

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ABSTRACT

The importance of *Jatropha curcas* as a feedstock for renewable energy is well recognized by developed nations in need of alternatives to petroleum. Although the plant is widespread mostly as a wild bush in many developing nations in the tropics, research on its genetics, agronomic characteristics and cultivation are presently being elucidated. This work discusses the importance of seed size and its relationship to germination, seedling development, and size of cuttings and their efficiency in establishing new plants. A seed lot was separated into 9 different ranges based on seed size (gram seed^{-1}) and seeds from each range were placed on moist blotting paper at room temperature to imbibe and were transferred to pots having Promix in a greenhouse environment. Seed size ranged from <0.10 to >0.90 but <1 gram seed^{-1} with most of the seeds being within ranges: **0.50 – 0.59g** (167), **0.60 – 0.69g** (270), and **0.70 - 0.79g** (268). In a different study, cuttings of different sizes based on number of nodes, length and diameter from four 1-year old plants were made to determine efficient production of new leaves, roots and callus from a stock of mother plants. Results showed a continuous distribution for both quantity and size of seeds within the investigational seed lot of 958 seeds. Germination was highest in the **0.80 - 0.89g** weight range, while no significant difference was found for development of root, stem, and true leaves of the seedlings. The establishment of new plants from cuttings of different size showed formation of callus as a precursor for root formation and was significantly greater with longer and smaller diameter cutting having 4 nodes and greater. Results from this work will provide information which will allow more efficient improvement, and establishment of jatropha plantation either from seeds or cuttings from genetically superior parent plants.

INTRODUCTION

Jatropha curcas L.:

- ✓Ranked sixth among crops as feedstock for biofuels
- ✓Not commercially grown in many regions where it can be productive
- ✓Drought resistant and can be cultivated on marginal land; no competition with food production (*RF 1998*)
- ✓Fast growing and produce seeds after approximately 1 – 3 years, depending on rainfall conditions and methods of propagation
- ✓Seeds contain about 35 percent of non-edible oil (*Henning 2002*)
- ✓Propagation by cuttings show a lower longevity, drought, and disease resistance than plants propagated by seeds (*Heller 1996*)
- ✓Pre-cultivation of Jatropha seedlings in poly-ethylene bags accelerate plantation by at least 3 months (*Henning 2002*).

Justification

- ➢Potential feedstock for biofuels in southern and western regions of U.S.
- ➢Research on cultivation and propagation of *J. curcas* L. is limited, especially in south eastern US

Objectives

Research the generative propagation of *J. curcas* L. from seeds and plant cuttings in greenhouse conditions for nursery growers by:

1) evaluating relationship of seed size to germination and seedling development and,

2) size of stem cuttings and its efficiency in establishing new plants

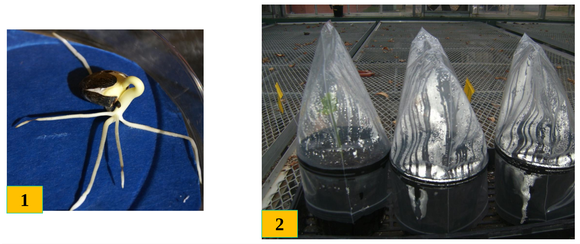
Materials and Methods

The methodology involved two stages:

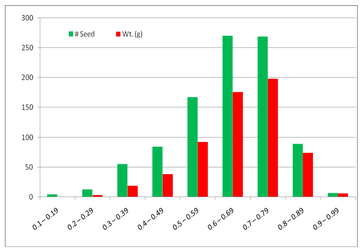
(i) general assessment of the viability of seeds based on germination and

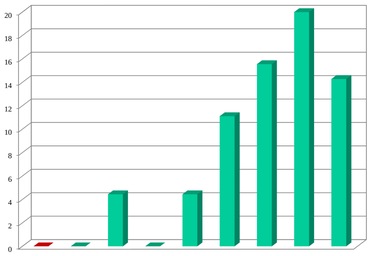
(ii) detailed assessment on germination and growth of plant cuttings during the first 8-10 weeks after planting.

- ▪Assessment on the basis of available seeds (958 - exact seed provenance is unknown) for seed weight and distribution
- ▪Seeds from each range were placed on moist blotting paper at room temperature and transferred to pots after establishment of roots
- ▪Cuttings of 3 -12cm and 3 - 6 nodes were made from ~1-year old plants and soaked in water to remove latex, cuttings were transferred to pots (Promix); observations were made for initiation of root formation (Callus), new leaves and new growth based on size of cuttings; data were analyzed (GLM, LSD & Graph-&-Go with SAS 9.1 (2008).



Progression of jatropha from seed germination to fruiting under greenhouse conditions (seed to fruits, ~410 days): (1) seed germination – date= Jan 21, 2009; (2) transfer to Promix in GH - date= Jan 29, 2009; (3) initiation of inflorescence – date = Jan 21, 2010; (6) matured fruits and harvested seed size – date = Mar 6, 2010





Figures 1 (top) and 2 (bottom). Distribution of seed size (0.1g to 0.9g from a random seed lot of 958 seeds (top), and percent germination (0 – 20%) among the ranges.

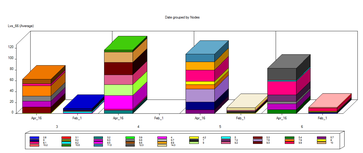


Figure 3. formation of new leaves in cuttings after 66 days based on planting dates, length and number of nodes .

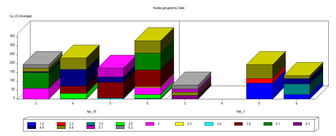


Figure 4. Initiation of roots based on callus formation in cuttings after 43 days based on planting dates and size of lower diameter of cutting.

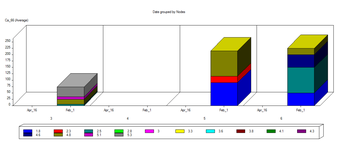


Figure 5. Initiation of roots based on callus formation in cuttings after 66 days; roots were fully developed on 2nd date.

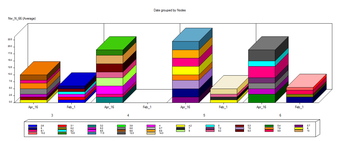


Figure 6. Growth based on callus formation of new nodes in cuttings after 66 days in relation to planting dates & length of cuttings.

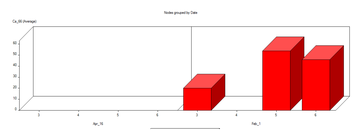


Figure 7. Callus as an indicator for root formation in cuttings by nodes after 66 days shows Feb 1 date slower to produce roots

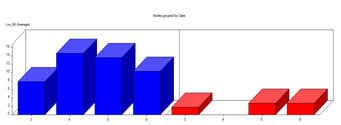


Figure 8. New leaves in cuttings by nodes after 66 days

Table 1. Development of plant cuttings planted in April for callus, leaves and new growth determined by nodes 43 and 66 days after planting.

No. of Nodes in cuttings	Callus @ 43-days	Callus @ 66-days*	Leaves@ 43-days	Leaves @ 66-days	new nodes @ 43-days	new nodes @ 66-days
3	25.00 A	Roots fully developed	4.500 A	7.875 A	1.2500 B	1.2500 B
4	37.13 A		6.875 A	14.625 A	2.1875 A	2.3750 A
5	40.63 A		8.250 A	13.625 A	2.6250 A	2.7500 A
6	41.88 A		6.375 A	10.375 A	2.2500 A	2.3750 A

Means with the same letter are not significantly different; p=0.05; N= 8/cutting
* Formation of roots

Table 2. Pearson Correlation Coefficients; N = 18, for Feb 1 planting; Prob > |r| under H0: Rho=0

	New Nodes 66 days	Leaves 43 days	Leaves 66 days	Callus 43 days	Callus 66 days	Length	Upper Diameter	Lower Diameter
New Nodes 43 days	**0.48241** **0.0426**	**0.93723** **<.0001**	**0.62102** **0.0059**	-0.24725 0.3226	-0.11253 0.6566	**0.68045** **0.0019**	-0.18032 0.4740	-0.01239 0.9611
New Nodes 66 days		0.40057 0.0995	0.61650 0.0064	0.14083 0.5773	0.27862 0.2629	**0.54178** **0.0202**	-0.32121 0.1937	-0.26862 0.2811
Leaves 43 days			0.66787 0.0025	-0.27307 0.2729	-0.11536 0.6485	**0.64208** **0.0041**	-0.16631 0.5095	-0.00765 0.9760
Leaves 66 days				0.05597 0.8254	0.36368 0.1379	**0.63714** **0.0045**	-0.26585 0.2863	-0.18038 0.4738
Callus 43 days					**0.87639** **<.0001**	0.10125 0.6893	-0.20110 0.4236	-0.24463 0.3279
Callus 66 days						0.23539 0.3471	-0.22147 0.3771	-0.23555 0.3467
Length							-0.45560 0.0574	-0.26177 0.2940
Upper Diameter								**0.96485** **<.0001**

Table 3. Pearson Correlation Coefficients for Apr 16 planting date Prob > |r| under H0: Rho=0 Number of Observations = 32

	New Nodes 66 days	Leaves 43 days	Leaves 66 days	Callus 43 days	Length	Upper Diameter	Lower Diameter
New Nodes 43 days	**0.92795** **<.0001**	**0.82411** **<.0001**	**0.66427** **<.0001**	**0.56755** **0.0007**	**0.47392** **0.0061**	**0.54631** **0.0012**	**0.35182** **0.0483**
New Nodes 66 days		**0.82957** **<.0001**	**0.74464** **<.0001**	**0.61831** **0.0002**	**0.49643** **0.0039**	**0.49071** **0.0044**	**0.32392** **0.0705**
Leaves 43 days			**0.85918** **<.0001**	**0.85239** **<.0001**	**0.58244** **0.0005**	**0.55435** **0.0010**	**0.43926** **0.0119**
Leaves 66 days				**0.81344** **<.0001**	**0.46211** **0.0078**	**0.54389** **0.0013**	**0.48032** **0.0054**
Callus 43 days					**0.49300** **0.0041**	**0.47944** **0.0055**	**0.38856** **0.0280**
Length						0.07712 0.6748	0.10652 0.5618
Upper Diameter							**0.84618** **<.0001**

Progression of jatropha plant cuttings under greenhouse conditions; 3 – 6 nodes samples of varying length ~ 3.1 to 12.3 cm: (1)Initiation of leaves in new cuttings– date = Jan 15, 2010; (2) a 4-node cutting with exposed roots and new leaves and nodes; (3 & 4) fully developed seedlings; not shown: matured fruits in less than one year.

Results

1. **Seed distribution (Figures 1 & 2): significant variation among seeds in the seed lot**
2. **Germination among the various ranges of seed size was higher in the higher seed size ranges**
3. **Figures 3 to 8 show that, formation of callus to produce roots, new leaves and growth based on development of new nodes respond to dependent factors such as number of nodes, length of cuttings, while response to planting dates is a temperature issue. Temperature was cooler for the Feb 1 date and significantly warmer during the April 16 planting date. These figures also indicate that seedling development is enhanced by longer cuttings with 4 and 5 nodes.**
4. **Development of growth variables (Tables 1 & 3,) was significantly better for Apr 16 compare to response from Table 2 for Feb1**

Conclusion

Development of jatropha to produce improved breeding lines and cultivars is practical in greenhouse conditions by using both seeds (~410 days from seeds to fruits) or >1 year from cuttings to seeds.

It is also feasible to generate large scale greenhouse production of genetically uniform seedlings for producers interested in the production of jatropha in the United States.

References

Heller, J. (1996). *Physic nut. Jatropha curcas L.* Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research, Gatersleben, International Plant Genetic Resources Institute, Rome.

Henning, R. (2002). Using the Indigenous Knowledge of Jatropha – The use of *Jatropha curcas* oil as raw material and fuel. IK Notes. No.47. August. Http://www.worldagroforestrycentre.org/Sites/TreeDBS/AFT/SpeciesInfo.cfm?SpID=10 13

RF (1998). The Potential of *Jatropha curcas* in Rural Development and Environment Protection –An Exploration. Concept paper. Rockefeller Foundation and Scientific & Industrial Research & Development Centre, Harare, Zimbabwe 1998.

SAS. 2008. Statistical Analysis System. Raleigh, N.C.

Acknowledgment

Thank you to Lee Hauck, Eric Obeng, Eric Kamya, Moses Semakula and Franck Bassong for their support and technical assistance.