Seedling Growth from Stem Cutting with Different Physiological Ages of *Jatropha curcas* L. of West Nusa Tenggara Genotypes

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Abstract

The use of accurate cutting age in vegetative propagation is imperative in order to improve seeding quality of some Jatropha genotypes. The objective of the research is to investigate the effect of cutting age from some jatropha genotypes on seedling growth and development. Cuttings from primary, secondary and tertiary branchs were grown and observed during seedling period and in the production field. The result showed that secondary branch as a semi-hard wood cuttings could be the most effective propagation material of *Jatropha curcas*, and followed by tertiary branch. All Jatropha genotypes of West Nusa Tenggara could be propagated using three types of stem cutting.

Keywords: propagation, branch, genotype, growth, development

1. Introduction

Due to severe energy crisis and escalation of petroleum prices, alternative energy sources are gaining importance. One of such alternative energy is a biodiesel from *Jatropha curcas* L., non edible oil seed. Therefore, to obtain a continuous and efficient production of the oil, it is important to have plants that produce large quantities of fruits and good quality of seed.

Adventitious root formation related to vegetative propagation has been of interest to nurseryman deal with *Jatropha curcas* since the plant was a heterozygotic cross-pollination plant. In addition, propagation by cutting is the only practical means of preserving a unique (high yielding) trait of plants or produce true-to-type clones (Ratree, 2004; Hartmant *et al*., 2002). On the other side, the seed viability depends on environmental conditions, and lose their viability after 15 months storage (Kobilke, 1989), or after five months if stored in an ambient room condition (Santoso *et.al*., 2013).

Therefore, preparing a good seedling by mean vegetative propagation techniques using an efficient and effective cutting is imperative in planting superior *Jatropha* successfully. Stem cutting is a traditional and promising method for multiplication of *Jatropha* crop (Gopale; Zunjarrao, 2011). Adventitious rooting of cutting may be directly or indirectly controlled by genetics and physiological age of stem or branch. Bijalwan and Thakur (2010) reported that the position of cutting and the age of mother plant played a significant role in rooting and sprouting. Rooting ability of cuttings from woody or perennial plants declined with increasing in mother plants age. Kraiem *et al*. (2010) added that the establishment and growth rate of stem cutting depend upon age variation, position in stem, and diameter of stem.

Parts of Jatropha crop generally used for propagation materials are primary, secondary and tertiary branch stem, and no longer than 1 m in length. To meet to this conditions, all branchs should be used. However, forming root ability of cutting is getting more difficult in accordance with the farther position from the apical shoot (Hartmann *et al*., 2002: Wilson, 1993) due to differences in the type and number of carbohydrates and other stored materials (Hartmann *et al*., 2002; Leakey, 1999). Henning (2003) also stated that the behavior of stem cuttings varies with age, genotypes, and physiological status of mother plant.

This article presents the results of experiment undertaken to propagate the West Nusa Tenggara genotypes of *Jatropha* plant through-out stem cutting with different physiological ages, as well as their growth during the two months after transplanting in the field.

2. Materials and Methods

2.1. Collecting, Grading of Cuttings, and Transplanting

This study was carried out at the screen house, Faculty of Agriculture, University of Mataram, Indonesia. Stem cutting of five selected superior genotypes of West Nusa Tenggara *Jatropha curcas* with three years old grown in the research station field were collected. Those genotypes were West Lombok, Middle Lombok, East Lombok, Sumbawa, and Bima. Healthy and uniform stem cuttings approximately 20-25 cm in length were obtained and grouped based on their physiological age: stem cut of primary branch (three years old) as a hard wood cutting; stem cut of secondary branch (about two years old) as a semi-hard wood; and stem cut of tertiary branch (about a year old) as a soft wood. Twenty five cuttings of each treatment were planted separately into polybag containing top soil mixture with manure as propagation media with three replications.

Two month-old seedling raised in polybags were planted in the field experiment to evaluate the adaptation performance. The plot size was 5 x 10 m, and number of plants per plot was 15 with 1.5 x 2 m spacing. All the five-teen plants in each plot constituted the measurement unit.

The soil was sandy loam Entisols and composed of sand (69%), silt (25%), and clay (5%), with 1.8% organic carbon, 0.2% total N, the pH 5.9-6.3, and cation exchange capacity of the soil measured 7.2-10.4 $cmol.kg^{-1}$.

2.2. Plant Maintenance

After planting the stem cutting, all polybag were watered once a week interval, and after field transplanting irrigation was applied weekly up to two month. Fertilizer was applied at the time of transplanting 5,000 kg manure ha^{-1} (2 kg $tree^{-1}$) and 25 kg Urea ha^{-1} (10 g $tree^{-1}$), 150 kg SP-36 ha^{-1} (60 g $tree^{-1}$), and 30 kg KCl ha^{-1} (12 g $tree^{-1}$). Second Urea application (25 kg Urea ha^{-1} (10 g $tree^{-1}$) was applied one month after planting.

2.3. Data Observation and Statistical Analysis

Seedling growth and development was recorded on 30 days and 60 days after planting. For assessing survival percentage, survive saplings were counted and expressed as a percentage after two months of transplanting. Root length of the seedling was measured from collar region to the root tip, expressed in centimeter. The number of fully opened leaves presented in each cutting was counted and expressed as number of leaves/cutting. Fresh weight of root and shoot was calculated and expressed in g/cutting. For analyzing dry weight of root and shoot, they were placed in paper bag, dried in a hot air oven maintained at $85 \pm 2^{O}C$ for 24 h (for root) and 48 h (for shoot), cooled in a desiccators, weighed and expressed as g/cutting.

The experiments were designed using a Completely Randomized Design (CRD) for seedling, and Randomized Completely Block Design (RCBD) for field adaptation. The data were analyzed using Minitab-14 Statistical software. A one-way ANOVA was applied to evaluate significant differences in the parameters. The Honestly Significant Difference (HSD at 5% level) was, then, subjected on treatment means.

3. Results and Discussion

Physiological age of stem cutting showed significant difference among the seedling growth variables. However, the genotypes, and its interaction with stem cutting did not affect on all growth seedling variables and their field survival at $p = 0.05$ (Table 1). It mean that the genetic base of *Jatropha curcas* genotype from West Nusa Tenggara was quite small, especially for seedling and early vegetative growth characteristics. Therefore, further discussion focused only on the effect of difference of physiological ages stem cutting.

Primary branch as a hard wood cutting significantly showed the longest days to root development compared to other two types of cuttings (Table 2). Whereas, secondary branch as a semi-hard wood cutting produced the highest number of roots, length of root, and dry weight of root (Table 2).

From the data of Table 2, our resume on the root growth that the secondary and tertiary branch were a better type of stem cutting than primary branch. Better growth of two stem cutting types might be due to the presence of higher concentration and influence of root promoting hormone from the upper part of stem cutting.

Hartmann *et al*. (2002) stated that reduced rooting potential of primary branch as hard wood stem due to lowering phenolic substances level. Furthermore, Kochhar *et al*. (2005) stated that under performances more woody, the stem cut might have converted most material for lignifications rooting and shooting proccesses slower.

Primary branch as hard wood cutting took the longerst day to develop a shoot compared two other types of cutting. The highest of number of leaves per cutting was observed in secondary branch. There was no significant difference on number of shoot and height of shoot among type of stem cuttings (Table 3). Earlier leaf opening and developing of new shoot in secondary and tertiary branch indicated that the presence of growth hormones in the tip of axils bud was ready to deliver to shoot.

Shoot development or sprouting of bud took place much later than rooting (see Table 2 and Table 3). This study was not in agreement with Kochhar *et.al*. (2005) that root formation in some cuttings took much longer than shoot development, or they did not have any visible roots, and showed only some callusings. In addition, they reported that in *Jatropha* species, shoots were formed much earlier than roots.

The biomass production from different grade of cutting (physiological age) was estimated in term of fresh shoot and root dry weight. In this study, secondary and tertiary branch produced shoot growth significantly better (Table 3 and Table 4), compared to primary branch. This phenomenon implied that the cutting develops more shoot probably because the carbohydrate reserve was easy to use for metabolic processes. It was not as late as differentiation organ or tissue of cutting. The low biomass production of hard-wood cutting (primary branch) may be attributed that cutting were more woody and might have converted most of the food materials for the lignification proccesses which resulted in over lignified stem, and then, caused lower rooting and shooting development. As Hartmann *et al*. (2002) stated that the age of plant from which stem cutting were taken as well as the stem position on plant determined rooting and shoot growth and survival percentage.

Plant survival percentage in the field and their growth in height and number of leaves achieved in two month after transplanting were shown in Table 5. Plants propagated using secondary and tertiary branch had higher survival percentage compared to that primary branch. This percentage was also followed by number of leaves and plant height after two month transplanting. The higher mortality observed on plant established by means of stem cutting from primary branch was attributed to failure on adaptation (stresses) to newly growth environment of the cultivation field.

In summary, seedlings propagated using secondary and tertiary branch cutting had better growth and quality components compared to that using primary branch. They also had higher survival percentages. The good root and shoot development system improved the survival potential from water and other stresses. Although they then lose leaves due to transplanting shock, the root and shoot organs still enabled them to develop new leaves quickly to increase the chances of survival.

4. Conclusion

Secondary branch as a semi-hard wood cuttings could be the most effective propagation material of *Jatropha curcas*, and followed by tertiary branch. All Jatropha genotypes of West Nusa Tenggara could be propagated using three types of stem cutting.

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Table 1: Analysis of Variance for Growth Variables of Stem Cuttings Seedling Influenced by Physiological Ages of Cuttings and Jatropha Genotypes

Growth variables	Repl.	Stem cutting (SC)	Genotypes (G)	SC x G	Error	CV (%)
Days to root development	0.63 ns	10.67**	3.20 ns	1.01 ns	0.12	2.35
Number of root at 1 month	0.55 ns	2.77 ns	3.08 ns	1.71 ns	1.02	2.22
Number of root at 2 month	0.73 ns	20.44*	3.77 ns	1.98 ns	0.38	2.01
Length of root at 1 month	0.98 ns	3.62 ns	5.01 ns	3.33 ns	0.33	5.51
Length of root at 2 month	0.67 ns	22.09*	5.33 ns	4.09 ns	0.41	5.15
Dry weight of root at 1 month	0.09 ns	1.68 ns	2.89 ns	2.77 ns	0.08	3.23
Dry weight of root at 2 month	0.12 ns	19.82*	2.76 ns	3.14 ns	0.13	3.02
Days to shoot development	1.02 ns	11.92*	4.11 ns	1.34 ns	1.12	7.71
Number of shoot at 1 month	0.71 ns	2.23 ns	2.36 ns	2.08 ns	0.04	4.95
Number of shoot at 2 month	0.66 ns	2.71 ns	2.58 ns	2.20 ns	0.11	4.14
Height of shoot at 1 month	0.24 ns	4.91 ns	2.61 ns	2.04 ns	0.21	5.45
Height of shoot at 2 month	0.46 ns	30.45*	2.44 ns	2.10 ns	0.19	5.05
Number of leaves at 1 month	0.71 ns	8.83 ns	4.88 ns	4.07 ns	0.22	4.50
Number of leaves at 2 month	0.68 ns	45.52*	5.17 ns	3.89 ns	0.31	4.76
Dry weight of shoot at 1 month	0.22 ns	2.21 ns	4.22 ns	3.07 ns	4.82	4.31
Dry weight of shoot at 2 month	0.35 ns	32.09*	4.07 ns	2.92 ns	0.55	5.33
Dry weight of shoot to root ratio at 1 month	0.49 ns	1.92 ns	3.02 ns	2.66 ns	0.78	3.54
Dry weight of shoot to root ratio at 2 month	0.55 ns	2.02 ns	3.24 ns	2.27 ns	1.15	3.99
Seedling existence percentage	1.27 ns	121.22*	8.82 ns	6.77 ns	1.08	6.31
Degree of freedom	2	2	4	8	28	-

Table 2: The Main Effect of Physiological Ages of Cuttings and Genotypes on Rooting Component of Stem Cutting Seedling

Treatment	Days to root development	Number of root		Length of root		Dry weight of root	
		1 month	2 month	1 month	2 month	1 month	2 month
Cutting							
Primary branch	12.2 a	9.8	10.6 b	13.3	14.3 b	0.69	1.12 b
Secondary branch	8.7 b	12.7	13.8 a	15.1	16.8 a	0.89	1.57 a
Tertiary branch	6.1 c	11.3	12.9 ab	12.9	14.4 b	0.85	1.31 ab
HSD 5%	2.2	-	2.8	-	2.1	-	0.42
Genotypes							
West Lombok	9.3	12.2	13.5	14.5	16.4	0.78	1.42
Middle Lombok	10.8	10.3	11.4	12.7	14.1	0.61	0.91
East Lombok	10.2	10.5	11.1	12.5	14.6	0.63	0.95
Sumbawa	9.8	11.9	12.9	14.1	16.1	0.75	1.31
Bima	9.9	11.7	13.2	14.4	15.7	0.72	1.28
HSD 5%	-	-	-	-	-	-	-

Table 3: Main Effect of Physiological Ages of Cuttings and Genotypes on Shooting Component of Stem Cutting Seedling

Treatment	Days to shoot development	Number of shoot		Height of shoot		Number of leaves	
		1 month	2 month	1 month	2 month	1 month	2 month
Cutting							
Primary branch	13.6 a	2.5	3.2	3.2	10.3 b	10.1	14.2 b
Secondary branch	10.7 b	3.5	4.1	4.7	14.6 a	12.2	16.9 a
Tertiary branch	9.1 b	3.8	4.2	4.9	12.7 a	11.4	15.5 ab
HSD 5%	2.78	-	-	-	2.11	-	2.08
Genotypes							
West Lombok	10.2	3.1	3.9	4.1	13.3	10.7	14.8
Middle Lombok	11.8	2.3	2.6	3.6	11.1	10.1	11.6
East Lombok	12.1	2.1	2.4	3.8	10.9	9.8	11.3
Sumbawa	10.7	2.8	3.7	3.9	13.1	9.9	13.9
Bima	11.1	3.2	3.3	4.2	12.8	10.2	13.5
HSD 5%	-	-	-	-	-	-	-

Table 4: Main Effect of Physiological Ages of Cuttings and Genotypes on Qualities Component of Stem Cutting Seedling

Treatment	Dry weight of shoot		Shoot root ratio		Percent seedling existence
	1 month	2 month	1 month	2 month	
Cutting					
Primary branch	3.2	4.7 b	3.8	3.6	84.2 b
Secondary branch	3.8	5.9 a	4.3	3.7	98.6 a
Tertiary branch	3.5	5.4 a	5.1	4.8	96.8 a
HSD 5%	-	1.12	-	-	11.5
Genotypes					
West Lombok	3.9	5.2	5.0	3.6	97.6
Middle Lombok	3.2	4.7	5.2	5.1	93.2
East Lombok	3.1	4.5	4.9	4.7	92.5
Sumbawa	3.7	5.1	4.9	3.8	98.1
Bima	3.6	4.9	5.1	3.9	96.7
HSD 5%	-	-	-	-	-

Table 5: Main Effect of Physiological Ages of Cuttings and Genotypes on Plant Survival and Growth After Transplanting

Treatment	Plant survival percent. in the field	Number of leaves		Plant height	
		1 month	2 month	1 month	2 month
Cutting					
Primary branch	80.3 b	12.1	16.3 b	14.5 b	22.7 b
Secondary branch	100.0 a	15.3	19.9 a	21.1 a	34.4 a
Tertiary branch	98.6 a	14.3	18.7 a	18.7 a	30.9 a
HSD 5%	12.72	-	2.21	3.8	7.53
Genotypes					
West Lombok	98.3	13.8	19.7	20.8	30.2
Middle Lombok	91.3	10.7	17.5	18.4	25.7
East Lombok	90.6	11.2	16.9	18.1	26.6
Sumbawa	96.6	12.9	19.1	19.2	28.5
Bima	96.3	12.5	18.2	19.5	29.9
HSD 5%	-	-	-	-	-