Agr. Nat. Resour. 56 (2022) 1215–1224

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Biomass yield stability of interspecific Jatropha hybrids through multiple harvest rotations with varying harvest ages

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Article Info

Article history:
Received 17 June 2022
Revised 19 September 2022
Accepted 8 November 2022
Available online

Keywords:
Harvest frequency,
Interspecific hybridization,
Jatropha curcas,
Pruning tolerance,
Short rotation woody crops

Abstract

Importance of the work: The consistency of biomass production is crucial for Jatropha cultivation as a short rotation woody crop for use as a biomass source.
Objectives: To investigate the effect of harvest age on Jatropha hybrid growth, assess the relationship between growth traits and biomass yield and evaluate the biomass yield stability of interspecific hybrids between *Jatropha curcas* and *J. integerrima*.
Materials & Methods: For 5 yr, 14 genotypes of Jatropha hybrids, were evaluated in the field, using three different harvest age rotations. Plant height, canopy width, stem and branch diameter and biomass yield were measured.
Results: Harvest ages and genotypes affected Jatropha hybrid growth traits. Jatropha hybrids grew larger with age. Plant height, canopy width and stem and branch diameter were all highly correlated with biomass yield (correlation coefficient range = 0.59 [$p < 0.05$] to 0.94 [$p < 0.01$]). At the first, second, third, fourth and fifth harvests at 12 mth intervals, the Jatropha hybrids had average biomass yields of 45.91 t/ha, 40.63 t/ha, 36.46 t/ha, 42.77 t/ha and 37.29 t/ha, respectively. Although harvest frequency affected biomass yield, the yield did not substantially decrease, suggesting pruning tolerance of the Jatropha hybrids. The optimal harvest age rotations for Jatropha hybrids were intervals of 12 mth and 18 mth, due to the highest five-yearly total biomass yields of 202.79 t/ha and 221.29 t/ha, respectively. Furthermore, the KUBJL 14 genotype had the highest growth and biomass production.
Main finding: The growth traits of the Jatropha hybrids could be used to assess biomass production. Jatropha hybrids have a high biomass potential and good biomass yield stability, even after multiple harvests.

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https://doi.org/10.34044/j.anres.2022.56.6.16

Introduction

Perennial crops planted on agricultural land and harvested in short rotations can offer substantial amounts of biomass for use as a renewable energy source. For example, Jatropha (*Jatropha curcas* L.) is an energy plant that garnered attention due to the usage of seed oil to produce biodiesel (Montes and Melchinger, 2016). Jatropha also has the potential to be used as a biomass source planting it involves the annual pruning of stems and branches for the plant to develop and generate new crops (Samsam, 2013). Every year, a considerable amount of biomass from Jatropha could be used as a raw material for energy generation. Furthermore, Jatropha is able to adapt to a wide range of environments due to its rapid growth, drought tolerance and tolerance of saline and poor fertility soils (Wani et al., 2012). Thus, Jatropha was appropriate for cultivation as a short rotation woody crop to supply biomass feedstock for power plants. On the other hand, the wood of Jatropha has a comparatively high moisture content. a low density and a poor calorific value when burning (Muakrong et al., 2014), resulting in less electricity being produced. However, breeding could improve the quality and quantitative traits of Jatropha biomass yield.

Interspecific hybridization is one method for developing plant genotypes; recently, it has been applied to enhance biomass yields in several short-rotation woody crops, such as willow (Gramlich et al., 2018), poplar (Barghini et al., 2015), eucalyptus (Sumathi and Ramasamy, 2017) and Leucaena (Brewbaker, 2013). Jatropha has a low genetic diversity (Laosatit et al., 2014), making it less likely to provide enough phenotypic variability to select new elite genotypes. However, Jatropha can cross-pollinate with plants of the same genus, specifically, *J. integerrima* Jacq., a genus of Jatropha (One et al., 2014; Fukuhara et al., 2016). The genetic background of Jatropha hybrids might be enlarged to be more broadly diversified, with hybrids potentially gaining certain useful traits from other plants (Muakrong and Srinives, 2020). Thus, t development of interspecific hybrids between *J. curcas* and *J. integerrima* is a method to increase the quantity and quality of Jatropha biomass (Muakrong et al., 2014). However, to further select the new hybrids, the biomass potential of the hybrids must be tested.

The cultivation of Jatropha hybrids as a source of raw materials for sufficient and sustainable energy production necessitates the availability of relevant management information. A fast-growing plant appropriate for planting as a biomass energy source should not only have a high biomass production per area but should also be pruning tolerant (Pleguezuelo et al., 2015) to facilitate gathering biomass repeatedly without replanting. Furthermore, several short-rotation woody crops, such as willow and poplar, require years of maturation before harvesting the biomass, and for some species, it is not possible to recoup the harvested biomass through new growth, while it could take years for biomass to regenerate before it can be harvested again (Pleguezuelo et al., 2015; Griffiths et al., 2019). However, numerous short-rotation woody crops, such as *Acacia mangium*, leucaena, and eucalyptus, can be grown and harvested in one year (Chotchutima et al., 2013; Sarmiento and Varela, 2015; Tudsri et al., 2019). In typical planting methods, Jatropha is pruned annually for Jatropha seed species that utilize the seed yield for biodiesel manufacturing (Tar et al., 2011; Samsam, 2013). However, the effect has not been studied of harvest age and harvest frequency on growth and biomass yield in biomass-utilized hybrid Jatropha genotypes. To cultivate hybrid Jatrophas as a short-rotation woody crop, it was important to examine the effect of harvest age on biomass production stability from recurrent biomass harvesting.

Breeding a hybrid Jatropha for biomass yield traits is a complicated and important procedure because biomass yield traits are quantitative and are controlled by multiple genes that are frequently sensitive to environmental effects as well as genetic and environmental interaction (Sixto et al., 2016). Thus, it may be difficult to select hybrids directly based on biomass yield. Consequently, as a selection criterion, the relationship between the traits of interest should be explored. The management approach for many short-rotation woody crops, such as willow (Berlin et al., 2017), poplar (Verlinden et al., 2013), eucalyptus (Santos et al., 2015) and leucaena (Chotchutima et al., 2013), has applied growth parameters, including stem size, height, and canopy width, to predict biomass yields and select genotypes. If a relationship between biomass yield and specific growth traits were discovered in Jatropha, these traits might be applied as an effective indirect tool for selecting Jatropha hybrids with high biomass yields.

A fundamental aspect of short rotation woody crops, in addition to having a high biomass yield and a short harvesting life, should be tolerance to pruning so that the plant can readily regrow to allow biomass harvesting multiple times. Furthermore, several growth traits might be used as an effective indirect tool for Jatropha hybrid selection. Thus, the goal of the current study was to investigate the effect of harvest age on Jatropha hybrid growth, to assess the relationship between growth traits and biomass yield and to evaluate the biomass yield stability of interspecific hybrids between *J. curcas* and *J. integerrima.*

Materials and Methods

Experimental design and treatments

The study was carried out in the field (14.01°N; 99.58°E). The experimental design was a randomized complete block split-plot with four replications and five plants in each plot. The main plots had harvest ages at intervals of 12 mth, 18 mth and 24 mth. The sub-plots consisted of 14 different Jatropha genotypes. F_1 interspecific hybrids of 13 genotypes (KUBJL 1, KUBJL 2, KUBJL 3, KUBJL 4, KUBJL 5, KUBJL 6, KUBJL 7, KUBJL 8, KUBJL 10, KUBJL 11, KUBJL 12, KUBJL 13 and KUBJL 14 (without KUBJL 9)) between *J. curcas* and *J. integerrima* were tested. *J. curcas* was also examined as the female parent JcM10, which had a large canopy size.

The experiment was conducted in the Jatropha Research Area at the Department of Agronomy, Kasetsart University, Kamphaeng Saen campus, Thailand. The soil type was classified as a sandy clay loam with a pH of 6.88. Electrical conductivity was typical (2.39 dS/m), organic matter (1.64%) was moderate and total nitrogen (0.073%) was low, while phosphorus (35.3 mg/kg), potassium (263.9 mg/kg), magnesium (144.2 mg/kg) and calcium (2,948 mg/kg) were high. The experiment lasted 5 yr, from April 2013 to March 2018. The weather conditions were recorded at Kasetsart University Kamphaeng Saen campus weather station. Annual rainfall was 981 mm, 761 mm, 1,175 mm, 791 mm and 825 mm in years 1–5, respectively, with average air temperatures in the ranges 16.5–37.4 °C, 15.5–37.3 °C , 17.3–37.5 °C , 20.0–38.9 °C and 19.2–38.9 °C in years 1–5, respectively.

The plots were prepared and planted with nursery-grown clones aged 2 mth. The plant spacing was 1 m between rows and 1.5 m between plants, resulting in a planting density of 6,666 plants/ha. Before transplanting, each plant was provided with 200 g of commercial compost. At 4 mth and 8 mth after transplanting and pruning, a 15-15-15 (N-P_2O_5-K_2O) compound fertilizer was applied at a rate of 20 g/plant. During the Jatropha planting season, hand weeding was done twice a year. After transplanting and pruning, the field was furrow-irrigated twice a month from months 1 to 4. Thereafter, depending on the environment, the plants were watered approximately every 30 d. The Jatropha hybrids were harvested five times at 12 mth, 24 mth, 36 mth, 48 mth and 60 mth (12 mth between harvests). In another series of plots, the Jatropha hybrids were harvested four times, with the first three at 18 mth, 36 mth, 54 mth (18 mth between harvests), with the fourth harvest at 60 mth (a 6 mth interval from the previous harvest). In another series of plots, the Jatropha hybrids were harvested three times, with the first two at 24 mth and 48 mth (24 mth between harvests), with the third harvest at 60 mth (a 12 mth interval from the previous harvest).

Data collection on growth traits of Jatropha hybrids

Growth data on the Jatropha hybrids were collected before the respective first harvest ages at 6 mth, 12 mth, 18 mth and 24 mth. Plant height, canopy width and stem and branch diameter were measured on two plants at random in each plot (Tar et al., 2011; Muakrong et al., 2014). The stem diameter was measured 5–10 cm above the ground, while the branch diameter was measured at the primary, secondary and tertiary branches in the middle of each branch length by randomly measuring four branches per position per plant. However, at age 6 mth, the diameter of the stem was not measured due to its small size.

Measurement of biomass yield, wood moisture content and wood density

The Jatropha hybrid plants were harvested for biomass yield at the designated harvest ages, with two plants per plot, to record biomass yield and wood properties. Plants were harvested 30 cm above the ground. Total fresh weights, including wood and leaves, were recorded as biomass weights. The aboveground biomass per plant was computed as biomass yield per hectare. Branches and stems were cut into pieces (15 cm long) with a diameter of 1.5–2.5 cm, with three pieces randomly selected per plant for wood moisture and density measurements. Wood samples were weighed and dried to a consistent weight for 72 h at 105 °C. The percentage of moisture was estimated using [(fresh weight - dry weight) / fresh weight] × 100. The water displacement method was used to measure the volume of wood samples and the dry wood density was computed using the oven-dry weight per dry volume. However, data on the moisture and density of the wood were not gathered during the final harvest year of each harvest age.

Statistical analysis

All data were subjected to analysis of variance that was appropriate for a split-plot in a randomized complete block design. A repeat measurement variance analysis was done on the original rootstock for biomass yields to examine the stability of biomass yields when many rotations were harvested on the original plant. The F statistics from these analyses were calculated. The total sum of squares was calculated as the percentage share of all the analysis effects under examination. This measure indicated the proportion of trait variance that was explained by individual factors. The variation in total biomass yields over the duration of the experiment (5 yr) was examined independently for each harvest age. Duncan's multiple range test was used for mean comparisons. Simple correlations between growth traits and biomass yield were computed.

Results and Discussion

Effect of harvest age on growth traits of Jatropha hybrids

Harvest age had a significant ($p < 0.01$) effect on all growth traits of the Jatropha hybrids (Table 1). Growth was higher in older Jatropha hybrids. Jatropha hybrid plant heights increased from 239.42 cm at 12 mth to 292.63 cm and 311.50 cm at 18 mth and 24 mth, respectively. The canopy width increased from 189.37 cm at 12 mth to 236.88 cm and 190.86 cm at 18 mth and 24 mth, respectively. The stem diameter increased from 55.37 mm at 12 mth to 72.01 mm and 73.19 mm at 18 mth and 24 mth, respectively. The primary branch diameter increased from 36.59 mm at 12 mth to 43.92 mm and 46.24 mm at 18 mth and 24 mth, respectively. The secondary branch diameter increased from 24.93 mm at 12 mth to 28.87 mm and 29.49 mm at 18 mth and 24 mth, respectively. The tertiary branch diameter increased from 17.86 mm at 12 months to 21.58 mm and 21.19 mm at 18 mth and 24 mth, respectively.

Table 1 Plant height, canopy width and diameter of stem and branches of interspecific hybrids between *Jatropha curcas* and *J. integerrima* at three harvest ages

Treatment	Plant height (cm)	Canopy width (cm)	Diameter (mm)			
			Stem	Primary branch	Secondary branch	Tertiary branch
Harvest age						
12 mth	239.42^{B}	189.37^{B}	55.37^{B}	36.59^{B}	24.93^{B}	17.86^{B}
18 mth	292.63^{A}	236.88^{A}	72.01^{A}	43.92^{A}	28.87^{A}	21.58^{A}
24 mth	311.50^{A}	190.86^{B}	73.19^{A}	46.24^{A}	29.49^{A}	21.19^{A}
F test	**	**	**	**	**	**
Genotype						
KUBJL 1	295.21^{bcd}	234.10^{abc}	71.11^{bcd}	43.84^{abc}	30.11^{ab}	21.70^{abc}
KUBJL 2	267.22^{ef}	210.90^{cd}	67.25^{cde}	44.71^{ab}	29.22^{a-d}	21.25^{abc}
KUBJL 3	302.78^{abc}	216.60^{bcd}	69.67^{bcd}	44.11^{abc}	29.81^{abc}	22.52^{a}
KUBJL 4	289.38^{cde}	197.71^{def}	62.53^{def}	37.88^{de}	26.60^{cde}	18.22^{de}
KUBJL 5	304.17^{abc}	252.81^{a}	76.75^{ab}	47.15^{a}	29.47^{abc}	22.05^{ab}
KUBJL 6	276.74^{def}	218.30^{bcd}	73.83^{bc}	46.87^{a}	31.93^{a}	22.24^{ab}
KUBJL 7	257.01^{fg}	178.85^{fg}	57.90^{fg}	37.06^{de}	25.41^{efg}	19.15^{cd}
KUBJL 8	279.44^{c-f}	205.59^{de}	65.28^{c-f}	40.56^{bcd}	25.58^{efg}	18.08^{de}
KUBJL 10	281.25^{c-f}	181.60^{ef}	57.16^{fg}	38.35^{de}	25.98^{def}	20.32^{a-d}
KUBJL 11	240.42^{g}	175.73^{fg}	52.13^{g}	34.65^{e}	22.38^{g}	16.11^{e}
KUBJL 12	270.63^{def}	182.50^{ef}	60.19^{efg}	39.55^{cde}	26.70^{b-e}	19.58^{bcd}
KUBJL 13	322.15^{a}	234.44^{abc}	73.25^{bc}	48.08^{a}	31.20^{a}	23.07^{a}
KUBJL 14	315.76^{ab}	235.97^{ab}	84.79^{a}	48.33^{a}	31.26^{a}	22.37^{ab}
JcM10	234.44^{g}	154.72^{g}	64.12^{def}	40.38^{bcd}	23.03^{fg}	16.25^{e}
F-test	**	**	**	**	**	**
Genotype × Harvest age						
F test	ns	**	*	*	ns	ns
CV (a)	27.22	29.64	17.60	19.15	18.80	22.28
CV (b)	10.95	14.93	15.90	14.84	15.51	17.70
Mean	281.19	205.70	66.85	42.25	27.76	20.21

CV = coefficient of variation

Mean values in the same column superscripted with different uppercase letters denote significant ($p < 0.05$) differences between harvest ages and different lowercase superscripts in the same column denote significant differences among the genotypes; ns = not significant ($p \geq 0.05$); * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

Pruning is a standard annual practice during the cultivation of Jatropha genotypes used for biodiesel production (Tar et al., 2011; Samsam, 2013). An interesting finding in the current study was that the growth of the Jatropha hybrids increased with age, with significantly higher growth at 18 mth than at 12 mth. However, there was no significant difference in Jatropha hybrid growth traits between 18 mth and 24 mth. These results showed that the Jatropha hybrids grew rapidly between the ages of 12 mth and 18 mth, then grew very slowly between the ages of 18 mth and 24 mth. Jatropha growth can be described by a nonlinear model, as do other plants, such as sugarcane, pepper and cocoa (Muniz et al., 2017; Jane et al., 2019, 2020). Miranda et al. (2021) described the Jatropha growth pattern as sigmoidal. The current findings suggested that the Jatropha hybrids might have had exponential growth from 12 mth to 18 mth, followed by stationary growth from 18 mth to 24 mth. Thus, Jatropha hybrids at age 18 mth could be the ideal age for biomass harvesting because this was the largest growth rate with no noticeable change in subsequent growth.

Based on the mean values for all three harvest ages, the Jatropha hybrids had significantly ($p < 0.01$) different growth traits between genotypes (Table 1). Plant heights of 322.15 cm and 315.76 cm were recorded for KUBJL 13 and KUBJL 14, respectively. KUBJL 5 and KUBJL 14 had the largest canopy widths (252.81 cm and 235.97 cm, respectively). The stem diameters of KUBJL 14 and KUBJL 5 were the largest (84.79 mm and 76.75 mm, respectively). KUBJL 14, KUBJL 13, KUBJL 5 and KUBJL 6 had the largest primary branch diameters (48.33 mm, 48.08 mm, 47.15 mm and 46.87 mm, respectively). The secondary branch diameters of KUBJL 6, KUBJL 14 and KUBJL 13 were the largest (31.93 mm, 31.26 mm and 31.20 mm, respectively), while KUBJL 13 and KUBJL 3 had the largest tertiary branch diameters (23.07 mm and 22.52 mm, respectively). Almost all the Jatropha hybrid genotypes had greater plant heights and canopy widths, as well as larger stem and branch diameters than the JcM10 Jatropha (the female parent genotype). These results indicated the heterosis of Jatropha hybrids. Muakrong et al. (2014) reported that interspecific hybrids of *J. curcas* and *J. integerrima* exhibited high heterosis in canopy size, branch number and wood weight. As a result, interspecific hybridization can extend the genetic diversity base of Jatropha and heterosis can be used to increase the biomass potential of Jatropha hybrids (Muakrong and Srinives, 2020). In the current investigation, the KUBJL 14 Jatropha hybrids outperformed the other hybrids in terms of growth. Furthermore, a genotype × harvest age interaction was discovered in canopy width, stem diameter, and primary branch diameter. Plant height, secondary branch diameter, and tertiary branch diameter were not affected by the genotype × harvest age interaction.

Relationship between biomass yield and growth traits of Jatropha hybrids

An analysis of the correlation between first-harvest biomass yield and growth traits in the Jatropha hybrids at the same harvest age revealed that biomass weight was positively correlated with plant height, canopy width, stem diameter and branch diameter at all three harvest ages (Table 2). The correlation coefficient (r) was 0.66 ($p < 0.01$) to 0.94 ($p < 0.01$) at 12 mth, 0.56 ($p < 0.05$) to 0.85 ($p < 0.01$) at 18 mth and 0.80 ($p < 0.01$) to 0.90 ($p < 0.01$) at 24 mth. Furthermore, when the three ages were combined, the correlation coefficient ranged between 0.66 ($p < 0.01$) and 0.90 ($p < 0.01$). Growth traits, such as stem size, height and canopy width, are commonly used in plant breeding because a relationship between these traits and biomass yield can be established. For example, growth traits were utilized to screen high biomass yielding genotypes in several short-rotation woody crops, including willow (Berlin et al., 2017), poplar (Verlinden et al., 2013), eucalyptus (Santos et al., 2015) and leucaena (Chotchutima et al., 2013). Based on the current results, the Jatropha hybrids with a large canopy size as well as a large stem and branch size had a high biomass production. Thus, plant height, canopy width, stem diameter and branch diameter could be used to predict Jatropha hybrid biomass yields. However, stem diameter was the best trait for evaluation because of its strong and consistent relationship with biomass yields. This is also a simple measurement method because it only requires measurement at one point.

Table 2 Correlation coefficients between fresh biomass yield with plant height, canopy width and diameter of stem and branches of interspecific hybrid between *Jatropha curcas* and *J. integerrima* at three harvest ages

Fresh biomass yield	Plant height	Canopy width	Diameter			
			Stem	Primary branch	Secondary branch	Tertiary branch
12 mth ($n = 14$)	0.66**	0.82**	0.80**	0.86**	0.94**	0.87**
18 mth ($n = 14$)	0.56*	0.85**	0.77**	0.81**	0.84**	0.71**
24 mth ($n = 14$)	0.87**	0.80**	0.83**	0.81**	0.90**	0.89**
Total ($n = 42$)	0.75**	0.66**	0.82**	0.83**	0.90**	0.85**

* = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

An analysis of the correlation between biomass yield at the three harvest ages and Jatropha hybrid growth traits at 6 mth found that biomass weight at harvest age was statistically positively correlated with plant height, canopy width, stem diameter and branch diameter at 6 mth (Table 3). In addition, the r values were between 0.55 ($p < 0.05$) to 0.90 ($p < 0.01$) at 12 mth, between 0.65 ($p < 0.05$) to 0.91 ($p < 0.01$) at 18 mth and between 0.62 ($p < 0.05$) to 0.79 ($p < 0.01$) at 24 mth. Furthermore, when data from the three ages were combined, the correlation coefficient was between 0.56 ($p < 0.01$) to 0.74 ($p < 0.01$). These results revealed that the growth traits of the Jatropha hybrids at 6 mth were related to biomass yield at 12 mth, 18 mth and 24 mth. Thus, biomass yield evaluation of Jatropha hybrids based on growth traits might begin as early as age 6 mth, which would speed up the selection of Jatropha hybrids for biomass yield.

Stability of biomass yields of Jatropha hybrids from repeated harvesting

The variance was investigated in biomass production of Jatropha hybrids harvested repeatedly on the original rootstock at different harvest age rotations. The Jatropha hybrids harvested on a 12 mth interval, genotypes and harvest frequency all had significant ($p < 0.01$) effects on biomass yield (Table 4). However, there was no effect of genotype × harvest frequency interaction on biomass yield. The highest levels of variation in biomass yields of Jatropha hybrids were attributed to genotype, accounting for 44.14% of the variance, followed by 2.52% for harvest frequency. Jatropha hybrids harvested on an 18 mth interval, genotypes and harvest frequency all had significant ($p < 0.01$) effects on biomass yield. However, there was no effect of genotype × harvest frequency interaction on biomass yield. The highest level of variation in the biomass yields of Jatropha hybrids was attributed to genotype (46.10%), followed by harvest frequency (10.76%). Furthermore, Jatropha hybrids harvested on a 24 mth interval, genotype, harvest frequency and genotype × harvest frequency interaction had significant ($p < 0.01$) effects on biomass yield. The biomass yields of the Jatropha hybrids varied the most due to genotype (51.17%), followed by harvest frequency (14.84%) and genotype × harvest frequency interaction (7.41%). The findings revealed that genotype and harvest frequency had an effect on the biomass yield of the Jatropha hybrids for all three harvest age rotations. Although the genotype × harvest frequency interaction was detected at 24 mth intervals, it was not observed at 12 mth or 18 mth intervals. Thus, the selection of Jatropha hybrids for high biomass could be done at the first harvest. The results of the selection of the Jatropha hybrids for the other harvest rotations produced selection results similar to the first harvest.

When the Jatropha hybrids were harvested frequently for all three harvest age rotations, their biomass yields tended to decline (Fig. 1). The Jatropha hybrids with a 12 mth interval had a biomass yield of 45.91 t/ha at the first harvest and biomass yields of 40.63 t/ha, 36.46 t/ha, 42.77 t/ha and 37.29 t/ha at the second, third, fourth and fifth harvests, respectively. Furthermore, compared to the first harvest, biomass yields decreased by 12.08%, 20.59%,

Table 3 Correlation coefficients between fresh biomass yield at three harvest ages with plant height, canopy width and diameter of stem and branches at age 6 mth of interspecific hybrid between *Jatropha curcas* and *J. integerrima*

Fresh biomass yield	Plant height	Canopy width	Diameter		
			Primary branch	Secondary branch	Tertiary branch
			(at 6 months)		
12 mth (n = 14)	0.55*	0.82**	0.85**	0.90**	0.73**
18 mth (n = 14)	0.65*	0.74**	0.84**	0.91**	0.70**
24 mth (n = 14)	0.62*	0.68**	0.70**	0.79**	0.70**
Total (n = 42)	0.56**	0.66**	0.66**	0.74**	0.67**

* = significant ($p < 0.05$); ** = significant ($p < 0.01$)

Table 4 F statistics from repeated measure variance analysis and percentage share of effects in total sum of squares of attribute for fresh biomass yield

Source of variation	Fresh biomass yield								
	12 mth intervals (harvested 5 times)			18 mth intervals (harvested 4 times)			24 mth interval (harvested 3 times)		
	df	F	Share (%)	df	F	Share (%)	df	F	Share (%)
Replication	3	2.87	5.61	3	0.49	0.83	3	1.65	1.70
Genotype (G)	13	5.21**	44.14	13	6.27**	46.10	13	11.5**	51.17
Error (a)	39		25.44	39		22.04	39		13.40
Harvest frequency (HF)	4	5.87**	2.52	3	31.5**	10.76	2	54.3**	14.84
G × HF	52	0.76	4.26	39	1.33	5.91	26	2.09**	7.41
Error (b)	168		18.03	126		14.36	84		11.48
Total			100.00			100.00			100.00

df = degrees of freedom; * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

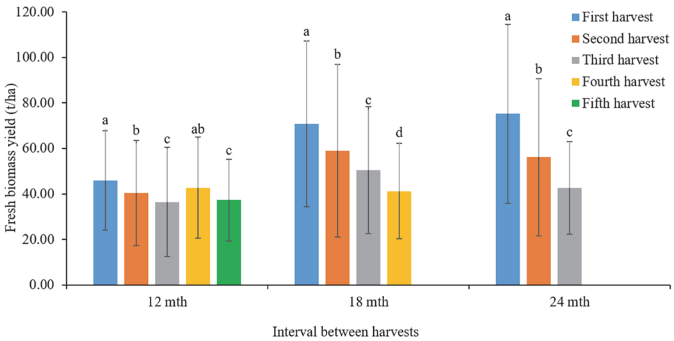

Fig. 1 Fresh biomass yield for three harvest age rotations of interspecific hybrids between *Jatropha curcas* and *J. integerrima* in multiple harvests, where error bars represent ± SD and different lowercase letters above bars denote significant ($p < 0.05$) differences among harvesting times for each harvest interval

6.83% and 18.77% for the second to fifth harvests, respectively. The Jatropha hybrid with an 18 mth interval produced a first harvest biomass yield of 70.73 t/ha and second, third and fourth harvest biomass yields of 58.90 t/ha, 50.39 t/ha and 41.27 t/ha, respectively. Compared to the first harvest, the subsequent biomass yields declined by 16.72%, 28.76% and 41.66%, respectively. However, there was only 6 mth of growth before the final (fourth) harvest. Furthermore, the Jatropha hybrids with a 24 mth interval had a first harvest biomass yield of 75.24 t/ha and second and third harvest biomass yields of 56.11 t/ha and 42.58 t/ha, respectively. Compared to the first harvest, subsequent biomass yields declined by 25.43% and 43.41%, respectively. However, there was only 12 mth of growth before the final (third) harvest. According to the experimental results, the Jatropha hybrids could produce multiple biomass yields on the same rootstock during different harvest age rotations. Furthermore, the Jatropha hybrids had reduced biomass yields when harvested frequently. However, compared to the other harvest age rotations, the Jatropha hybrids showed only a slight drop in biomass yield for the 12 mth harvest interval.

When the Jatropha hybrids were harvested multiple times at all three harvest age rotations, the wood moisture content increased (Fig. 2). At the first, second, third and fourth harvests of the Jatropha hybrids with the 12 mth harvest interval, the wood moisture contents were 57.02%, 57.81%, 62.04% and 64.02%, respectively. In the first, second and third harvests of the Jatropha hybrids with the 18 mth harvest interval, the wood moisture contents were 56.09%, 60.05% and 63.07%, respectively. Finally, the first and second harvests of the Jatropha hybrids with the 24 mth harvest interval had wood moisture contents of 57.82% and 62.96%, respectively.

On the other hand, the dry wood density of the hybrids changed in the opposite way. For the three harvest age rotations, repeated harvesting resulted in a reduction in dry wood density (Fig. 3). At the first, second, third and fourth harvests, the dry wood densities of the Jatropha hybrids with the 12 mth harvest interval were 0.59 g/cm^3, 0.57 g/cm^3, 0.50 g/cm^3 and 0.50 g/cm^3, respectively. At the first, second and third harvests, the dry wood densities of Jatropha hybrids with the 18 mth harvest interval were 0.58 g/cm^3, 0.52 g/cm^3 and 0.52 g/cm^3, respectively. Furthermore, with the 24 mth harvest intervals, the dry wood densities of the Jatropha hybrids were 0.56 $g/cm^3$and 0.52 g/cm^3 at the first and second harvests, respectively. According to the findings, frequent harvesting affected the wood quality of the Jatropha hybrids, with higher wood moisture content and decreased dry wood density. This could have been because the wood of the Jatropha hybrids that regenerated on the original rootstock was more juvenile than the wood of the previous harvest. Pruning delays the development of Jatropha. For example, Valdes-Rodriguez et al. (2020) reported that pruning delayed the flowering of Jatropha by 2 mth, while pruning the Jatropha plant could increase the number of new shoots and branches (Ghosh et al., 2011; Rajaona et al., 2011), resulting in high moisture content in the wood of the Jatropha hybrids.

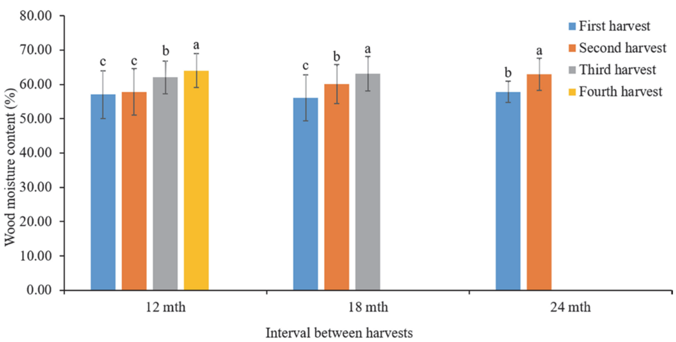

Fig. 2 Wood moisture content for three harvest age rotations of interspecific hybrids between *Jatropha curcas* and *J. integerrima* in multiple harvests, where error bars represent ± SD and different lowercase letters denote significant ($p < 0.05$) differences among harvesting times for each harvest interval

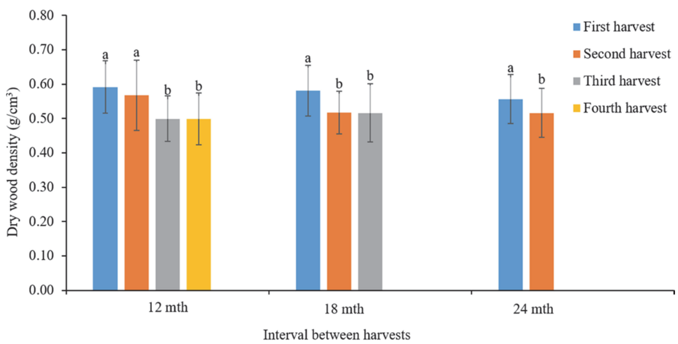

Fig. 3 Dry wood density for three harvest age rotations of interspecific hybrids between *Jatropha curcas* and *J. integerrima* in multiple harvests, where error bars represent ± SD and different lowercase letters denote significant ($p < 0.05$) differences among harvesting times for each harvest interval

Short-rotation woody crop cultivation is a strategy for sustainable biomass fuels. Several species of short rotation woody crops are currently planted for biomass sources, including poplar (*Populus* sp.), willow (*Salix* sp.), leucaena, acacia and eucalyptus, which have been developed to provide higher yields using agricultural technology (Aref et al., 2003; Prasad et al., 2011; Djomo et al., 2015). Rapid growth and short harvest rotations are desirable characteristics of fast-growing plants ideal for use as energy crops (Djomo et al., 2015; Bergante et al., 2016). The current research revealed that interspecific Jatropha hybrids have high biomass yield potential and short harvest rotations. The Jatropha hybrids in the current study provided excellent biomass yields from the first harvest, with average biomass yields of 45.91 t/ha, 58.90 t/ha and 75.24 t/ha at harvest age rotations of 12 mth, 18 mth and 24 mth, respectively (Fig. 1). Compared with other woody crops, commercial plantations of poplars and willows might generate up to 25 t/ha of dry biomass per year, and eucalypts and leucaena could attain around 40 t/ha (Sixto et al., 2015; Fernández et al., 2018, 2020). The, Jatropha hybrids in the current study were harvested for biomass yields several times on the same rootstock with varying harvest age rotations (12–24 mth intervals), confirming their tolerance to pruning. While some woody crops, such as poplar, have been developed, several commercially available poplar cultivars may not tolerate frequent harvesting or short harvest rotation without a loss in productivity or resprouting capability (Dillen et al., 2013). On the other hand, willow displayed strong pruning tolerance and good biomass yield stability over repeated harvest rotations, with a tendency for higher biomass yield after the first harvest (Stolarski et al., 2018).

The biomass production of the Jatropha hybrids increased with harvest age. However, the shortest harvest period was the 12 mth interval, allowing it to be harvested every year without greatly reducing biomass yields. The aging of the Jatropha hybrids resulted in increased biomass yields, although the 18 mth interval produced the highest growth and biomass yield, which was comparable to harvesting at the 24 mth interval. Furthermore, in the final harvest (after only 6 mth growth from the previous harvest), the 18 mth interval for the Jatropha hybrids provided yields comparable to the Jatropha hybrids harvested at intervals of 12 mth or 24 mth. Thus, an interval of 18 mth would be biomass harvest regime ideal for Jatropha hybrid cultivation.

Total biomass yield potential of Jatropha hybrids

The total biomass yield with the 12 mth interval (harvested three times) was not significantly different from that with the 18 mth interval (harvested twice) over a three-year period (Fig. 4), with total biomass yields of 122.73 t/ha and 129.64 t/ha, respectively. However, over a four-year period, the total biomass yield with the 12 mth interval (harvested four times) was higher than the total biomass yield with the 24 mth interval (harvested twice), with total biomass yields of 165.50 t/ha and 131.35 t/ha, respectively. The current study revealed that two harvests with the 18 mth interval provided a total biomass yield comparable to three harvests with 12 mth intervals, whereas two harvests with the 24 mth interval produced a lower total biomass yield than four harvests with 12 mth intervals.

A five-year assessment of total biomass yields of the Jatropha hybrids showed there was a significant difference among the harvest age rotations (Table 5). The Jatropha hybrids with 12 mth and 18 mth intervals produced total biomass yields of 202.79 t/ha and 221.29 t/ha, respectively, which were greater than the total biomass yield of 173.93 t/ha for the 24 mth interval. Furthermore, at 12 mth, 18 mth and 24 mth intervals, there were significant ($p < 0.01$) differences among total biomass yields across genotypes. For all harvest age rotations, mostly the Jatropha hybrids produced more total biomass than the non hybrid (JcM10). Furthermore, comparing the total biomass yields of the Jatropha hybrids, it was observed that biomass weights were in the ranges 118.93–377.02 t/ha for the 12 mth interval, 89.94–379.26 t/ha for the 18 mth interval and 70.54–313.47 t/ha for the 24 mth interval. Across all harvest age rotations, the KUBJL 14 Jatropha hybrids consistently had the highest biomass yields, with 377.02 t/ha, 379.26 t/ha and 313.47 t/ha at 12 mth, 18 mth and 24 mth intervals, respectively. The harvest rotation of short rotation woody crops is in the range 7–15 yr. However, many short-rotation woody crops have reduced the rotation age from 3 yr to 4 yr to enable faster utilization (Griffiths et al., 2019). For example, poplar had a harvest rotation of 2 to 8 years (Oliveira et al., 2020), willow, acacia, and eucalyptus has a harvest rotation of 3–4 yr (Moya et al., 2019; Stolarski et al., 2020) and leucaena has a harvest rotation of 1–3 yr (Fernández et al., 2020). According to the findings from the current study, Jatropha hybrids can be harvested after 1–2 yr of rotation; however, the recommended

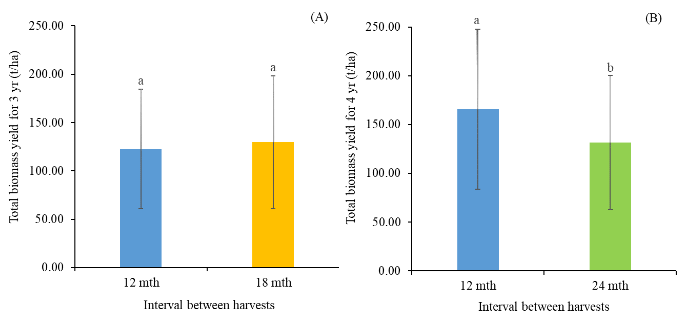

Fig. 4 Comparisons of total biomass yield for interspecific hybrids between *Jatropha curcas* and *J. integerrima* in multiple harvests: (A) between 12 mth and 18 mth intervals over 3 yr; (B) between 12 mth and 24 mth intervals over 4 yr, where error bars represent ± SD and different lowercase letters denote significant ($p < 0.05$) differences among harvesting interval

Table 5 Total biomass yield over 5 yr in different harvest age rotations of interspecific hybrids between *Jatropha curcas* and *J. integerrima*

Genotype	Total biomass yield (t/ha)		
	12 mth intervals (harvested 5 times)	18 mth intervals (harvested 4 times)	24 mth intervals (harvested 3 times)
KUBJL 1	243.52[b]	252.92[bcd]	244.43[bc]
KUBJL 2	268.91[b]	253.09[bcd]	201.54[cd]
KUBJL 3	261.42[b]	246.35[b-e]	209.45[cd]
KUBJL 4	179.31[bcd]	146.99[d-g]	157.24[de]
KUBJL 5	191.21[bc]	336.38[ab]	276.99[ab]
KUBJL 6	239.76[b]	341.37[ab]	159.90[de]
KUBJL 7	132.09[cd]	205.54[c-f]	99.61[efg]
KUBJL 8	190.08[bc]	89.94[g]	101.94[efg]
KUBJL 10	118.93[cd]	142.83[efg]	99.77[efg]
KUBJL 11	131.67[cd]	130.09[fg]	70.54[g]
KUBJL 12	176.47[bcd]	195.34[c-g]	155.57[def]
KUBJL 13	242.19[b]	283.24[abc]	253.51[abc]
KUBJL 14	377.02[a]	379.26[a]	313.47[a]
JcM10	86.53[d]	94.77[g]	91.02[fg]
F test	**	**	**
CV	32.81	33.68	26.31
Mean	202.79[A]	221.29[A]	173.93[B]

CV = coefficient of variation
Means in the same row superscripted with different uppercase letters denote significant differences between harvest age rotations; different lowercase superscripts in the same column denote significant differences among the genotypes; ** = highly significant ($p < 0.01$)

harvest rotation for Jatropha hybrids is 12–18 mth intervals to obtain the highest total biomass yield. The Jatropha hybrids outperformed Jatropha in terms of biomass yield potential and pruning tolerance. KUBJL 14 was the Jatropha hybrid with the highest potential biomass yield.

In conclusion, the growth and biomass yield of Jatropha hybrids increased with age, while the growth and biomass yield were highest with the 18 mth interval. Growth traits and biomass yields varied greatly among the Jatropha hybrid genotypes, with KUBJL 14 having the highest growth and biomass yield. Because biomass yield was substantially correlated with plant height, canopy width, stem diameter and branch diameter, these traits could be utilized as an effective indirect tool for selecting hybrid Jatropha genotypes for high biomass yields. The Jatropha hybrids with high biomass yields could be selected during the first harvest because no effect of genotype × harvest frequency interaction on biomass yield was discovered. Furthermore, the Jatropha hybrids could be harvested multiple times for biomass on the same rootstock at different harvest ages. The Jatropha hybrids could be harvested at any time between 12 mth and 24 mth intervals; however, the optimal harvest age rotation was between 12 mth and 18 mth intervals due this producing the highest overall biomass yield. Thus, the findings of the current study can be applied to the breeding and management of Jatropha hybrids for use as a sustainable biomass source.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was funded by the Chair Professor Grant of Thailand's National Science and Technology Development Agency, project number P-11-00599. The Department of Agronomy, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen campus, Thailand provided support.

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