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Research article

Genotype performance and relationship between leaf traits, biomass yield and wood quality on interspecific hybrids of Jatropha

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Abstract

Importance of the work: Pruning tolerance of fast-growing trees is critical for biomass energy crop cultivation. Leaf traits might be used to identify Jatropha genotypes with high biomass yields.
Objectives: To evaluate the effects of pruning and performance of genotypes for leaf traits, biomass yield and wood quality in interspecific hybrids between *Jatropha curcas* and *J. integerrima*, as well as the relationships among these traits.
Materials & Methods: In total, 16 Jatropha genotypes were planted in the field for 2 yr and pruned each year for biomass harvesting. Leaf traits, biomass yield, wood quality, chemical composition, and calorific value of wood were all assessed.
Results: After pruning, there was no significant reduction in the overall biomass yield and leaf traits, such as leaf size, average leaf weight, specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR). Furthermore, the hemicellulose and ash contents, moisture, density and calorific value of Jatropha genotype wood were not reduced. Leaf traits, biomass yield and wood quality of a Jatropha genotype showed significant genotype variation. Interspecific hybrids KUBJL 1, KUBJL 3, KUBJL 4, KUBJL 6, KUBJL 11, and KUBJL 13 showed improved leaf traits after pruning. Pruning had no effect on the chemical composition of KUBJL 2, KUBJL 3, KUBJL 8, KUBJL 10, KUBJL 5, KUBJL 11, KUBJL 12, and KUBJL 14. Moreover, KUBJL 5, KUBJL 11, KUBJL 5, KUBJL 4, and KUBJL 2 had the highest pruning tolerance index (PTI), with fresh weight PTIs of 106.25%, 104.07%, 98.27%, and 94.57%, respectively. There were significant and highly significant correlations between biomass yield and leaf size (Pearson's coefficient [r] = -0.57 [$p < 0.05$] to -0.75 [$p < 0.01$]), average leaf weight (r = -0.58 [$p < 0.05$] to -0.74 [$p < 0.01$]), and SCMR (r = 0.64 [$p < 0.05$] to 0.79 [$p < 0.01$]). Wood calorific value was also related to leaf moisture content (r = -0.33^{ns} to -0.69 [$p < 0.01$]), wood moisture content (r = -0.73 [$p < 0.01$] to -0.77 [$p < 0.01$]), wood density (r = 0.25^{ns} to 0.75 [$p < 0.01$]), lignin (r = 0.71 [$p < 0.01$] to 0.74 [$p < 0.01$]) and ash (r = -0.73 [$p < 0.01$] to -0.82 [$p < 0.01$]).
Main finding: These traits could be used to select Jatropha hybrids with high biomass yield and heat value. Furthermore, Jatropha interspecific hybrids with pruning tolerance could be selected from this study.

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Introduction

There is global concern to pursue sustainable alternative energy sources. Jatropha (*Jatropha curcas* L.) is a potential biomass energy plant because it is fast-growing and tolerant of drought, salinity and low fertility soils (Wani et al., 2012). Thus, Jatropha could be grown as a fast-growing plant with a high biomass yield as the tree responds readily to annual pruning of its stems and branches by regrowing and producing new biomass (Samsam, 2013). As a result, there was a large amount of biomass from the stems, branches, and leaves of Jatropha each year that could be used as raw materials for renewable energy production. On the other hand, Jatropha wood has a low density and high moisture content, resulting in a low calorific value when burned (Muakrong et al., 2014). However, both qualitative and quantitative traits of Jatropha biomass could be improved through breeding.

Jatropha could be cross-pollinated with plants of the same genus, specifically *J. integerrima* Jacq. (Laosatit et al., 2014; One et al., 2014; Fukuhara et al., 2016). Despite the F_1 generation plants of interspecific hybrids having very little seed set, they have a high biomass potential. Muakrong et al. (2014) found that interspecific hybrids between *J. curcas* and *J. integerrima* had a high biomass potential, with the hybrids being notably superior to their parents (heterosis) in terms of biomass yields. Thus, the production of Jatropha hybrids could fully exploit their heterosis because the hybrids could be propagated using clones. Consequently, interspecific hybridization could be used as a breeding strategy for developing the biomass potential of Jatropha hybrids.

The cultivation of Jatropha hybrids as a source of raw materials for sufficient and sustainable energy production requires relevant management information. While a fast-growing tree may be suitable for planting as a source of biomass energy, it should have a high biomass per area as well as the ability to regrow quickly after pruning (Pleguezuelo et al., 2015; Arunyanark et al., 2022) as this allows harvesting multiple biomass yields without having to replant. Many fast-growing trees, such as willow and Populus, require years of maturation before being pruned to harvest their biomass, with some cultivars not recovering and producing new growth or there may be several unproductive years until sufficient regrowth has developed to warrant harvesting (Pleguezuelo et al., 2015; Griffiths et al., 2019). However, several fast-growing tree species, such as *Acacia mangium* and Leucaena, with Eucalyptus being harvested for biomass after only 1 yr of growth (Chotchutima et al., 2013; Sarmiento and Varela, 2015; Tudsri et al., 2019). The normal biomass system for Jatropha cultivars used for biodiesel production involves annual pruning (Tar et al., 2011; Samsam, 2013). Wood as raw material source of heat can be subjected to high temperatures during the energy production process. Because of the presence of a diverse range of organic and inorganic compounds in varying types and quantities, wood is a highly volatile material (Nasser and Aref, 2014). The calorific value of raw materials is critical to their ability to produce energy (Domingos et al., 2020). The heat energy of wood is related to its properties and chemical composition, including its cellulose, hemicellulose, lignin and ash contents (Börcsök and Pásztory, 2021). The effects of pruning on biomass yield and wood quality in biomass-utilized Jatropha hybrid has not been published. Thus, cultivating Jatropha hybrids as a biomass energy crop is required to assess the regrowth and stability of biomass yield and wood quality after pruning.

The selection process for breeding Jatropha hybrids for biomass yield traits is critical and difficult because the biomass yield traits of fast-growing trees are quantitative and regulated by multiple genes, with frequent environmental effects and the effect of genetic and environmental interactions (Sixto et al., 2016). Thus, the direct selection of biomass yield traits in plant breeding projects is difficult. Therefore, the relationship between the traits of interest should be investigated to use them as selection criteria. The physiological and morphological traits of plant leaves were found to be related to photosynthetic capacity (Rezai et al., 2018), a process important for plant biomass accumulation. Several plants had been studied for the relationship between leaf traits and biomass yield (Qu et al., 2017; Chen et al., 2018; Konôpka et al., 2020). However, the relationship between leaf trait and biomass yield in Jatropha hybrids has not been reported. The measurement methods for some leaf traits are simple, quick and low cost, which would make them suitable in the breeding of Jatropha hybrids where a large number of samples must be evaluated for selection.

Jatropha hybrids with high biomass yields and pruning tolerance are important for use as biomass energy crops, with the hybrids regrowing so that biomass can be harvested on multiple occasions. Furthermore, if a correlation between leaf traits and biomass yield were discovered, some leaf traits could be used as an effective indirect tool for Jatropha hybrid selection. Thus, the objectives of this study were to assess the effects of pruning and relative performance of genotypes at the first and second pruning for leaf traits, biomass yield and wood quality, as well as to determine the relationship between biomass yield, leaf traits and wood quality in interspecific hybrids of *J. curcas* and *J. integerrima*.

Materials and Methods

Experimental design and treatments

The study was conducted in a research field (14.01°N, 99.58°E) at the Department of Agronomy, Kasetsart University, Kamphaeng Saen Campus, Thailand. The field experiment was performed for two years, from April 2013 to March 2015. The soil type was described as a sandy clay loam with a pH of 6.88. The electrical conductivity (2.39 dS/m) was normal, organic matter (1.64%) was moderate, total nitrogen (0.073%) was low and phosphorus (35.3 mg/kg), potassium (263.9 mg/kg), magnesium (144.2 mg/kg) and calcium (2,948 mg/kg) were high. Climatic data were collected at the weather station Kamphaeng Saen campus. The rainy season started in June and lasted until the end of November. There were 981 mm of rain from April 2013 to March 2014, and 761 mm of rain from April 2014 to March 2015. The average air temperature over the study period was in the range 15.5–37.4 °C.

The experiment was set out using a randomized complete block design with four replications and five plants in each plot. In total, 16 Jatropha genotypes were studied including 13 genotypes of F_1 interspecific hybrids between *J. curcas* and *J. integerrima* (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]), *J. curcas* (a large canopy size) as the female parent (JcM10), *J. integerrima* (a tall tree shape) as the male parent (Ji 2), and *J. integerrima*, Ji 1 line, which is common in Thailand. Cuttings were used to propagate all Jatropha genotypes.

The plots were prepared and planted with clones aged 2 mth that had been cultured in nurseries. The spacing between plants was 1 m and between rows was 1.5 m, resulting in a planting density of 6,666 plants/ha. Each plant received 200 g of commercial compost before transplanting. A 15:15:15 (N-to-P_2O_5-to-K_2O) compound fertilizer was applied at 20 g/plant at 4 and 8 mth after transplanting and at 4 and 8 mth after pruning. Hand weeding was performed twice a year during the trial period. During the first 4 mth, the field was furrow-irrigated twice a month after transplanting and pruning. Then, the plants were watered approximately every 30 d, depending on the climate. The biomass yields of the Jatropha were measured twice (in March 2014 and March 2015). Pruning was done one year after planting for biomass harvesting in the first year. Then, the rootstock was allowed to regrow for one year after pruning, with the plant then being pruned again in the second year to harvest the biomass.

Measurement of leaf traits

The leaf traits were collected when the Jatropha genotypes were aged 8 mth, in both the first and second years, by randomly selecting the fifth fully expanded leaves from the top of shoots or branches, with five leaves per plant. Data were collected from all five plants in each plot. The chlorophyll content of leaves was determined using a SPAD chlorophyll meter (SCMR, model Minolta SPAD-502 meter; Tokyo, Japan). The measurements were taken on the left and right positions of the leaves, with two points per leaf and the average value of each plot was determined. In addition, leaf area data were collected by taking a leaf sample for chlorophyll content measurement and measuring the leaf area using a leaf area meter (Li-3100C Area Meter; Licor Inc.; Lincoln; USA). Then, the average area per leaf was computed and the leaf size was recorded before the leaf samples were dried in a hot-air oven at 80 °C for 72 hr before being removed from the oven and weighed. The average leaf dry weight per leaf was calculated and the average leaf weight was recorded. The ratio of average leaf size-to-leaf weight (measured in square centimeters per gram) was used to compute the specific leaf area (SLA).

Measurement of biomass yield, moisture content and wood density

The Jatropha plants were pruned to produce biomass at age 1 and 2 yr (two pruning events at one year interval). At random, two plants per plot were selected to record the biomass yield and wood traits. The plant was pruned at 30 cm above the ground and the leaves were separated from the wood in the pruned material. Therefore, the stem and branches were used to represent wood. Fresh weight, wood, and leaf sections were all measured immediately after harvesting. Aboveground biomass per plant was calculated as the biomass yield per hectare. Branches and stems with a diameter of 1.5–2.5 cm were cut into 15 cm long pieces; three parts were randomly collected per plant for wood moisture and density measurements. The leaf moisture measurement was based on a 500 g subsample of total leaf weight from each plot. The wood and leaf samples were weighed separately and dried to constant weight for 72 hr at 105 °C. The percentage of moisture was calculated using the fresh weight and dry weight of the wood and leaves = [(fresh weight - dry weight) / fresh weight] × 100. Then, the dry weight of the biomass yield was calculated. The water displacement method was used to determine the volume of wood samples; dry wood density was calculated using the oven-dry weight per dry volume.

For each genotype, the pruning tolerance index (PTI) was calculated as follows: PTI = (biomass obtained from the second pruning /biomass obtained from the first pruning) × 100.

Measurement of chemical composition and heat value in wood

The entire wood yield from each plot was chopped into small pieces; a 2 kg sub-sample of wood fresh weight was taken from each plot and oven-dried at 105 °C for 72 hr. After that, the wood samples were chosen at random for chemical composition and heat value measurements. The percentages of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using Fiber bag technology and ANKOM 200 Fiber Analysis. Van Soest's method was used to determine the levels of cellulose, hemicellulose, lignin and lignocellulose (Van Soest et al., 1991). The method of Association of Official Analytical Chemists (1990) was used to determine the crude ash content. A standard bomb calorific combustion method was used to determine the heat value (calorific value) of the wood (Association of Official Analytical Chemists, 1990).

Statistical analysis

Two-way analysis of variance (2 years × 16 genotypes) was performed on the two-year data for leaf traits, biomass yield and wood quality to assess genotypic variability and effects of consecutive pruning. Mean comparisons were based on Duncan's multiple range test. In addition, simple correlations were calculated between leaf traits, biomass yield and wood quality, as well as correlations between the calorific value and chemical composition of wood. The tests were considered significant at $p < 0.05$.

Results and Discussion

Genotype variability on leaf traits and biomass yield

On the same plant, the performance of the Jatropha genotypes was evaluated twice, before pruning in the first year and after pruning in the second year. The results showed that pruning did not affect the leaf traits and biomass yield of Jatropha. In all the leaf traits studied, there were no significant differences between the first and second years. The two-year mean leaf size was 38.85 cm^2/leaf (Table 1), average leaf weight was 0.24 g, SLA was 161.72 cm^2/g and SCMR was 37.58. The values for the wood fresh weight, leaf fresh weight and total fresh weight of the Jatropha genotypes were not significantly different between the first and second years, with the mean values for the two-year period being 32.11, 6.34, and 38.46 t/ha, respectively. In addition, the wood dry weight and total dry weight of the Jatropha genotypes were not significantly different between the first and second years, with mean values for the two-year period being 13.67 and 15.08 t/ha, respectively. However, there was a significant difference in the leaf dry weight between the two years. The leaf dry weight was 1.74 t/ha in the first year, which was higher than the leaf dry weight of 1.09 t/ha in the second year. The results revealed that pruning did not affect the biomass yield of Jatropha genotypes. Furthermore, pruning did not affect leaf traits, such as leaf size, average leaf weight, SLA and SCMR.

The leaf traits and biomass yields of the Jatropha genotypes varied between genotypes. The results showed that the mean values for the leaf size and average leaf weight were significantly different between genotypes (Table 1) and appeared in the following order: *J. curcas* (JcM10) > interspecific hybrids (KUBJL 1 to KUBJL 14) > *J. integerrima* (Ji 1 and Ji 2). Different Jatropha genotypes were significantly different in SLA, with the order being JcM10 > KUBJL 1 to KUBJL 14 > Ji 1 and Ji 2. In addition, there was a significant difference in SCMR between genotypes in the Jatrophas genotypes whereby Ji 1 and Ji 2 > KUBJL 1 to KUBJL 14 > JcM10. These results indicated that interspecific hybrids inherited small, thick, dark green leaves from *J. integerrima*. Consequently, the interspecific hybrids had smaller leaf sizes than *J. curcas*, but the leaves were thicker and the chlorophyll content in the leaves was greater.

Furthermore, there was a significant difference in the biomass yield between the genotypes of Jatropha and the order according to the total fresh weight and total dry weight is KUBJL 1 to KUBJL 14 > JcM10 > Ji 1 and Ji 2. The interspecific hybrid of KUBJL 14 had the highest biomass yield, with an average total fresh weight of 75.00 t/ha and a total dry weight of 29.05 t/ha per year. Its biomass yield was comparable to that of other woody crops. Commercial poplar and willow plantations can yield up to 25 t/ha of dry biomass per year, while eucalypts and leucaena yield around 40 t/ha (Sixto et al., 2015; Fernández et al., 2018, 2020). These results showed that the interspecific hybrids had higher biomass yields than the parent species, indicating that heterosis had a strong effect on the biomass yield traits of the interspecific hybrids.

Table 1 Biomass yield, leaf size, leaf weight average, specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) of interspecific hybrids between *Jatropha curcas* and *J. integerrima* measured in biomass obtained from the first and second pruning (with one year interval)

Treatment	Leaf size (cm²/leaf)	Average leaf weight (g)	SLA (cm²/g)	SCMR	Fresh weight (t/ha)			Dry weight (t/ha)		
					Wood	Leaf	Total	Wood	Leaf	Total
Pruning event										
First year	38.17	0.23	161.89	37.60	34.08	7.06	41.14	14.83	1.74	16.57
Second year	39.52	0.24	161.55	37.55	30.15	5.61	35.77	12.50	1.09	13.59
F-test	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
Genotype										
KUBJL 1	36.76^{def}	0.23^{c-f}	161.60^{cde}	37.44^{c-f}	43.84^{bcd}	8.04^{bcd}	51.89^{bcd}	17.97^{b-e}	1.77^{cd}	19.72^{cde}
KUBJL 2	29.03^{gh}	0.19^{efg}	158.11^{d-g}	39.46^{c}	46.89^{bc}	7.78^{cde}	54.69^{bc}	20.09^{bc}	1.49^{de}	21.60^{bc}
KUBJL 3	35.14^{d-g}	0.23^{cde}	153.83^{d-g}	39.58^{c}	52.68^{ab}	10.91^{ab}	63.59^{ab}	22.91^{ab}	2.52^{ab}	25.41^{ab}
KUBJL 4	28.86^{gh}	0.20^{ef}	148.39^{fg}	38.43^{cde}	29.27^{efg}	4.75^{ef}	34.03^{ef}	13.00^{e-h}	1.08^{ef}	14.09^{e-h}
KUBJL 5	37.06^{de}	0.22^{c-f}	171.16^{bc}	36.29^{def}	33.35^{def}	6.05^{def}	39.39^{def}	14.15^{d-g}	1.34^{de}	15.48^{d-g}
KUBJL 6	24.31^{hi}	0.15^{gh}	163.42^{cd}	37.80^{cde}	43.52^{bcd}	8.91^{bcd}	52.42^{bcd}	18.43^{bcd}	1.93^{bcd}	20.33^{bcd}
KUBJL 7	36.52^{def}	0.22^{c-f}	164.59^{cd}	35.80^{efg}	22.94^{fgh}	4.41^{fgh}	27.36^{fg}	9.88^{ghi}	0.91^{e-h}	10.79^{ghi}
KUBJL 8	41.54^{d}	0.26^{c}	160.23^{c-f}	36.63^{def}	37.90^{cde}	8.48^{bcd}	46.38^{cde}	15.59^{c-f}	1.88^{bcd}	17.48^{c-f}
KUBJL 10	65.26^{b}	0.36^{b}	184.67^{a}	33.40^{gh}	24.65^{e-h}	4.12^{fgh}	28.76^{fg}	10.54^{fgh}	0.89^{e-h}	11.43^{gh}
KUBJL 11	57.28^{c}	0.35^{b}	162.33^{cd}	36.06^{d-g}	20.53^{gh}	4.70^{efg}	25.23^{fg}	8.70^{hi}	1.03^{efg}	9.72^{hi}
KUBJL 12	37.72^{de}	0.25^{cd}	155.97^{d-g}	34.89^{fg}	27.36^{efg}	6.96^{c-f}	34.31^{ef}	11.78^{fgh}	1.54^{cde}	13.32^{fgh}
KUBJL 13	24.11^{hi}	0.15^{gh}	165.19^{cd}	35.78^{efg}	45.18^{bcd}	9.24^{abc}	54.41^{bc}	20.52^{bc}	2.19^{bc}	22.70^{bc}
KUBJL 14	29.37^{fgh}	0.19^{fg}	159.83^{c-f}	38.78^{cd}	62.66^{a}	12.33^{a}	75.00^{a}	26.11^{a}	2.95^{a}	29.05^{a}
JcM10	87.26^{a}	0.49^{a}	181.36^{ab}	31.18^{h}	14.83^{hi}	1.63^{gh}	16.47^{gh}	5.17^{ij}	0.34^{h}	5.53^{ij}
Ji 1	30.98^{e-h}	0.21^{def}	146.69^{g}	47.10^{a}	4.63^{i}	1.56^{h}	6.19^{h}	2.11^{j}	0.35^{gh}	2.46^{j}
Ji 2	20.32^{i}	0.14^{h}	150.13^{efg}	42.65^{b}	3.59^{i}	1.58^{h}	5.18^{h}	1.73^{j}	0.44^{fgh}	2.17^{j}
F test	**	**	**	**	**	**	**	**	**	**
Genotype × Year										
F test	**	**	**	*	ns	ns	ns	ns	ns	ns
Mean	38.85	0.24	161.72	37.58	32.11	6.34	38.46	13.67	1.41	15.08

Means in same column superscripted with different lowercase letters are significantly ($p < 0.05$) different. ns = non-significant ($p > 0.05$); * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$); SLA = specific leaf area; SCMR = SPAD chlorophyll meter reading

Interestingly, there was an interaction effect between years (before and after pruning) and genotypes on leaf size, average weight average, SLA and SCMR, but not on the fresh weight and dry weight of the Jatropha genotypes.

Genotype variability on quality traits and chemical composition of wood

The pruning did not affect the quality of the Jatropha wood. The moisture content and density of the Jatropha wood samples did not significantly differ between the first and second years (Table 2). The wood moisture content averaged 57.15% over the two years, and the wood density was 0.59 g/cm^3. However, there was a significant difference in the leaf moisture content between the two years. The leaf moisture content in the first year was lower (75.58%) than the leaf moisture content in the second year (80.63%). The chemical composition of the Jatropha wood was influenced by the pruning. Wood harvested in the first year had significantly higher cellulose, lignin and lignocellulose contents than the second year. In the first year, the cellulose, lignin and lignocellulose contents were 44.61%, 16.38%, and 75.92%, respectively, and 41.99%, 15.61%, and 72.05%, respectively, in the second year. However, there was no effect of pruning on the content of hemicellulose and ash in wood, with two-year mean values of 14.76% and 4.38%, respectively. In fact, the calorific value of the Jatropha wood in the first year (4,406 cal/g) was significantly lower than in the second year (4,568 cal/g). The results showed that pruning did not affect the moisture content nor the density of the Jatropha wood samples, nor the content of hemicellulose or ash in the wood. However, pruning reduced the cellulose, lignin and lignocellulose contents of the wood. Pruning did not reduce the calorific value of hybrid wood. Furthermore, before reaching the second year of pruning, the Jatropha genotypes were subjected to drought and aphid infestation, resulting in major leaf fall of the new leaves that had not yet fully developed. Thus, the Jatropha genotypes had lower values for leaf weight and higher values of leaf moisture content in the second year than in the first year.

The quality and chemical composition of wood from the Jatropha genotypes varied between genotypes. The wood moisture, leaf moisture, wood density, chemical composition and calorific values of wood were significantly different among the genotypes of Jatropha genotypes (Table 2). The order of wood moisture and leaf moisture was JcM10 > KUBJL 1 to KUBJL 14 > Ji 2, while the order of wood densities was

Table 2 Moisture content, wood density, wood chemical compositions and calorific value of interspecific hybrids between *Jatropha curcas* and *J. integerrima* measured in biomass obtained from the first and second pruning (with one year interval)

Treatment	Moisture content (%)		Wood density	Cellulose	Hemi-cellulose	Lignin	Lignocellulose	Ash	Calorific value
	Wood	Leaf	(g/cm^3)	(%)	(%)	(%)	(%)	(%)	(cal/g)
Pruning event									
First year	56.96	75.58	0.61	44.61	14.93	16.38	75.92	4.62	4,406
Second year	57.34	80.63	0.58	41.99	14.60	15.61	72.05	4.14	4,568
F test	ns	**	ns	**	ns	**	**	ns	**
Genotype									
KUBJL 1	58.29^{b}	78.03^{bcd}	0.55^{cd}	43.91^{abc}	13.68^{f}	15.34^{cde}	72.93^{c-f}	3.76^{ef}	4,501^{bcd}
KUBJL 2	57.17b^{c}	80.65^{a}	0.60^{bc}	42.27^{b-e}	14.40^{c-f}	15.32^{cde}	71.98^{ef}	4.50^{cd}	4,448^{d}
KUBJL 3	56.60^{bcd}	77.27^{bcd}	0.59^{bcd}	42.48^{b-e}	14.22^{ef}	16.07^{cd}	72.77^{c-f}	3.82^{ef}	4,476^{bcd}
KUBJL 4	54.50^{cd}	77.42^{bcd}	0.63^{b}	43.23^{b-e}	13.66^{f}	15.10^{de}	71.98^{ef}	4.07^{cde}	4,455^{d}
KUBJL 5	57.16^{bc}	78.30^{bc}	0.61^{bc}	44.57^{ab}	15.04^{a-e}	15.79^{cd}	75.40^{bcd}	4.11^{cde}	4,494^{bcd}
KUBJL 6	56.59^{bcd}	78.14^{bcd}	0.53^{d}	43.36^{b-e}	13.85^{f}	15.25^{cde}	72.46^{def}	3.87^{def}	4,503^{bcd}
KUBJL 7	57.17^{bc}	79.19^{ab}	0.60^{bc}	41.08^{de}	15.38^{abc}	15.89^{cd}	71.11^{f}	4.55^{c}	4,471^{cd}
KUBJL 8	57.05^{bcd}	77.56^{bcd}	0.60^{bc}	41.89^{cde}	15.45^{ab}	16.19^{cd}	73.53^{c-f}	3.81^{ef}	4,545^{b}
KUBJL 10	57.69^{bc}	78.27^{bcd}	0.58^{bcd}	40.83^{e}	14.62^{b-f}	15.79^{cd}	71.23^{f}	5.35^{b}	4,483^{bcd}
KUBJL 11	56.89^{bcd}	78.5^{abc}	0.57^{cd}	42.96^{b-e}	14.90^{a-e}	17.62^{a}	75.47^{bc}	4.64^{c}	4,506^{bcd}
KUBJL 12	57.05^{bcd}	78.06^{bcd}	0.59^{bc}	43.20^{b-e}	14.33^{def}	16.31^{bc}	73.84^{c-f}	4.04^{c-f}	4,524^{bc}
KUBJL 13	54.11^{cd}	76.68^{cd}	0.60^{bc}	43.69^{a-d}	15.33^{a-d}	15.61^{cd}	74.64^{cde}	4.07^{cde}	4,487^{bcd}
KUBJL 14	56.96^{bcd}	76.70^{cd}	0.61^{bc}	44.35^{abc}	14.91^{a-e}	15.95^{cd}	75.21^{bcd}	4.33^{cde}	4,468^{cd}
JcM10	66.55^{a}	80.72^{a}	0.46^{e}	44.41^{abc}	15.23^{a-e}	14.37^{e}	74.01^{c-f}	7.47^{a}	4,319^{e}
Ji 1	57.30^{bc}	78.21^{bcd}	0.70^{a}	44.61^{ab}	15.84^{a}	17.37^{ab}	77.82^{ab}	4.32^{cde}	4,488^{bcd}
Ji 2	53.33^{d}	76.04^{d}	0.70^{a}	45.99^{a}	15.38^{abc}	18.00^{a}	79.37^{a}	3.39^{f}	4,625^{a}
F test	**	**	**	*	**	**	**	**	**
Genotype x Year									
F test	ns	*	ns	ns	**	**	**	ns	*
Mean	57.15	78.11	0.59	43.30	14.76	16.00	73.98	4.38	4,487

Means in same column superscripted with different lowercase letters are significantly ($p < 0.05$) different. ns = non-significant ($p > 0.05$); * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

the reverse Ji 1 and Ji 2 > KUBJL 1 to KUBJL 14 > JcM10. Interspecific hybrids had lower cellulose, hemicellulose and lignocellulose contents in the wood samples than did *J. integerrima* but they were not significantly different from *J. curcas*. However, the following was the order for the lignin content and calorific value in wood Ji 2 > KUBJL 1 to KUBJL 14 > JcM10, while it was reversed for the ash content of the wood JcM10 > KUBJL 1 to KUBJL 14 > Ji 2. Furthermore, the effect of years × genotypes interaction was found in leaf moisture, hemicellulose, lignin and lignocellulose contents and in the heat value, but not in cellulose and ash contents in the wood samples from the Jatropha genotypes. These results showed that *J. integerrima* had better wood quality than *J. curcas* and the interspecific hybrids. However, the interspecific hybrids had lower values for wood moisture content and higher values for wood density than *J. curcas*. In addition, the wood of the interspecific hybrids contained more lignin and had a higher calorific value than for *J. curcas* but with less ash. These findings demonstrated that the quality and chemical composition of the good wood sourced from *J. integerrima* as the male parent (Ji 2) could be used to improve the wood properties of interspecific hybrids.

In the cultivation of Jatropha hybrids, the stems and branches must be pruned each year to harvest biomass and then allowing the rootstock to regrow in the following year (Arunyanark et al., 2022). Thus, concern has been raised that pruning might affect the growth and biomass yield of Jatropha hybrids. Another important characteristic of growing fast-growing trees for use in biomass plants with short harvesting cycles is their ability to regrow after pruning (Pleguezuelo et al., 2015). Although pruning reduced the cellulose, lignin and lignocellulose contents of the Jatropha hybrid wood samples, it did not affect the biomass yield, nor the quality and calorific value of the wood. These findings suggested that Jatropha hybrids could be pruned for multiple biomass harvests. However, the growth and biomass yield stability of Jatropha hybrids pruned for several years and grown in different environments should be investigated.

Pruning tolerance of Jatropha genotypes

Significant interaction between year and genotypes of jatropha was observed for leaf traits and wood chemical composition and thus implied that response to pruning was different among genotypes. Most Jatropha genotypes showed no difference in leaf size, leaf weight average, SLA, and

SCMR between the first and second years (Fig. 1). However, in the second year, the leaf size of KUBJL 1, KUBJL 3, KUBJL 6, KUBJL 13, and Ji 1 increased (Fig. 1A) while the leaf weight average increased in KUBJL 3, KUBJL 6, KUBJL 13, JcM10, and Ji (Fig. 1B). Furthermore, in the second year, SLAs of KUBJL 11, KUBJL 13, and JcM10 were lower than the first year (Fig. 1C) whereas higher SCMR was observed only in KUBJL 4 and Ji 1 (Fig. 1D). The results show that after pruning, these Jatropha genotypes had improved leaf traits.

Almost all Jatropha genotypes have hemi-cellulose, lignin, and lignocellulose content in the wood that decreased or did not differ between the first and second years (Fig. 2). However, hemicellulose content increased in KUBJL 2, KUBJL 3, KUBJL 8, KUBJL 10, and Ji 2 in the second year (Fig. 2A). In the second year, Ji 1 had an increase in lignin, whereas KUBJL 5, KUBJL 11, KUBJL 12, KUBJL 14, JcM10, and Ji 2 had no difference in lignin between the first and second years (Fig. 2B). Furthermore, lignocellulose content increased in KUBJL 11 in the second year, whereas lignin content in KUBJL 5, KUBJL 8, Ji 1, and Ji 2 did not differ between the first and second years (Fig. 2C). In terms of wood calorific value, almost all Jatropha genotypes increased in the second year (Fig. 2D) with Ji 1, KUBJL 1, Ji 2, and KUBJL 2 had the greatest increase in calorific value, 7.82%, 4.91%, 4.82%, and 4.81% increased, respectively. The results showed that pruning had no effect on the wood chemical composition of these Jatropha genotypes. Moreover, pruning increases the calorific value of the wood of these Jatropha genotypes.

When the total fresh weight and total dry weight of Jatropha genotypes were changed due to pruning, JcM10 had the highest pruning tolerance index (PTI) of fresh weight and dry weight, 118.02% and 109.39%, respectively (Fig. 3A and 3B). KUBJL 5, KUBJL 11, KUBJL 5, KUBJL 4, and KUBJL 2 were the interspecific hybrids with the highest PTI, with fresh weight PTIs of 106.25%, 104.07%, 98.27%, and 94.57%, respectively (Fig. 3A), and dry weight PTIs of 107.10%, 96.35%, 92.46%, and 96.05%, respectively (Fig. 3B). These Jatropha genotypes exhibited high pruning tolerance, with no or very slight reduction in biomass yield due to pruning.

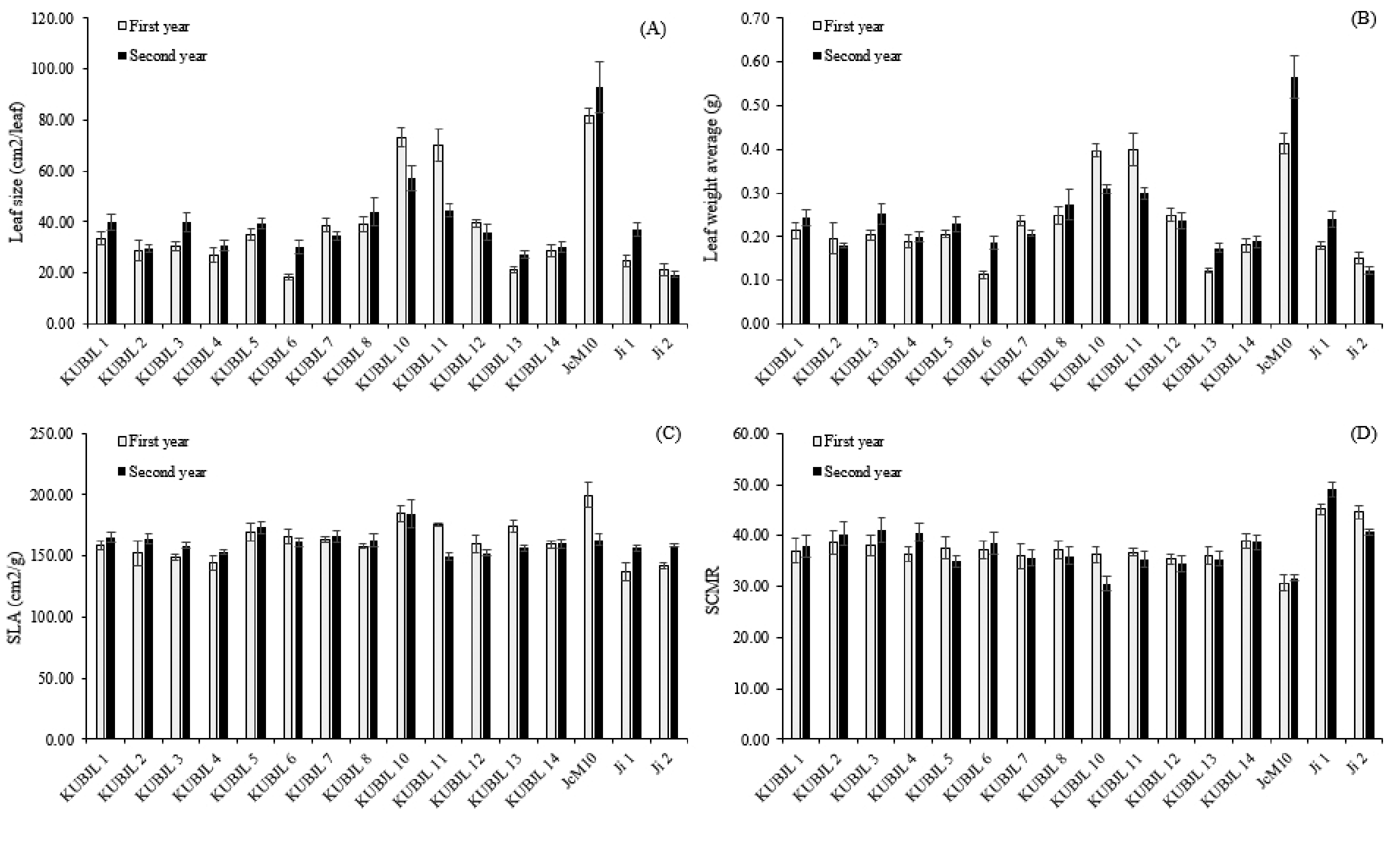

Fig. 1 Comparison between the first and second year of: (A) leaf size; (B) leaf weight average; (C) specific leaf area (SLA); (D) SPAD chlorophyll meter reading (SCMR) of interspecific hybrids between *Jatropha curcas* and *J. integerrima*, where error bars represent ± SE

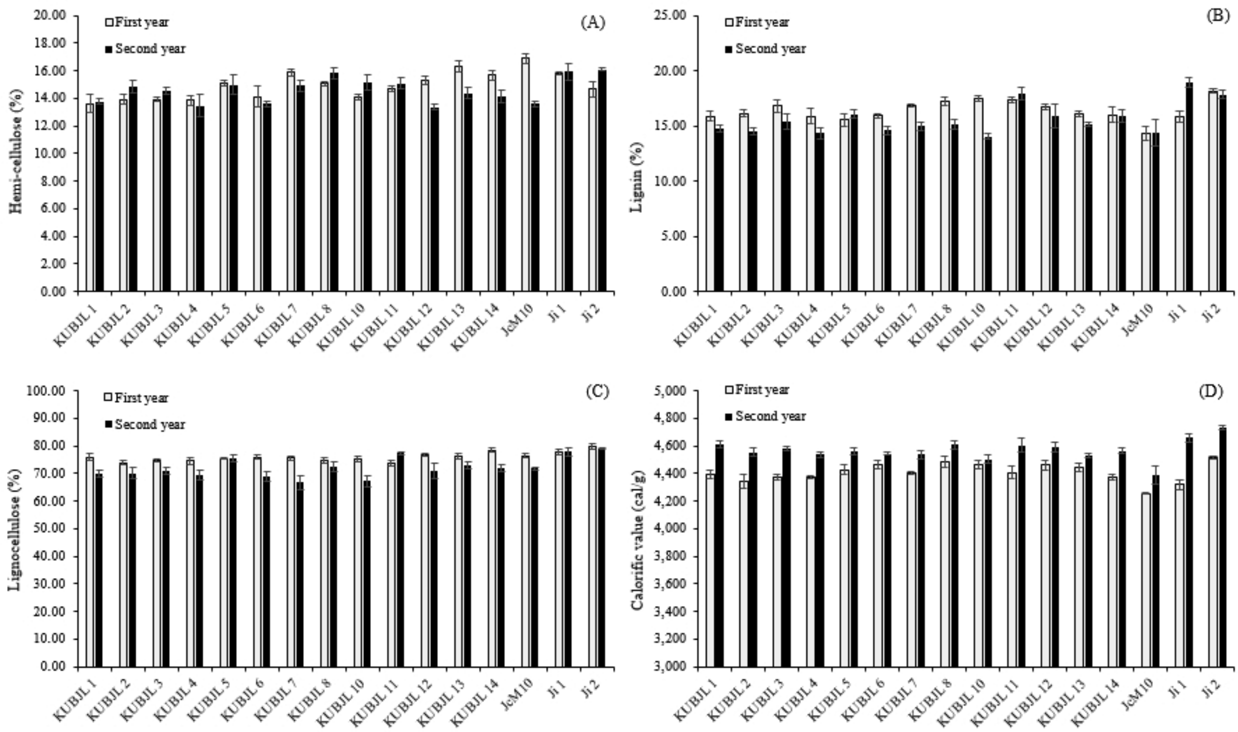

Fig. 2 Comparison between the first and second year of: (A) hemi-cellulose; (B) lignin; (C) lignocellulose; (D) calorific value of interspecific hybrids between *Jatropha curcas* and *J. integerrima*, where error bars represent ± SE

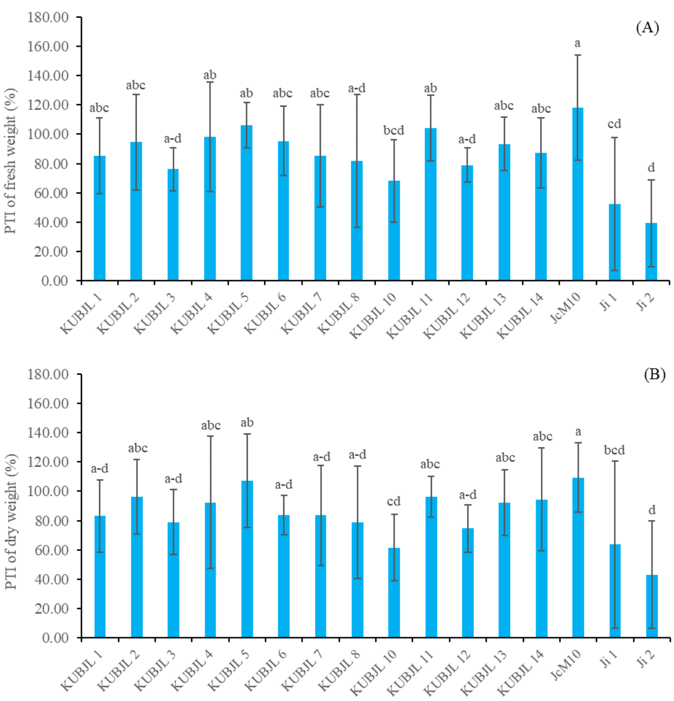

Fig. 3 Pruning tolerance index (PTI) of: (A) fresh weight; (B) dry weight of interspecific hybrids between *Jatropha curcas* and *J. integerrima*, where error bars represent ± SD and different lowercase letters denote significant ($p < 0.05$) differences among Jatropha genotypes

Relationship between leaf traits and biomass yield of Jatropha genotypes

The current results showed that there was a significant, negative correlation between SCMR and leaf size (r = -0.80**), average leaf weight (r = -0.74**) and SLA (r = -0.83**), as shown in Fig.4A – 4C, respectively, indicating that SCMR could be used to estimate the leaf size, average leaf weight and SLA of the Jatropha genotypes. In particular, SCMR and SLA demonstrated a strong correlation, with SLA being the inverse of leaf thickness. Plants with lower SLA values had thicker leaves. The SLA and chlorophyll contents in the leaves of many plant species have been reported to be negatively correlated (Marenco et al., 2009). Nyi et al., 2012) reported a relationship between the SCMR and chlorophyll content, as well as a relationship between the SCMR and photosynthetic efficiency in leaves of *J. curcas*. SCMR could also be used to assess the genotype variability of chlorophyll content in *J. curcas* (Senger et al., 2014). According to recent research, the SCMR could be used to assess the SLA of Jatropha genotypes. Hybrids with higher SCMR values had lower SLA values. Thus, SCMR and SLA are traits that could be used to evaluate the chlorophyll content and photosynthetic efficiency of Jatropha genotypes.

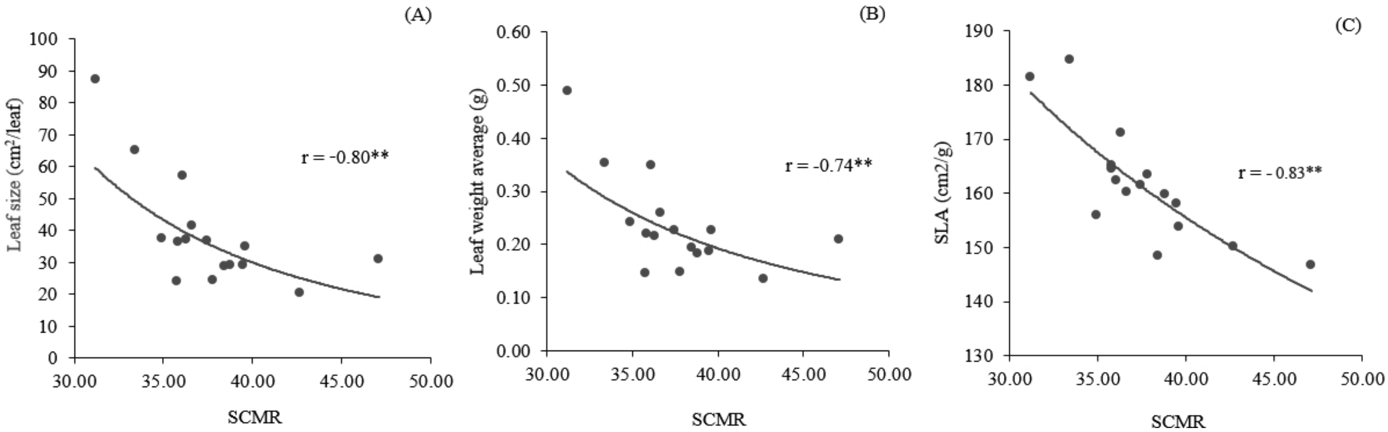

Fig. 4 Relationship between SPAD chlorophyll meter reading (SCMR) and: (A) leaf size; (B) average leaf weight; (C); specific leaf area (SLA), where average is over 2 yr, r = Pearson's correlation coefficient (n = 16) and ** = highly significant ($p < 0.01$)

The two genotypes of *J. integerrima* were not included in the calculation of the correlation between leaf trait and biomass yield because *J. integerrima* has small, thick, dark-green leaves but a very low biomass yield that could influence the correlation between leaf traits and the biomass yield of the Jatropha genotypes. The results showed that leaf size was significantly negatively correlated with the wood fresh weight, leaf fresh weight and total fresh weight, with correlation coefficients (r) ranging from -0.57* to -0.71** (Table 3). The leaf size was also significantly negatively correlated with wood dry weight, leaf dry weight and total dry weight, with correlation coefficients ranging from -0.60* to -0.75**. In addition, the average leaf weight was significantly negatively correlated with the wood fresh weight, leaf fresh weight and total fresh weight, with correlation coefficients ranging from -0.58* to -0.70**. The average leaf weight was also significantly negatively correlated with wood dry weight, leaf dry weight and total dry weight, with correlation coefficients ranging from -0.62* to -0.74**. Furthermore, a strong positive correlation was found between the SCMR and wood fresh weight, leaf fresh weight and total fresh weight, with correlation coefficients ranging from 0.65* to 0.77**. The SCMR had a high positive correlation with wood dry weight, leaf dry weight and total dry weight, with correlation coefficients ranging from 0.64* to 0.79**. However, a low and intermittently negative correlation was observed between SLA and biomass yield, with correlation coefficients ranging from 0.00 to 0.61*. According to these correlations, leaf traits, such as leaf size, average leaf weight and SCMR could be used as indirect characters to select Jatropha genotypes with high biomass yields. The findings revealed that Jatropha genotypes with small leaf sizes and high leaf chlorophyll contents were associated with high biomass yields due to the possibility that the small leaf size would reduce light obstruction between the leaves within the plant canopy, thereby improving light distribution

Table 3 Pearson's correlation coefficients (r; n = 14) between biomass yield and leaf size, leaf weight average, specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) of interspecific hybrids aged 2 yr

Traits	Year	Fresh weight			Dry weight		
		Wood	Leaf	Total	Wood	Leaf	Total
Leaf size	First	-0.70**	-0.68**	-0.70**	-0.75**	-0.65*	-0.74**
	Second	-0.57*	-0.62*	-0.58*	-0.60*	-0.62*	-0.61*
	Average	-0.69**	-0.71**	-0.70**	-0.73**	-0.68**	-0.73**
Average leaf weight	First	-0.68**	-0.66**	-0.68**	-0.73**	-0.64*	-0.73**
	Second	-0.58*	-0.63*	-0.60*	-0.62*	-0.62*	-0.62*
	Average	-0.70**	-0.70**	-0.70**	-0.74**	-0.67**	-0.74**
SLA	First	-0.57*	-0.55*	-0.57*	-0.61*	-0.49	-0.60*
	Second	0.00	-0.08	-0.02	-0.01	-0.09	-0.02
	Average	-0.36	-0.45	-0.38	-0.39	-0.43	-0.39
SCMR	First	0.74**	0.72**	0.74**	0.75**	0.66**	0.75**
	Second	0.68**	0.65*	0.68**	0.68**	0.64*	0.68**
	Average	0.77**	0.73**	0.77**	0.79**	0.68**	0.78**

ns = non-significant ($p > 0.05$); * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

within the canopy (Valencia et al., 2016). Furthermore, the high chlorophyll content of Jatropha could improve photosynthesis efficiency (Nyi et al., 2012), enabling plants to accumulate more biomass. Therefore, leaf traits, such as leaf size, average leaf weight and SCMR could be used for indirect characterization to select Jatropha genotypes with high biomass yields.

Relationship between wood quality, chemical composition and calorific value

The calorific value of Jatropha hybrid wood was highly negatively correlated with its moisture content, with correlation coefficients ranging from -0.73** to -0.77** (Table 4). The calorific value was moderately negatively correlated with leaf moisture, with correlation coefficients ranging from -0.33 to -0.69**. However, the calorific value was positively correlated with wood density, with correlation coefficients ranging from 0.25 to 0.75**. In addition, there was a strong positive correlation between calorific value and lignin content, with correlation coefficients ranging from 0.71** to 0.74**. There was also a strong negative correlation between calorific value and ash content, with correlation coefficients ranging from -0.73** to -0.82**. However, the correlations between calorific value and cellulose (r = -0.10 to 0.29), hemicellulose (r = -0.29 to 0.58*) and lignocellulose (r = 0.15 to 0.59*) contents were low and intermittent. These results revealed that the high calorific value of the wood of Jatropha genotypes was due to its low moisture content and high density. The moisture and density of the wood were quality traits of the wood. Wood with a low moisture content and a high density would burn with a high heat energy (Al-Sagheer and Prasad, 2010). In addition, the high calorific value of the Jatropha genotypes wood was due to its high lignin content but low ash content. The calorific value of raw materials is critical in the production of energy (Domingos et al., 2020). The chemical composition of wood, specifically the lignin and ash contents, was found to be strongly associated with the heat energy of wood (Börcsök and Pásztory, 2021). As a result, these wood traits could be used to indicate wood quality and in the selection of Jatropha genotypes for high calorific value in wood.

The current study found a negative correlation between the wood moisture content and wood density (r = -0.72**), as shown in Fig. 5. This result demonstrated that the two traits were inversely related, with high moisture content wood having a low density. Thus, one of the traits might be chosen for Jatropha genotypes selection. Both the moisture content of the wood and the density of the dry wood could be measured easily and correlated with the calorific value of the wood. Therefore, both traits could be useful for selecting Jatropha genotypes, where there are large sample sizes to be measured. Furthermore, the current study discovered a correlation between the leaf moisture content and the wood moisture content (r = 0.72**), as shown in Fig. 6A and wood density (r = -0.53*, as shown in Fig. 6B, indicating that the leaf moisture content could be used to assess the moisture content and density of Jatropha hybrid wood. Although wood moisture content and density are important quality traits, measuring them is time-consuming, expensive, destructive and inconvenient for a large number of samples. These constraints preclude its use in plant breeding programs involving large numbers of samples (Arunyanark et al., 2008, 2009). The assessment of wood moisture content and density

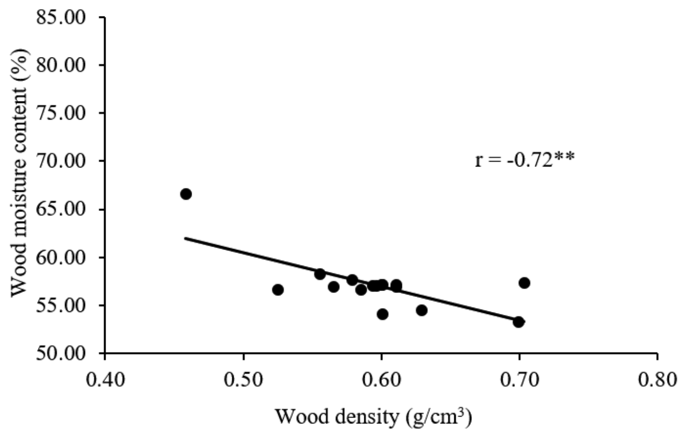

Fig. 5 Relationship between wood density and wood moisture content (average at age 2 yr), where r = Pearson's correlation coefficient (n = 16) and ** = highly significant ($p < 0.01$)

Table 4 Pearson's correlation coefficients (r, n = 16) between calorific value and wood moisture content, leaf moisture content, wood density and wood chemical composition of interspecific hybrids aged 2 yr

Calorific value	Wood moisture content	Leaf moisture content	Wood density	Cellulose
First year	-0.73**	-0.51*	0.25	-0.10
Second year	-0.76**	-0.33	0.75**	0.29
Average	-0.77**	-0.69**	0.63**	0.15
Calorific value	Hemi-cellulose	Lignin	Lignocellulose	Ash
First year	-0.29	0.74**	0.15	-0.74**
Second year	0.58*	0.71**	0.59*	-0.73**
Average	0.08	0.73**	0.43	-0.82**

ns = non-significant ($p > 0.05$); * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

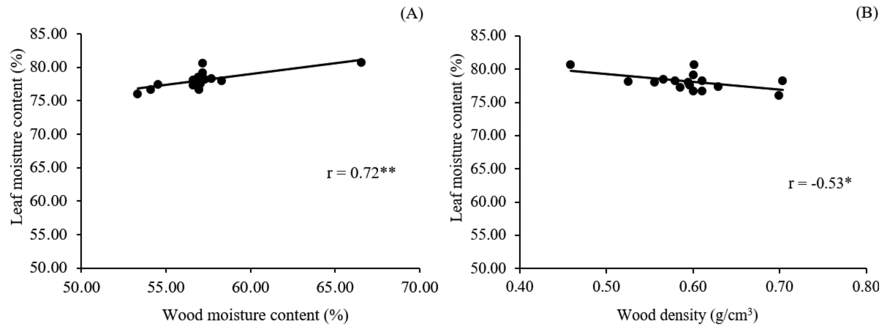

Fig. 6 Relationship between leaf moisture content and: (A) wood moisture content; (B) wood density (average for 2 yr), where r = Pearson's correlation coefficient (n = 16), * = significant ($p < 0.05$) and ** = highly significant ($p < 0.01$)

requires an indirect trait that is easy and quick to measure, economical and effective. Leaf sampling is less damaging to plants than wood sampling. Leaf moisture measurement is less disruptive to plants than either wood moisture or wood density measurements; it was also easier and faster. The moisture content of the leaves was related to the calorific value of the wood. Therefore, the leaf moisture content was identified as a trait that could be effectively used in large plant breeding projects.

In summary, after pruning in the first year, the wood of the Jatropha genotype in the second year had lower cellulose, lignin and lignocellulose contents. However, the hemicellulose, ash, moisture, density, and calorific value of the wood were not reduced in the second year. Furthermore, there was no reduction in biomass yield and leaf traits, such as leaf size, average leaf weight, SLA and SCMR. Based on these findings, Jatropha genotypes could be pruned for multiple biomass harvests. Leaf traits, biomass yield and wood quality of a Jatropha genotype showed significant genotype variation. Interspecific hybrids KUBJL 1, KUBJL 3, KUBJL 4, KUBJL 6, KUBJL 11, and KUBJL 13 showed improved leaf traits after pruning. Pruning had no effect on the chemical composition of KUBJL 2, KUBJL 3, KUBJL 8, KUBJL 10, KUBJL 5, KUBJL 11, KUBJL 12, and KUBJL 14. Furthermore, the interspecific hybrids with the highest pruning tolerance were KUBJL 5, KUBJL 11, KUBJL 5, KUBJL 4, and KUBJL 2. The relationship between the SCMR and leaf size, average leaf weight and SLA demonstrated that the SCMR could be used to evaluate leaf size and leaf thickness in Jatropha genotypes. Relationships were identified between biomass yield and leaf size, average leaf weight and the SCMR, indicating that these leaf traits could be used as indirect traits to select Jatropha genotypes with high biomass yield. Furthermore, the calorific value in wood was related to the wood density and to the leaf moisture, wood moisture, lignin and ash contents in wood. As a result, these traits could be used to indicate the wood quality of Jatropha genotypes and in the selection of Jatropha hybrids with high calorific value.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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