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Identification and characterization of tetraploid and octoploid *Jatropha curcas* induced by colchicine

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ABSTRACT

Jatropha curcas is considered to be one of the greatest potential biofuel plants. However, the application of J. curcas is limited by a lack of high yielding varieties. Polyploids often generate useful germplasm for genetic improvement in many plants. Here, tetraploid and octoploid J. curcas were obtained by treating the shoot tips of seedlings with colchicine. The ploidy of J. curcas was determined by measuring the nuclear DNA content with flow cytometry and by counting chromosomes. Treatment with 0.1% colchicine for seven days was the most effective condition for producing polyploid plants, yielding 15% tetraploids. There was no significant plant height difference between tetraploid and diploid plants, but octoploid plants grew slowly and were about one-third the height of diploid plants. With increased ploidy, the stomata and pollen grains became larger, stomatal density decreased, the leaves became thicker, and the net photosynthetic rate and stomatal conductance decreased. Compared with diploid plants, the tetraploid plants had larger leaves, male and female flowers and pollen, but the octoploid leaves were about half the size of the diploid leaves. The fruits and seeds of the tetraploid plants were larger than those of the diploid, but the single seed weight and oil content were decreased. The octoploid plants were sterile, with no flower buds on three-year-old plants. The polyploid plants obtained in this study could be useful for breeding programs and for studies of flower development and abiotic stress tolerance in *J. curcas*.

Introduction

The huge potential of plant biomass has generated a significant interest towards the sustainable production of renewable energy in recent years (Benning and Pichersky 2008; Tilman et al. 2009; Somerville et al. 2010; Laimer et al. 2015). The most appropriate choice of bioenergy feedstock often depends on technical, economical, and socio-environmental aspects of the plants' cultivation, but perennial grasses and trees are the most sustainable future sources of biofuels (Ohlrogge et al. 2009; Papini and Simeone 2010; Alves et al. 2015). Bioethanol and biodiesel are the two major renewable biofuels in current use, and biodiesel has several advantages over bioethanol as a liquid fuel (Durrett et al. 2008; Ohlrogge, et al. 2009).

Jatropha curcas is a perennial woody oil plant belonging to the family Euphorbiaceae, and is considered one of the most promising biodiesel feedstocks because of the high oil content (30–40%) in its seeds (Achten et al. 2008; Meher et al. 2013). *J. curcas* can grow on arid and barren hills where food crops do not grow well (Kandpal and Madan 1995; Fairless 2007; Makkar and Becker 2009; Zuleta et al. 2012). However, *J. curcas* has not been commercialized because its yield is too poor (Sanderson 2009; Divakara et al. 2010). Thus, breeding of high yielding J. curcas varieties is considered the highest priority for study at the present (Pan et al. 2010; Pan and Xu 2011; Edrisi et al. 2015; Kumar et al. 2015). Current research results show that the genetic diversity of J. curcas is too low for cross-breeding, so it is necessary to create new germplasms, such as mutants and polyploids (Ram et al. 2008; Sun et al. 2008). Remarkable achievements have been made in mutation breeding of J. curcas (Dhakshanamoorthy et al. 2011; Mukherjee et al. 2011), e.g. Dwimahyani and Ishak (2004) selected early maturing, greater branching and large-seeded J. curcas plants by mutation breeding. Only one study of polyploidy breeding in J. curcas has been conducted: tetraploid J. curcas seedlings were produced through tissue culture (de Oliveira et al. 2013), but the method requires complex equipment and sophisticated techniques, and takes a long time (more than 150 d) to get tetraploid seedlings. Thus, it is necessary to develop a simple method for polyploidy induction of J. curcas (Divakara, et al. 2010; de Oliveira et al. 2013).

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Biofuel; colchicine; flow cytometry; flower; physic nut; polyploidy

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Polyploidy breeding is an effective breeding method, with a short duration and easy operation compared with traditional cross-breeding and mutation breeding, and can increase breeding germplasm resources (Kashkush et al. 2002; Thao et al. 2003; Madon et al. 2005). Polyploidy breeding has 70 years of history; it has been applied to create improved varieties of many plants (Blakeslee and Avery 1937), such as watermelon, kiwi, banana, and oil palm (Compton and Gray 1993; Van Duren et al. 1996; Madon et al. 2005). The somatic cells of polyploid plants are larger than those of diploid plants, and the size of vegetative and reproductive organs increases correspondingly. Polyploid plants have been induced from seeds, apical meristems, apical buds, and tissue culture by treatment with colchicine or oryzalin (Shao et al. 2003; Wu et al. 2007; Omidbaigi et al. 2010; Rêgo et al. 2011).

The size of stomata increases with increased ploidy level, so the size of stomata can be used to test the ploidy of plants (Beck et al. 2003, Zlesak 2009). In addition, flow cytometry (FCM) analysis offers several advantages for determination of plant nuclear DNA content, and is a rapid and reliable method for ploidy identification (Dolezel et al. 1989; Carvalho et al. 2008).

In this study, polyploid plants of *J. curcas* were induced by treating the shoot tips of seedlings with colchicine. The polyploidy of *J. curcas* plants was determined by stomatal size and then flow cytometry analysis of the nuclear DNA content, and finally confirmed by counting the chromosomes of the next generation. The morphological and physiological characteristics of the tetraploid and octoploid *J. curcas* were characterized by comparing with those of diploid plants.

Materials and methods

Plant materials

Seeds of *J. curcas* were collected in September 2011 from five trees that were grown at Xishuangbanna Tropical Botanical Garden (XTBG, 21°54′N, 101°46′E, 580 m asl) of the Chinese Academy of Sciences located in Mengla County, Yunnan Province, southwest China.

Polyploid plant induction and identification

Mature seeds were planted in seeding bags (8 × 10 cm), with two seeds to each bag, and germinated in a growth chamber (25°C, 8 h light, illumination intensity 5–8 µmol $m^{-2} s^{-1}$). When the seedlings were at the two cotyledons and one true leaf stage, the apices of the seedlings were covered with absorbent cotton before treatment with colchicine. Various concentrations of colchicine solution (0.1%, 0.2%, 0.4%, 0.6% and 0.8% (w/v), formulated by 1% dimethyl sulfoxide) were applied to the absorbent cotton three times each day at 8:00, 16:00 and 23:00 for 1, 4, 7 or 10 d. Each group contained approximately 40 seedlings. The absorbent cotton was removed from the seedlings and they were grown in a greenhouse at 25°C for two weeks, and then planted in the field with a density of 2 m \times 2 m.

When the plant height reached 50 cm, the polyploid plants were identified by stomatal size and flow cytometry, and further confirmed by counting of chromosomes of the first filial generation of the tetraploid plants.

Plant height measurement and analysis of leaf characteristics

Five diploid and tetraploid plants and three octoploid plants, all one year old, were selected to measure plant height. Only three octoploid *J. curcas* plants were obtained. These trees were used for all biological trait analysis.

From each plant, five mature leaves were selected to measure the petiole length, and leaf length and width. Stomatal density and length were measured by nail-varnish impressions of abaxial surfaces of fresh mature leaves (Fernandez et al. 1998) and the epidermal imprints were observed using a microscope at 400× magnification. From each leaf, six horizons were taken to analyze stomatal density. From each horizon, six mature stomata were selected to measure size. Temporary mounts were made from leaves that were cross-sectioned and observed at 100× magnification to analyze leaf thickness. A 1.8 cm² hole punch was used to remove three 1.8 cm² leaf segments from the center and edge of the leaves, and these were put in an oven at 65°C for 48 h and dried to constant weight. The leaf segments were weighed with an analytical balance and the specific leaf weight was calculated (Mamrutha et al. 2010). The total chlorophyll, chlorophyll a (Chl a) and chlorophyll b (Chl b) contents were determined according to Arnon (1949).

Stomatal conductance and net photosynthetic rate determination

The stomatal conductance and net photosynthetic rate were measured between 09:00 and 11:00 am for the leaves of the diploid and tetraploid plants with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) under 1500 μ mol m⁻² s⁻¹ photosynthetic photon flux density supplied by a fluorescence leaf chamber LI-6400-40 LCF (Li-Cor) (Yong et al. 2010). CO₂ concentration, atmospheric pressure and relative humidity of the air in the reference chamber and leaf temperature was controlled by the equipment at 400 μ mol mol⁻¹, 95 kPa, 70% and 25°C, respectively. Before the measurement, each leaf was illuminated with the saturating light for about 10 min to achieve full photosynthetic induction. Two leaves were selected from each tree, and each leaf was measured 10 times.

Analysis of flower, fruit and seed characteristics

Thirty inflorescences were selected from diploid and tetraploid plants. The total number and number of female flowers/inflorescence, fruits/inflorescence, and seeds/fruit were counted. Female and male flower diameter, fruit diameter, and seed diameter and length were measured with a vernier caliper. Thirty blooming male flowers from diploid and tetraploid plants were selected, and fresh pollen was collected to make a temporary slide, which was observed through microscope at 400× magnification. One hundred pollen grains were selected for each sample to measure the pollen diameter. The weight per seed was determined with an analytical balance. The oil contents of the seeds were measured using a minispec mq-one Seed Analyzer (Bruker Optik, Ettlingen, Germany) (Pan and Xu 2011).

Flow cytometry (FCM) analysis

Nuclei were released from 1 cm² of young leaf tissue, in which the stomata were larger than those of the diploid plant, and then stained with propidium iodide. The leaf tissue was placed in a Petri dish with 750 µl of chopping buffer (44.8 mmol l⁻¹ MgCl₂, 46 mmol l⁻¹ sodium citrate, 20 mmol l⁻¹ (pH 7.2) 3-morpholinopropanesulfoinc acid, 0.1% Triton X-100, 50 µg ml⁻¹ RNaseA, 1% PVP) (Galbraith et al. 1983) and chopped with a sharp razor blade. Then, the suspension was transferred into a 1.5 ml Eppendorf tube after filtration with a 50 μ m disposable nylon filter. Propidium iodide solution was added into the Eppendorf tube to a final concentration of 50 µg ml⁻¹. The tube was kept on ice in the dark for 30 min. The samples were then put into an Accuri C6 flow cytometer (BD Biosciences Franklin lakes, New Jersey, USA; FL2 channel 585 ± 20 nm (PE/PI)), and 10,000 events were recorded per sample (Omidbaigi et al. 2010; de Oliveira et al. 2013).

Counting of chromosomes

The diploid and tetraploid plant seeds were germinated, and when the young roots were 3-5 cm, the root tips (2–3 mm) were removed, fixed in a methanol/acetic acid (3:1) solution, and kept at 4°C for at least 1 h. Then, the root tips were washed with distilled water three times to remove the fixative solution, and dissociated in 1 M hydrochloric acid at 60°C for 5 min. The root tips were washed again to remove the dissociation solution, and stained with carbol fuchsin for 10 min (Gu et al. 2005). The samples were transferred to glass slides, one drop of 45% acetic acid was added, and they were observed through a microscope at 1000× magnification. At least 20 cells were sampled and analyzed per root tip. 15 diploid and tetraploid plants were used, respectively, for chromosome counts. More than 50 root tips were analyzed.

Statistical analysis

The data (plant height, net photosynthetic rate, stomatal conductance and characteristics of leaf, flowers, fruits and seeds) were analyzed using the Statistical Product and Service Solution version 16.0 software (SPSS Inc., Chicago, USA). Differences between the groups were tested at significance levels of p < 0.05 and p < 0.01 using one-way ANOVA with Tukey's post hoc tests.

Results

Polyploid J. curcas induction and identification

The *J. curcas* seedlings were treated with different concentrations of colchicine solution for different times. The survival rates of the seedlings decreased with increasing colchicine concentration and the extension of treatment time; the highest mortality rate was 30% in the 0.4% colchicine treatments for 4 and 10 d (Table 1). At the same colchicine concentration, the efficiency of induction increased at first and then decreased as the treatment time increased. Our results showed that the highest induction rate was 15%, in the 0.1% colchicine treatment for 7 d, while the lowest induction rate was 2.5% at colchicine concentrations of 0.2%, 0.4% and 0.8% applied for 1, 10 and 7 d, respectively (Table 1).

Several studies have shown that with increased ploidy of plants, the stomatal size was greater, and stomatal density was lower than in the diploid plants (Srivastava and Srivastava 2002; Beaulieu et al. 2008; Ye et al. 2010). In this study, as shown in Figure 1A–C, we found that the stomatal lengths of the tetraploids and octoploids increased 0.68 and 1.47 times, respectively. The number of stomata per area unit of leaf decreased by 51.48% and 71.63% respectively, compared with the diploid plants.

The DNA content of the nuclei can be measured by flow cytometry through detection of the fluorescence emitted by stained nuclei, and the DNA content of the nuclei is positively correlated with the fluorescence value (Hedley et al. 1983). By comparison with the fluorescence of G1 nuclei of diploid J. curcas, the ploidy level of the colchicine-induced plants was determined. The results showed that there were two kinds of polyploid J. curcas plants, tetraploid and octoploid, which exhibited twofold and fourfold fluorescence intensity compared to that of diploid plants (Figure 1D-F). To further confirm the derived tetraploids and octoploids, the chromosomal number was analyzed. As shown in Figure. 1G and H, the root tip cells of the tetraploid seedlings contained 4n = 44 chromosomes (Figure 1H), which was double the 2n = 22 chromosomes (Figure 1G) of the diploid plants (Carvalho et al. 2008). The chromosomes in the root tip cells of the octoploid were too crowded to count with a microscope.

Table	 The effect of 	f colchicine	treatments on inc	luction of	[:] polyp	loidy in .	J. curcas
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Colchicine con- centration (%)	Treatment duration (days)	Survival rate (%) ^a	No. of tetraploids	No. of octoploids	No. of mixoploids	Polyploidization efficiency (%) ^b
0.1	1	100	0	0	0	0
	4	77.5	1	0	3	10
	7	80	4	0	2	15
	10	72.5	1	0	3	10
0.2	1	100	0	0	1	2.5
	4	77.5	1	0	1	5
	7	82.5	2	0	1	7.5
	10	75	1	1	0	5
0.4	1	95	1	1	1	7.5
	4	70	2	0	2	10
	7	82.5	1	0	1	5
	10	70	0	0	1	2.5
0.6	1	95	1	0	1	5
	4	75	1	0	1	5
	7	90	4	0	1	12.5
	10	77.5	2	0	0	5
0.8	1	92.5	3	0	1	10
	4	80	3	0	1	10
	7	77.5	1	0	0	2.5
	10	75	4	0	0	10

^aEach treatment contained 40 plants.

^bPolyploidization efficiency was calculated as the percentage of the total number of the tetraploids, octoploids and mixoploids out of the 40 treated plants.



Figure 1. Identification of polyploid *J. curcas.* (A–C) Stomata of (A) diploid, (B) tetraploid, and (C) octoploid leaves. Bars = 50 μ m. (D–F) Flow cytometry (FCM) analysis of (D) diploid, (E) tetraploid, and (F) octoploid nuclei. (G, H) Chromosomes of (G) the diploid and (H) the tetraploid. Bars = 5 μ m.

Morphological differences among diploids, tetraploids and octoploids

The diploid, tetraploid and octoploid plants were transplanted into the field. The heights of the diploid and tetraploid plants showed no significant difference, but the octoploid plants were shorter, reaching just 1/3 the height of the diploid plants (Figure 2A–C, Table 2). As the ploidy of *J. curcas* increased, the petiole length of the plants significantly decreased; the petioles of the tetraploid and octoploid plants were 8.3% and 55.6% shorter than those of the diploid plants. However, the leaf length and width of the tetraploid plants were significantly greater than those of diploid plants, with increases of 8.36% and 21.36% respectively (Table 2). The



Figure 2. Plants and leaves of diploid and tetraploid *J. curcas.* (A) Diploid, (B) tetraploid, and (C) octoploid plants. Bars = 10 cm. (D) From left to right, diploid, tetraploid, and octoploid leaf. Bars = 5 cm. (E–G) Leaf cross section of (E) diploid, (F) tetraploid, and (G) octoploid. Bars = $200 \,\mu$ m.

Table 2. Plant height and leaf characteristics in diploid (2x), tetraploid (4x), and octoploid (8x) J. curcas. Values are mean ± standard deviation.

Characteristic	Diploid	Tetraploid	Octoploid
Plant height (cm)	123.86 ± 7.73	124.43 ± 6.59	43.00 ± 5.77^{b}
Petiole length (cm)	23.54 ± 0.58	$21.58 \pm 0.52^{\circ}$	10.45 ± 0.85^{b}
Leaf length (cm)	14.84 ± 0.43	16.08 ± 0.29^{a}	8.27 ± 0.48^{b}
Leaf width (cm)	16.34 ± 0.58	19.83 ± 0.44^{a}	7.55 ± 0.47^{b}
Leaf thickness (µm)	259.44 ± 2.91	332.42 ± 5.97^{b}	491.47 ± 11.48 ^b
Stomata length (µm)	25.76 ± 0.24	43.19 ± 0.31^{b}	63.56 ± 0.86^{b}
Stomata number (mm ⁻²)	110.50 ± 2.27	53.61 ± 1.23 ^b	31.35 ± 1.39 ^b
Specific leaf weight (mg/cm ⁻²)	6.31 ± 0.352	5.95 ± 0.19	8.07 ± 0.35^{b}
Content of chlorophyll (mg cm ⁻²)	3.32 ± 0.22	3.15 ± 0.18	3.10 ± 0.24

 $^{\rm a}$ Statistically different from the diploid at 5% level. $^{\rm b}$ Statistically different from the diploid at 1% level.

Table 3. Net photosynthetic rate and stomatal conductance in diploid (2x), tetraploid (4x), and octoploid (8x) J. curcas. Values	are
mean \pm standard deviation.	

Characteristic	Diploid	Tetraploid	Octoploid
Net photosynthetic rate(μ mol CO ₂ m ⁻² s ⁻¹)	21.74 ± 0.41	20.77 ± 0.33	$10.72 \pm 0.35^{\circ}$
Stomatal conductance (μ mol H ₂ O m ⁻² s ⁻¹)	0.62 ± 0.01	0.47 ± 0.01^{a}	$0.20\pm0.00^{\rm a}$

^aStatistically different from the diploid at 1% level.

leaf length and width of the octoploid plants were significantly lower than in the diploid plants, with decreases of 44.27% and 53.79% respectively (Figure 2D, Table 2). There was a significant difference in leaf thickness

among different polyploidy levels (Figure 2E–G); the tetraploid and octoploid leaves were 0.28 and 0.89 times thicker than the diploid leaves respectively (Table 2). The specific leaf weight of the tetraploid



Figure 3. Flower, pollen, fruit and seed of diploid and tetraploid *J. curcas.* (A) Female (left panel) and male (right panel) flowers of the tetraploid (upper panel) and diploid (lower panel). Bar = 1 cm. (B, C) Pollen of (B) the diploid and (C) the tetraploid. Bars = 25 μ m. (D) Fruit of the diploid (left) and tetraploid (right). Bar = 1 cm. (E, F) Fruit shell and seeds of (E) the diploid and (F) the tetraploid. Bar = 1 cm.

was not significantly different to the diploid, but the octoploid specific leaf weight was increased 0.28 times. The total chlorophyll contents per unit area unit of leaf

were not significantly different among the diploid, tetraploid and octoploid plants (Table 2).

Variability in net photosynthetic rate and stomatal conductance of the polyploid plants

As shown in Table 2, the height of octoploid plants was less than that of diploid plants. Plant growth is usually correlated with the photosynthetic rate (Cai et al. 2009). We supposed that photosynthetic rate of the octoploid plants would be lower than that of diploid plants. Thus, the net photosynthetic rates of diploid, tetraploid and octoploid plants were examined. The results showed that the net photosynthetic rate of the octoploid was indeed significantly lower than the diploid, as it decreased by 50.69%; the net photosynthetic rate of the tetraploids showed no significant difference to the diploid plants (Table 3). In addition, the stomatal conductance of the tetraploid and octoploid *J. curcas* plants was significantly lower than the diploid plants; it decreased by 24.19% and 67.74%, respectively (Table 3).

Variability in flower, fruit and seed characteristics

Compared with the diploid plants, the female and male flower diameter, fruit diameter, seed diameter and length, and pollen diameter were significantly larger in the tetraploids, by 28.12%, 32.62%, 15.90%, 2.46%, 6.33% and 29.00%, respectively (Figure 3, Table 4). The total numbers of flowers per inflorescence, fruits per infructescence, and seeds per fruit were significantly decreased 19.50%, 81.92% and 47.74% respectively compared with the diploid. Unexpectedly we found that the single seed weight and oil content were significantly decreased by 6.99% and 7.58% respectively, compared with diploid plants (Table 4).

Discussion

In this study, we demonstrated that polyploid *J. curcas* plants could be obtained by treating seedlings with colchicine. Concentration of colchicine and treatment duration are two key parameters for successful polyploidization by colchicine treatment (Moore and Janick 1983). As shown in Table 1, at the same concentration of colchicine, the frequency of polyploid induction rose at first, and then fell with increasing treatment duration. This result was consistent with previous studies in *Platanus acerifolia* and *Ocimum basilicum* (Liu et al. 2007; Omidbaigi et al. 2010).

The plant height and flowering time of tetraploid and diploid plants grown in the field was not significantly different. However, the octoploid *J. curcas* plants were shorter, and only 1/3 the height of diploid plants. One of the reasons for the slow growth of the octoploid *J. curcas* may be that the stomatal conductance and net photosynthetic rate of the octoploid was significantly

Table 4. Flower, fruit and seed characteristics in diploid (2x) and tetraploid (4x) J. curcas. Values are mean ± standard deviation.

Diploid	Tetraploid
7.02 ± 0.07	9.31 ± 0.10^{b}
8.71 ± 0.14	11.16 ± 0.22^{b}
237.63 ± 12.37	191.28 ± 12.77^{a}
11.93 ± 1.97	8.11 ± 1.09
85.29 ± 0.54	110.43 ± 0.85^{b}
11.23 ± 0.6	2.03 ± 0.26^{b}
29.84 ± 0.3	34.58 ± 0.45^{b}
2.87 ± 0.06	1.50 ± 0.09^{b}
18.97 ± 0.08	19.44 ± 0.13^{b}
11.28 ± 0.06	12.00 ± 0.15^{b}
0.84 ± 0.01	0.78 ± 0.02^{a}
37.28 ± 0.36	$34.45\pm0.30^{\rm b}$
	$\begin{array}{r} \mbox{Diploid} \\ \hline 7.02 \pm 0.07 \\ 8.71 \pm 0.14 \\ 237.63 \pm 12.37 \\ 11.93 \pm 1.97 \\ 85.29 \pm 0.54 \\ 11.23 \pm 0.6 \\ 29.84 \pm 0.3 \\ 2.87 \pm 0.06 \\ 18.97 \pm 0.08 \\ 11.28 \pm 0.06 \\ 0.84 \pm 0.01 \\ 37.28 \pm 0.36 \end{array}$

^aStatistically different from the diploid at 5% level.

^bStatistically different from the diploid at 1% level.

lower than in diploid plants (Table 3). Unexpectedly, the octoploid plants were sterile, with no flower buds on three-year-old plants, whereas diploid plants flowered within one year. This is inconsistent with polyploid sunflower; despite tetraploid and octoploid sunflower plants growing more slowly than the diploid, they could flower normally (Srivastava and Srivastava 2002).

With increased ploidy of J. curcas, the size of the stomatal apparatus and leaf thickness was greater, and stomatal density was lower than in the diploid plants; these phenomena are consistent with polyploid crape myrtle, sunflower and other species (Srivastava and Srivastava 2002; Beaulieu et al. 2008; Ye et al. 2010). Similar to alfalfa (Setter et al. 1978), the specific leaf weight of the tetraploid was not significantly different to the diploid (Table 2). However, the leaf thickness was increased 0.89 times compared with the diploid, leading to an increase of specific leaf weight in the octoploid. The total chlorophyll per area unit of leaf showed no significant differences among the diploid, tetraploid and octoploid plants. These results were opposite to black wattle (Mathura et al. 2006), and similar to soybean (Sen and Vidyabhusan 1960; Mathura et al. 2006).

Cell size is often correlated with ploidy (Malladi and Hirst 2010), and organ size can be correlated with the degree of polyploidy and cell size (Cheniclet et al. 2005). Our results showed that the sizes of leaf, flower, pollen, fruit and seed of the tetraploid J. curcas were significantly increased compared with diploid plants (Figures 2 and 3, Tables 2 and 4). Similar results were also reported in crape myrtle, basil, muskmelon and Actinidia chinensis (Omidbaigi et al. 2010; Wu et al. 2012; Q. Zhang et al. 2010; W. Zhang et al. 2010). Unlike Dendranthema nankingense and Dracocephalum kotschyi (Liu et al. 2011; Zahedi et al. 2014), the total number of flowers per inflorescence, fruits per infructescence and seeds per fruit of the tetraploid J. curcas were all significantly decreased compared with the diploid (Table 4). This may have been because the pollen fertility of the tetraploid was low (He et al. 2010, 2011), or because the tetraploid plants did not produce enough photosynthetic product (Rekika

et al. 1998). Energy deficiency or unbalanced distribution may also lead to low weight and oil content in the tetraploid *J. curcas* seeds (Table 4).

The octoploid *J. curcas* showed unexpected phenotypes. Compared to the diploid *J. curcas* plants, the octoploid plants have smaller leaves, but with bigger cells. In addition, after growing for three years in the field, the octoploid *J. curcas* did not produce flower buds. These plants may be a good material for further study of the mechanisms of regulation of cell and organ size, and flowering in polyploid plants. The polyploid plants obtained in this study could also be useful for breeding programs and for studies of abiotic stress tolerance (Liu et al. 2011; Chao et al. 2013) in *J. curcas*.

Conclusion

In this study, we obtained tetraploid and octoploid *J. curcas* by treating the shoot tips of seedlings with colchicine. Compared with diploid *J. curcas* plants, the sizes of stomata, leaves, male and female flowers, pollen, fruits and seeds of tetraploid plants became larger, but stomatal density, the stomatal conductance, single seed weight and oil content decreased. The octoploid plants were sterile, with no flower buds on three-year-old plants. Compared with those of diploid plants, the stomata became larger, and leaves became thicker in octoploid plants, but the plant height, leaf size, stomatal density, the net photosynthetic rate, and stomatal conductance decreased.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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