Occurrence of Tetraploidy in Colchicine-Treated Physic Nut (*Jatropha curcas* Linn.)

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ABSTRACT

A study on the effect of colchicine on the physic nut (*Jatropha curcas* Linn.) was carried out at a research site in Pathum Thani province. Colchicine was used to treat plants of physic nuts of clones FF25B-14 and FF20SBr-3 at 500–8,000 ppm for 10 d. The results showed that colchicine concentrations at 5,000–6,000 ppm affected the size of vegetative and reproductive parts such as the leaves, inflorescence and staminate flowers compared with normal plants. The treated plants showed larger stomatal size than nontreated plants. Study of the chromosome number revealed that the treated plants had doubled chromosomes (44) compared to the normal plants. These plants were assumed to be 4n or tetraploids while the normal plants were 2n or diploids (2n = 22). Both the vegetative growth and yield of the tetraploid plants were lower than in normal plants.
Keywords: colchicine, tetraploid, physic nut, growth, yield

INTRODUCTION

Physic nut (*Jatropha curcas* Linn.) is well known as an energy plant. The oil can be used for low speed engines without adding any substances and is suitable for agricultural engines such as in plowing machinery, water pumps and farm trucks (Sidhinew, 2002). Although the physic nut can be grown under various climatic and edaphic conditions and can resist drought, it has a poor yield of 100–300 $kg.rai^{-1}.yr^{-1}$ (Pasbhut and Sutiponpiboon, 2001) which equates to 625–1,875 $kg.ha^{-1}.yr^{-1}$. Therefore, study on increasing the yield is necessary to solve this problem. In the current research, the aim was to increase the yield of physic nut by using colchicine to develop a new variety that would have double the number of chromosomes and affect production by producing a bigger seed. Many researchers have reported that different concentrations of colchicine increase the chromosome number in many plants causing bigger flowers and bigger seeds. Henderson (1977) reported for watermelon that double the chromosome (4n) number caused thicker seed coats and bigger seeds than in normal plants (2n). Sinchai (1978) applied one drop of a colchicine concentration at 0.2% on the mature cotyledon of watermelon in the morning and one drop in the evening over 10 d and reported an increase in the number of tetraploid (4n) plants. Adaniya and Shirai (2001) used a colchicine concentration at 0.2% on the shoot tip of ginger and found the highest percentage of tetraploid plants on day 8.

The objective of the current experiment was to study the effect of colchicine on physic nut plants, the occurrence of tetraploid plants and

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to study the characteristics and yield of the new plants compared with the normal variety.

MATERIAL AND METHODS

Materials

Two physic nut clones with high yields (FF25B-14 and FF20SBr-3) were provided by Assistant Professor Sanan Khamlert, a former lecturer in the Horticulture Department, Kasetsart University, Bangkok, Thailand. Colchicine (97 % analytical grade) was obtained from Sigma-Aldrich Quimica SA; Madrid, Spain and used at concentrations of 0, 500, 1,000, 2,000, 4,000, 5,000, 6,000, 7,000 and 8,000 ppm.

Preparation of rootstock cutting and budding

Piece of stem (10–15 mm wide and 15–20 cm long) of normal physic nut were used for cutting as rootstocks. The stems were inserted to a depth of about 25–38 mm in 10 × 15 cm-sized plastic bags. The rooting medium filling the bag contained rice husk charcoal and coarse sand in a ratio of 3:1. Each stem cutting (rootstock) was rooted in a bag for 30–45 d. Rooted stocks were budded using the chip budding method (Hartman et al., 1990) with physic nut clones FF25B-14 and FF20SBr-3 for experiment I and experiment II, respectively. Bud wood samples (scions) succeeded in budding in about 30 d and were then ready for the colchicine treatment.

Preparation of grafted plants for colchicines treatment

Grafted plants were prepared for the colchicine treatment using cotton wool and plastic tape. A small piece of cotton wool was placed closely under the bud to absorb the colchicine solution in each treatment. Then, the scion bud and cotton wool were wrapped with budding tape, leaving a space for placing colchicine drops. Colchicine was dropped onto the scion bud and absorbed by the cotton wool. Shoots and leaves emerged from the scion bud by 30 d after the treatments had finished. Rooted stock samples with scion shoots were planted in a bed for selection.

Experiments

I. Study of the effect of colchicine concentrations at 500, 1,000, 2,000 and 4,000 ppm on physic nut clone FF25B-14.

A complete randomized design was used with four replications and five plants in each experimental unit. A sample of 100 budding plants of physic nut clone FF25B-14 was used with four replications. Five plants in each replication were randomly selected and each plant was treated with a colchicine concentration of 0 (control), 500, 1,000, 2,000, or 4,000 ppm at two drops per scion bud for 10 d at around 0900–1100 hours. The experiment started in February 2006.

Π. Study of the effect of colchicine concentrations at 0, 5,000, 6,000, 7,000 and 8,000 ppm on physic nut clone FF20SBr-3.

A sample of 100 grafted plants of clone FF20SBr-3 was selected for the experiment. A complete randomized design was used with four replications and five plants in each experimental unit. Five plants in each replication were randomly selected and each plant was treated with a colchicine concentration of 0 (control), 5,000, 6,000, 7,000, or 8,000 ppm at two drops per scion bud for 10 d at around 0900–1100 hours. The experiment started in April 2006.

Data collection

The same parameters were measured in both experiments. The length and width were measured of stomata of colchicine-treated plants and control plants on mature leaf no.4. The chromosome number was counted from the root tip of colchicine-treated plants that appeared to have bigger stomata. Growth and production were measured in both the colchicine-treated and normal plants using the parameters of height, diameter, fruit number, seed number and seed weight.

Chromosome technique

The roots of selected plants were cut from stem air layering in May and June 2009. White root tips about 0.5 cm long were collected during 1100 to 1200 hours and kept in 8-hydroxyparabenzene at 5–10 °C for 3–4 hr. After that, the roots were kept in 50% formalin-aceto-alcohol at room temperature before studying the chromosome number. Samples of roots were dipped in haematoxylin and dehydrated with an ethanol series and were then dipped in acetocarmine. The root tips were examined under a microscope.

RESULTS AND DISCUSSION

There was no significant difference in the size of the stomata among treatments and the control for clone FF25B-14 (Table 1).

The results for clone FF20SBr-3 of the measurements of stomatal size from each colchicine treatment and for the control are shown in Table 2. The measurements of stomatal length showed that a colchicine concentration of 5,000 ppm produced the longest stomatal length of 37.6 µm while concentrations of 6,000 ppm, 7,000 ppm and 8,000 ppm resulted in lower stomatal lengths of 36.9µm, 32.5µm and 33.1µm, respectively (Table 2). The stomatal width of treated plants was the largest (25.9 µm) with a colchicine concentration of 5,000 ppm and decreased with a concentration of 6,000 ppm to 7,000 ppm to 23.6 µm and 21.9 µm, respectively, and was higher at a concentration of 8,000 ppm (24.5µm) as shown in Table 2 and Figures 1.

Table 1 Stomatal size of physic nut clone FF25B-14 treated with colchicine.

Colchicine treatment (ppm)	Stomatal length (µm)	Stomatal width (µm)
0	29.45	21.15
500	30.77	22.58
1,000	28.85	22.8
2,000	30.35	23.18
4,000	28.68	23.75
F-test	NS	NS
Coefficient of variation (%)	6.96	9.29

NS = Not significantly different at ($P \leq 0.05$).

Table 2 Average size of stomata of physic nut clone FF20SBr-3 treated with 4 colchicine concentrations and control.

Colchicine treatment (ppm)	Stomatal length (µm)	Stomatal width (µm)
0 (control)	27.8^{a}	18^{a}
5,000	37.6^{d}	25.9^{c}
6,000	36.9^{cd}	23.6^{bc}
7,000	32.5^{b}	21.9^{b}
8,000	33.1^{bc}	24.5^{bc}
F-test	*	*
Coefficient of variation (%)	12.82	14.55

Values in the same column with different superscript letters are significantly different ($P \leq 0.05$) by least significant difference.
* = Means are statistically significantly different ($P \leq 0.05$).

Size of stomata of colchicine-treated plants

Colchicine concentrations at 5,000–8,000 ppm in some treated plants stimulated vegetative buds and resulted in new leaves that differed from normal leaves by having bigger stomata than the control (0 ppm). The colchicine-treated plants in experiment II produced two plants that had characteristics completely different from the normal plants. These two plants had an average width and length of stomata that were bigger than in normal plants (Table 2 and Figure 2).

Flowers

In August 2006, one of the colchicine-treated plants flowered and was compared to a normal plant. It was observed that the colchicine-treated plant had bigger male flowers than a normal plant (Figure 3).

Fruits

The fruits of the colchicine-treated plant were observed and showed a thicker peel compared to fruit from a normal plant (Figure 4).

Other characteristics

It was found that the leaves of the colchicine-treated plant with bigger stomata were wider and dark green in color while normal plant leaves were narrow and light green in color. In addition, the plant with bigger stomata had more clearly visible veins compared to a normal plant. (Figure 5).

These characteristics were the same as those reported by Suqiong *et al.* (2008) for tetraploid plants of *Citrus grandis* [*C. maxima*] cv. Shantianyou which had small stems, short

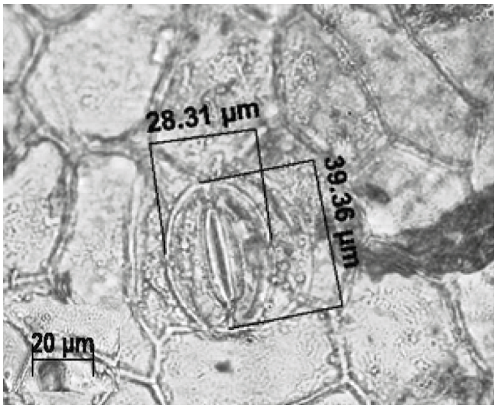

Figure 1 Stomatal size of control plants.

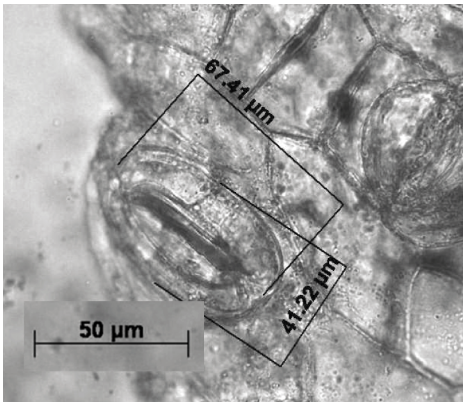

Figure 2 Stomatal size in plants treated with colchicine at 5,000 ppm.

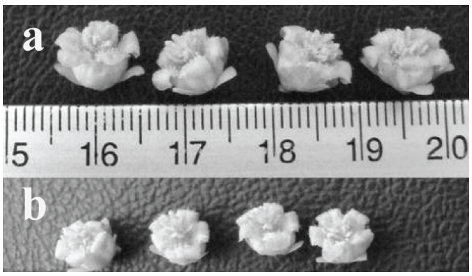

Figure 3 Male flowers: (a) colchicine-treated plant; (b) normal plant.

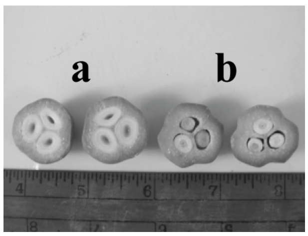

Figure 4 Comparison of cross section of fruit: (a) normal plant; (b) colchicine-treated plant.

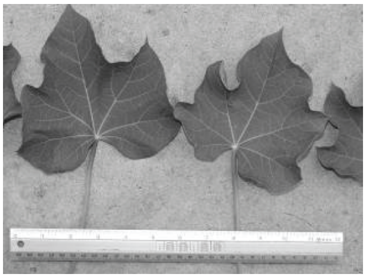

Figure 5 Larger leaf and more clearly visible veins with dark green leaf of colchicine-treated plant (left) compared to normal leaf (right).

branches and thick, dark green leaves. Other experiments have also shown the same results including Gu *et al.* (2005) who studied *Ziziphus jujube*, Kadota and Niimi (2002) who studied *Pyrus pyrifloria* and Hamill *et al.* (1992) with banana (*Musa* spp.).

The normal method of use of colchicine is in a plant tissue culture technique as conducted by Gu *et al*. (2005) whose used shoot tips of *Ziziphus jujube* and a low concentration of colchicine (500–1,000 ppm) by dipping for 24–72 hr while Kadota and Niimi (2002) used colchicine at concentrations of 100–1,000 ppm in *Pyrus pyrifloria* by dipping for 1–8 d. Hamill *et al.* (1992) used higher concentrations of colchicine at 5,000 ppm but a shorter time (2 hr) which differed from the current experiment which used field budding propagation and colchicine treatment of the scion bud after successful budding. The current experiment was simple and less expensive than the tissue culture technique which is complex and may easily result in failure from contamination. However, the conventional method used in the current study might also be at risk from disease and insect attack.

Chromosome number

The roots of selected plant no.1 were cut from stem air layering in May and June 2009. It was found that the chromosome number of selected plant no.1 was doubled to 44 chromosomes (Figure 6), while Whongchana (2008) reported that the normal plant had 22 chromosomes (2n = 22) as shown in Figure 7.

Growth and yield

The growth of tetraploid physic nut plants (chromosome 2n = 44) as indicated by plant height and stem diameter were lower than in normal plants (diploid, 2n = 22) when compared at the same harvest time (Figure 8a). The yields of the tetraploid plant and normal plants are shown in Figure 8b.

Normal plants (average 2.52 m) were higher than tetraploid plants (average 1.53 m). The stem diameter of normal plants (average

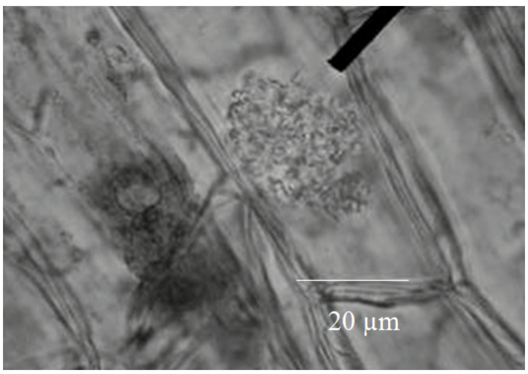

Figure 6 Colchicine treated physic nut cell with chromosome 2n = 44 (400×).

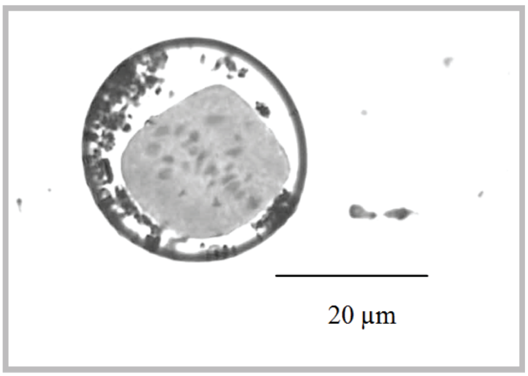

Figure 7 Normal physic nut cell with chromosome 2n = 22 (Whongchana, 2008).

7.62 cm) was greater than in tetraploid plants (5.22 cm). The tetraploid plants produced lower yields than normal plants, which might have been caused by low growth vigor, female flowers always dropping easily and a low fruit set of about 1–2 fruits per bunch compared to normal plants. Fruit development in tetraploid plants always included an incomplete fruit set and aborted seeds, with only one or two seeds per fruit while there was a complete fruit set on normal plants having three seeds per fruit. These were important reasons for the lower yield in the tetraploid plants. Several reports on colchicine treatment showed similar results to this experiment such as Rattanapan (2001) who studied *Curcuma longa* Linn. and found that normal plants had more vigor than polyploid plants. In watermelon, tetraploids had a bigger and thicker seed coat than normal plants but produced smaller amounts of seed and lower yields than normal plants (Sinchai, 1978). However, these tetraploid plants were grown by budding onto normal plant rootstocks, Therefore, the growth of the tetraploid plant might have been controlled or influenced by the rootstock which itself was of higher vigor than the tetraploid. Thus, own-root propagation such as by air layering or stem cutting

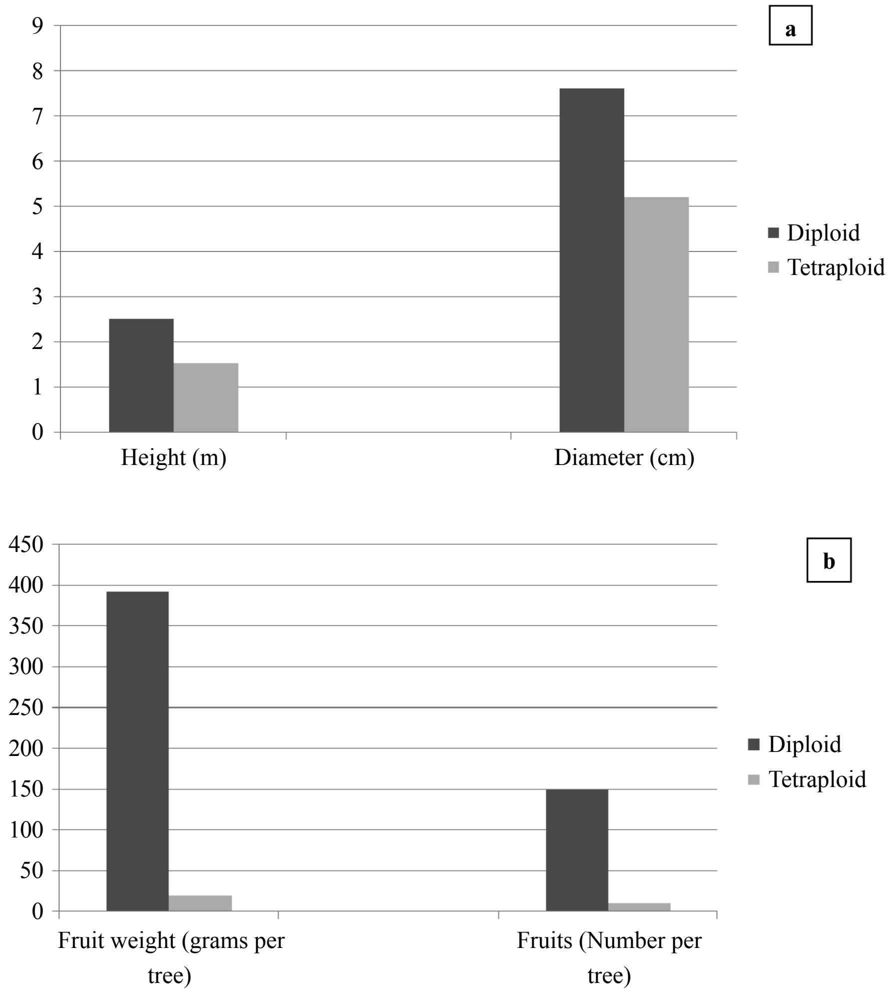

Figure 8 Growth (a) and yield (b) of the tetraploid (2n = 44) and normal (2n = 22) plants. Growth data were collected at age 2 y and yield data were collected between June and July 2009. Average growth and yield of two plant groups, each group with 5 plants.

should be used and compared to scion budwood on rootstock for further study of growth and yield.

CONCLUSION

Colchicine concentrations at 5,000–6,000 ppm could affect the width and length of stomata of physic nut clone FF20SBr-3. Two plants of physic nut clone FF20SBr-3 showed different characteristics such as leaf size and leaf shape and especially inflorescence size, with the male flowers bigger than in normal plants. The chromosome number was found to be 2n = 44 chromosomes indicating that these plants were tetraploid. The growth of the tetraploid plant was lower than for a normal plant and the yield was very low compared to a normal plant.

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