RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.060

ScienceAsia 50 (3): 2024: ID 2024060: **1–7**

Cytogenetic analysis of progeny from interspecific hybridization between *Jatropha curcas* and *Jatropha integerrima* using the GISH method

Matiya Changjalern[a], **Nobuko Omido**[b], **Penjit Srinophakun**[c,d], **Vipa Hongtrakul**[a,d,*]

[a] Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900 Thailand
[b] Graduate School of Human Development and Environment/ Department of Human Environmental Science, Kobe University, Kobe 657-8501 Japan
[c] Chemical Engineering Department, Faculty of Engineering, Kasetsart University, Bangkok 10900 Thailand
[d] Center of Excellence for Jatropha, Kasetsart University, Bangkok 10900 Thailand

*Corresponding author, e-mail: fscivph@ku.ac.th

Received 20 Dec 2023, Accepted 12 Apr 2024
Available online 30 Jun 2024

ABSTRACT: Vigorous interspecific hybrids have been obtained from cross-pollination between two tropical species of *Jatropha*, *J. curcas* and *J. integerrima*, in a project improving *J. curcas* for use as a biodiesel source. The F_1 hybrids have been confirmed using co-dominant DNA markers and some can be successfully backcrossed to *J. curcas*. These interspecific hybrids show segregation of leaf, seed, and flower characters compared to their parents. Although co-dominant DNA markers can be used to effectively verify the F_1 interspecific hybrids, it is difficult to confirm the hybridity of the backcross hybrids using DNA markers after many backcross generations. Therefore, this study used the genomic *in situ* hybridization (GISH) method, using genomic DNA probes from *J. curcas* and *J. integerrima*, to analyze the chromosome composition of the interspecific *Jatropha* hybrids from various generations. Seven samples, including one sample of F_1, one sample of open pollination of F_1 (OPF_1), two samples of BC_1, two samples of BC_2, and one sample of open pollination of BC_1 ($OPBC_1$), were selected based on the presence of high seed yield or disease resistance. The GISH chromosome composition results indicated that the proportions of chromosomes of *J. curcas* : *J. integerrima* : crossover event assessed in meristematic tissues at the root tip of seeds from F_1#36, OPF_1#36, BC_1#201, BC_1#202, BC_2#524, BC_2#525, and $OPBC_1$#VH1 were 13:9:1, 20:2:1-2, 17-5:2, 21:1:1-2, 22:0:4, 22:0:3, and 22:0:2, respectively. The GISH method proved a valuable tool for genomic analysis as well as monitoring chromatin transfer and introgression in the interspecific hybrids, enhancing the *Jatropha* breeding program considerably.

KEYWORDS: *Jatropha curcas*, *Jatropha integerrima*, genomic *in situ* hybridization, cytogenetic analysis

INTRODUCTION

Jatropha curcas L. (*J. curcas*) is a plant native to Mexico, and was introduced to Asia and Africa by Portuguese traders who used it as a hedge plant. Belonging to the family Euphorbiaceae, this plant has been recognized for its potential as a biodiesel source, primarily due to its oil-rich seeds. A significant challenge in biofuel production is the need for large plots of land, which are becoming an increasingly limited resource in the face of the growing global population [1]. However, *J. curcas* stands out as a drought-tolerant perennial crop capable of thriving in wild and arid soils whose poor soil quality renders them unsuitable for farming or crop cultivation [2].

J. curcas has two sets of genomes and a chromosome number of $2n = 2x = 22$, with a genome size of about 370 Mbp [3]. The species is known to have a narrow genetic base [4–6] and low seed yield, prompting research to develop *J. curcas* lines with higher yields.

The improvement of the cultivar through conventional breeding within *J. curcas* may be challenging due to the close genetic similarities between conspecifics; the likelihood of obtaining new traits from such a combination is minimal. Therefore, recent research has focused on broadening the genetic diversity of *J. curcas* through crossbreeding (interspecific hybridization) with other *Jatropha* species [7–9]. The underlying principle of interspecific hybridization is to combine favorable characteristics—such as oil quantity and quality, yield, as well as resistance to insects (for example, army worms, aphids, mealy bugs) and diseases (for example, Anthracnose, Passalora leaf spot, Cercospora/Pseudocercospora leaf spot)—from parental plants in their progeny [10].

Genomic *in situ* hybridization (GISH) is a method that utilizes whole-genomic DNA as a probe to investigate the relationships, divergence, and evolution of the genome in plants [11]. It is also employed to identify chromosome fragments from alien species that have integrated into the chromosomes of a particular plant species [12]. GISH was initially employed to differentiate the genomes of the intergeneric hybrids between *Hordeum chilense* and *Secale africanum* [13] and in Tricolor and Wheat-Rye Migration Line [14, 15]. Furthermore, the GISH technique has been utilized for karyotype analysis in numerous plant species [16–19].

Well-developed genetic *in situ* hybridization analysis allows for the cytological discrimination of closely related genomes in interspecific hybrids. This approach to investigate genome similarity is a powerful tool for biosystematic and evolutionary studies. More importantly, it often allows for the analysis of species interrelationships where conventional methods cannot be performed.

Gene flow in birch (*Betula*) was studied via interspecific hybridization between the diploid dwarf *B. nana* and the tetraploid downy *B. pubescens* using different approaches, from cytogenetic and botanical, to molecular and palynological [20]. Backcrossing of the obtained triploid F_1 hybrids to either male or female parent produced the progenies, belonging to diploid, triploid and tetraploid, which were successfully confirmed by genome size analysis based on flow cytometry and DNA densitometry. Variations in plant and pollen morphology as well as chloroplast DNA haplotype in the progenies supported the cytogenetic study. Genetic variation permits flexibility and survival of a population in the face of environmental changes. Other cytogenetic approach, like GISH, can be used for supporting the study of introgressive hybridization and determining the chromosome composition of the interspecific hybrids from different generations.

DNA markers have been widely used to confirm the F_1 hybrid. Different alleles from both male and female parents can be detected effectively in the F_1 hybrid using the co-dominant DNA marker. During backcrossing, the donor genome will typically be reduced by half in each generation, causing difficulties in using DNA markers to confirm the hybridity of the backcross generation hybrids.

The aim of the present study was to use the GISH technique to distinguish chromosomes from the different parental genomes (*J. curcas* and *J. integerrima*) in the interspecific hybrids from the F_1 generation, backcross generation, and open pollination. This technique will be a useful tool for verifying hybrids for future breeding of *J. curcas* and other plants.

MATERIALS AND METHODS

Plant materials

Seven hybrid samples originating from interspecific hybridization between *J. curcas* and *J. integerrima* in the *Jatropha* breeding program were selected from different generations. Sample selection was mainly based on these hybrids' potential to generate high seed yield or moderately high disease resistance. The samples included one sample of the first filial generation of *J. curcas* and *J. integerrima* (F_1#36); one sample originating from the open pollination of F_1, referring to pollination by insect pollinators in the experimental field (OPF_1#325); two samples of the first backcross generation, meaning the F_1 crossing back to the recurrent parent *J. curcas* (BC_1#201 and BC_1#202); two samples of the second backcross generation, meaning the BC_1 crossing back to the recurrent parent *J. curcas* (BC_2#524 and BC_2#525); and one sample of the open-pollination of BC_1 ($OPBC_1$#VH1). The breeding scheme of the samples is shown in Fig. 1, and the pedigree and characters of the seven selected hybrids are listed in Table 1. Seeds of the seven selected hybrids were collected and used for the GISH analysis. Seeds of F_1#36, OPF_1#325, BC_1#201, BC_1#202, BC_2#524, BC_2#525, and $OPBC_1$#VH1 were F_2#36, F_1 of #325, BC_1F_1#201, BC_1F_1#202, BC_2F_1#524, BC_2F_1#525, and F_1 of #VH1, respectively.

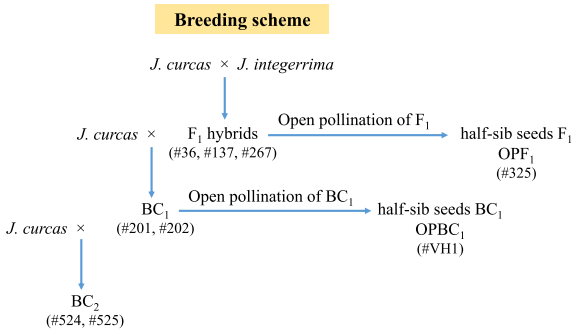

Fig. 1 Breeding scheme of selected hybrid samples from different generations of interspecific hybridization, the seeds of which were used in the GISH analysis. *J. curcas* is the recurrent parent (OP = open pollination, F_1 = first filial generation, BC = backcross).

Slide preparation

Seeds (five seeds/sample) were germinated at a controlled room temperature of 25 °C. Root tips about 1 cm long were collected and pretreated with 0.002 M 8-hydroxyquinoline (Sigma, MO, USA) solution at 20 °C for 3 h and then fixed in 3:1 absolute ethanol:glacial acetic acid for 16 h. Root tips were washed in distilled water prior to digestion in 20 µl of an enzyme mixture containing 2% cellulase (Yakult, Tokyo, Japan) and 5% pectolyase (Sigma, MO, USA) at 37 °C for 1.5–2 h, then washed in distilled water for 1 min. Root tips of only about 0.3–0.5 mm long were macerated and pressed onto a slide with a drop of 3:1 absolute ethanol:glacial acetic acid, then exposed to steam and air dried.

DNA probes and *in situ* hybridization

Total genomic DNA from *J. curcas* and *J. integerrima*, as the parental generation, was extracted from young leaves using the standard cetyltrimethylammonium bromide (CTAB) method with some modifications [21]. Genomic DNAs were digested and labeled by nick translation with digoxigenin-dUTP (Roche, Lewes, UK) (for *J. curcas*) and Cy3-dUTP (Cytiva, Carnegie Mellon University, USA) (for *J. integerrima*) and were used as GISH probes. Probe

Table 1 Pedigree and specific characters of selected hybrids whose seeds were used in the GISH analysis.

Hybrid sample		Pedigree	Sample character
F_1	#36	*J. curcas* × *J. integerrima* ↓ F_1#36	Flowers (V-shaped and pink color), successfully backcrossing to *J. curcas* and complete seed setting, leaves similar to *J. curcas** and seeds similar to *J. integerrima***, semi-hard woody stem, moderate disease resistance (F_1 = first filial generation).
OPF_1	#325	*J. curcas* × *J. integerrima* ↓ F_1#267 × ↓ OPF_1#325	Flowers (bell shaped and greenish-yellow color) similar to *J. curcas*, leaf shape and seeds similar to *J. curcas*, thick leaves, moderately high disease resistance (OPF_1 = open pollination of F_1).
BC_1	#201	*J. curcas* × *J. integerrima* ↓ *J. curcas* × F_1#137 ↓ BC_1#201	Flowers (bell-shaped and pinkish-white color), long pedunculated flowers, leaves and seeds similar to *J. curcas*, moderate disease resistance (BC = back-cross generation).
	#202	*J. curcas* × *J. integerrima* ↓ *J. curcas* × F_1#137 ↓ BC_1#202	Flowers (bell-shaped and greenish-white color), long pedunculated flowers, leaves and seeds similar to *J. curcas*, moderate disease resistance.
BC_2	#524	*J. curcas* × *J. integerrima* ↓ *J. curcas* × F_1#137 ↓ BC_1#202 × *J. curcas* ↓ BC_2#524	Flowers (bell-shaped and greenish-yellow color) similar to *J. curcas*, leaves and seeds similar to *J. curcas*, high number of fruit per plant, moderate seed yield, moderate disease resistance.
	#525	*J. curcas* × *J. integerrima* ↓ *J. curcas* × F_1#137 ↓ BC_1#202 × *J. curcas* ↓ BC_2#525	Flowers (bell-shaped and greenish-yellow color) similar to *J. curcas*, leaves and seeds similar to *J. curcas*, high number of fruit per plant, moderate seed yield, moderate disease resistance.
$OPBC_1$	#VH1	*J. curcas* × *J. integerrima* ↓ *J. curcas* × F_1#137 ↓ BC_1#202 × ↓ $OPBC_1$#VH1	Flowers (bell-shaped and greenish-yellow color) similar to *J. curcas*, leaves and seeds similar to *J. curcas*, high number of fruit per plant, moderate seed yield, moderate disease resistance. (OPBC1 = open pollination of BC_1)

*J. curcas**: tree/shrub, highly branching, cordate-palmately lobed leaves, bell-shaped and greenish-yellow flowers, and tardily dehiscent fruits with black seeds.

*J. integerrima***: shrub, sparse branching, ovate fiddle-shaped leaves, star-shaped and crimson-red flowers, dehiscent fruits with small brown seeds with spots, semi-hard woody stem and disease resistance.

labeling and *in situ* hybridization followed the steps previously described by Ohmido and Fukui [22] and Ohmido et al [23], with minor modifications. Briefly, the hybridization mixture containing labeled probes in hybridization buffer (50% deionized formamide, 2 × SSC (saline-sodium citrate), and 10% sodium dextran sulphate) was denatured for 10 min at 80 °C and then immediately put on ice. Each slide was filled with 10 µl of hybridization mixture and covered with a slip. Then, slides were left overnight at 37 °C in a moist chamber, followed by three 5-min immersions of the slides in 20% formamide in 2 × SSC and 0.1 × SSC at 42 °C. After that, 100 µl of anti-digoxigenin-fluorescein isothiocyanate (FITC) (Roche, Lewes, UK) in 4 × SSC were added and incubated in a humid dark box for 1–2 h at 37 °C. The slides were then briefly washed with 2 × SSC three times for 5 min each time. Then, 70 µl of 4′6-diamidino-2-phenylindole (Serva, Heidelberg, Germany) (DAPI, 0.5 µg/ml) was added to all slides for 10 min at room temperature. Finally, 10 µl of VS (Vectashield mounting medium) was applied to the slides and they were examined under a fluorescent microscope.

RESULTS AND DISCUSSION

Seeds of the seven hybrids (Table 1) selected from the F_1, OPF_1, BC_1, BC_2, and $OPBC_1$ generations of the cross between *J. curcas* and *J. integerrima*

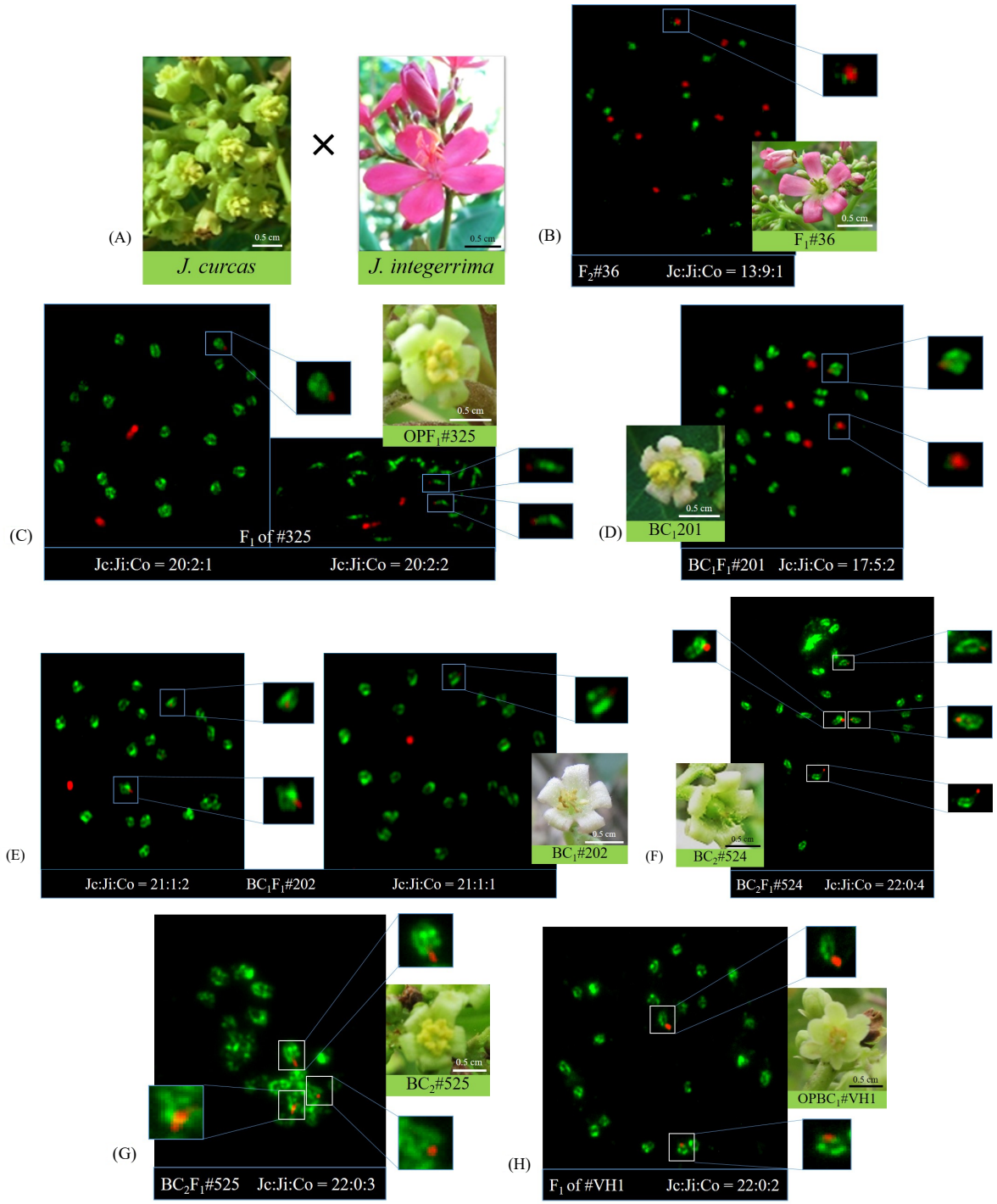

Fig. 2 GISH images of chromosomes at the root tip of seed samples of selected hybrids from different generations of interspecific hybridization; (A) flowers of the parental generation: *J. curcas* (bell-shaped and greenish-yellow flowers) and *J. integerrima* (star-shaped and crimson-red flowers); flower of (B) F_1#36/chromosomes of F_2#36, (C) OPF_1#325/chromosomes of F_1 of #325, (D) BC_1#201/chromosomes of BC_1F_1#201, (E) BC_1#202/chromosomes of BC_1F_1#202, (F) BC_2#524/chromosomes of BC_2F_1#524, (G) BC_2#525/chromosomes of BC_2F_1#525, and (H) $OPBC_1$#VH1/chromosomes of F_1 of #VH1, with chromosomes of *J. curcas* in green and *J. integerrima* in red. Small images at the side of each figure show an enlarged view of the chromosomes containing a crossover event (Jc:Ji:Co = *J. curcas* : *J. integerrima* : crossovers, F_1 = first filial generation, BC = backcross, OP = open pollination).

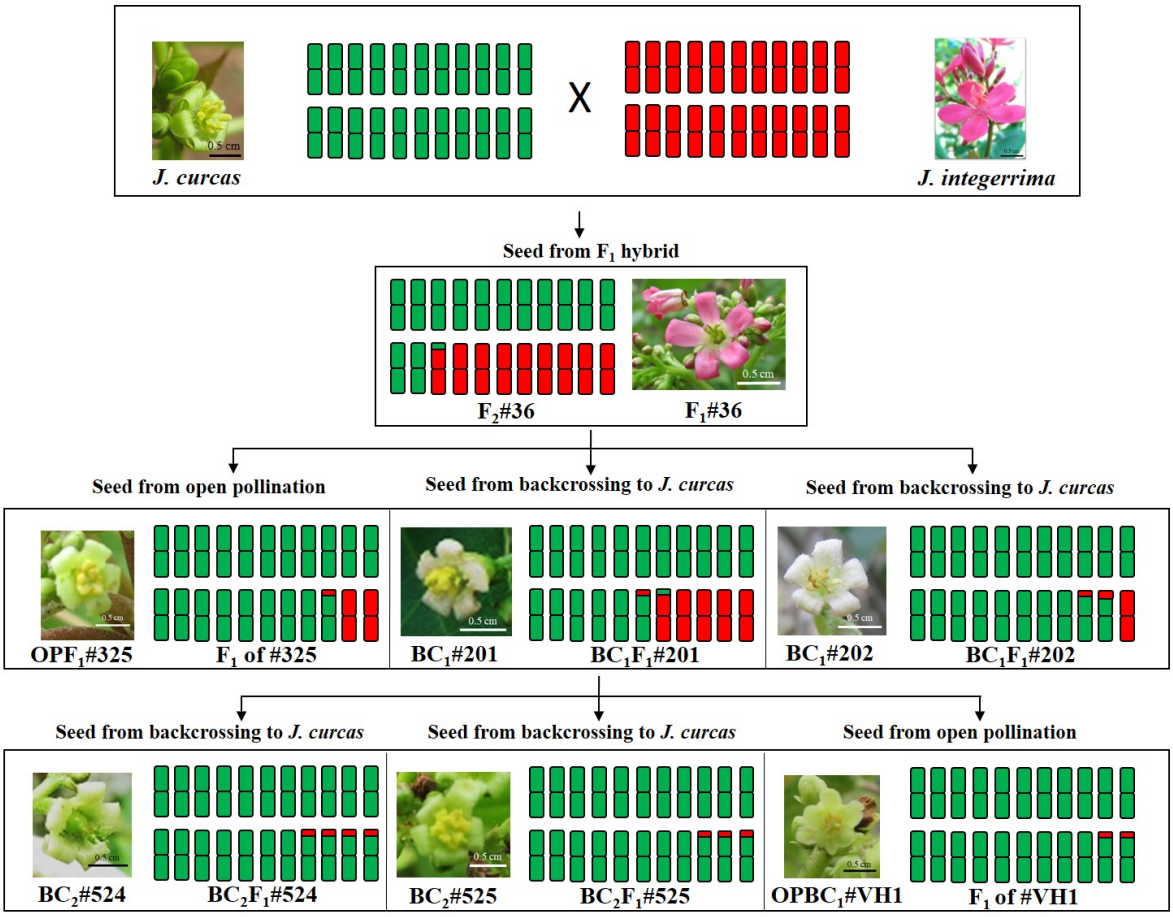

Fig. 3 Chromosome patterns inherited from parents *J. curcas* (green chromosome) and *J. integerrima* (red chromosome), based on the GISH analysis of chromosomes at the root tip of seed samples of selected hybrids from different generations of interspecific hybridization, where flower color of the hybrids varied significantly.

(Fig. 2A) (the parental generation) were investigated using GISH. *J. curcas*, used as the recurrent parent, was selected from a previous *J. curcas* survey project in the country. The selected line produces moderate seed yield but has low disease resistance. The morphological characteristics of *J. curcas* are as follows: it grows as a tree/shrub and is highly branching, with cordate-palmately lobed leaves, greenish-yellow flowers, and tardily dehiscent fruits with black seeds. On the other hand, *J. integerrima* is an ornamental shrub whose morphological characteristics include sparse branching, ovate fiddle-shaped leaves, crimson-red flowers, and dehiscent fruits containing small brown seeds with spots. *J. integerrima* has the distinct characteristics of a semi-hard woody stem and disease resistance [24]. The hybrids obtained from the interspecific hybridization exhibited a wide range of variation in vegetative traits, such as stem type, branching habit, and leaf size and shape, which were similar to those of the parent breeds. The F_1 hybrids of *J. curcas* and *J. integerrima* have pink or white flowers. Some F_1 can successfully backcross to *J. curcas* and complete seed setting to produce the first backcross (BC_1) generation, and subsequently to the second backcross (BC_2) generation. After many generations of backcrossing as well as open pollination, the obtained progeny had similar characteristics to the recurrent parent, *J. curcas*. The pedigree and specific characteristics of selected hybrids whose seeds were used in the GISH analysis are listed in Table 1. Changjalern et al [25] developed three specific DNA markers and used them effectively to confirm the hybrids from interspecific hybridization in *Jatropha* species. However, using DNA markers to trace donor genomes becomes increasingly difficult with progressive backcrossing due to the theoretical reduction by half of the donor genome in each generation. Therefore, we conducted a GISH analysis to visualize the presence of the donor parent genome and the recurrent parent genome in the hybrids from different generations of interspecific hybridization. In combination with previously used methods of genomic analysis, the results of our study will enhance the

success of the *Jatropha* breeding program.

The contribution of the parental genome to that of each hybrid sample could clearly be distinguished by GISH, and its configuration at the metaphase was also revealed. Images of the GISH results (Fig. 2B-H) show the chromosomes from *J. curcas* (in green) and *J. integerrima* (in red) of each hybrid sample. Hybridization distribution in the 22 chromosomes of the F_2#36 hybrid indicated that 13 chromosomes came from *J. curcas*, while the other nine chromosomes came from *J. integerrima*. One small fragment of *J. curcas* chromosome located on the distal end of a *J. integerrima* chromosome was seen from this sample, indicating a crossover event (Fig. 2B). The proportion of chromosomes from the GISH result of *J. curcas* : *J. integerrima* : crossovers in the F_2#36 hybrid was therefore 13:9:1.

For the result from seeds of the open-pollinated F_1 hybrid, F_1 of #325, the chromosomes consisted of two types of hybrid cell: (1) 20 *J. curcas* chromosomes, two chromosomes from *J. integerrima*, and one crossover, and (2) 20 *J. curcas* chromosomes, two chromosomes from *J. integerrima*, and two crossover events, as shown in Fig. 2C. Consequently, the proportion of chromosomes from the GISH result of *J. curcas* : *J. integerrima* : crossovers in F_1 of #325 hybrid was 20:2:1-2.

For the GISH result from seeds of BC_1 hybrids, the chromosomes of BC_1F_1#201 consisted of 17 *J. curcas* chromosomes, five chromosomes from *J. integerrima*, and two crossovers (Fig. 2D), while the chromosomes of BC_1F_1#202 consisted of two types of hybrid cell: (1) 21 chromosomes from *J. curcas*, one chromosome from *J. integerrima*, and one crossover, and (2) 21 *J. curcas* chromosomes, one chromosome from *J. integerrima*, and two crossovers (Fig. 2E). The proportion of chromosomes of *J. curcas* : *J. integerrima* : crossovers was 17:5:2 in BC_1F_1#201, and 21:1:1-2 in BC_1F_1#202.

The GISH result from seeds of the BC_2 and $OPBC_1$ samples indicated all hybrid chromosomes came from *J. curcas*, together with some small fragments of *J. integerrima* chromosome on some *J. curcas* chromosomes. The chromosomes of BC_2F_1#524, BC_2F_1#525, and F_1 of #VH1 consisted of 22 chromosomes from *J. curcas* and four, three, and two fragments of *J. integerrima* chromosomes located at the distal end of *J. curcas* chromosomes, respectively (Fig. 2F-H). The proportions of chromosomes of *J. curcas* : *J. integerrima* : crossovers in BC_2F_1#524, BC_2F_1#525, and F_1 of #VH1 were 22:0:4, 22:0:3, and 22:0:2, respectively. The genomic constitution of meristematic cells at the root tip of seed samples of the seven selected hybrids from different generations of the interspecific hybridization between *J. curcas* and *J. integerrima* indicated by the GISH analysis are summarized in Fig. 3, with *J. curcas* chromosomes depicted in green and *J. integerrima* chromosomes in red.

Seed germination after self-pollination of F_1 hybrids was low, as previously observed by Muakrong et al [7], causing difficulties in the advance selection of the next generation. However, backcrossing to the production of BC_1 of some F_1 hybrids was successful in the *J. curcas* breeding program. GISH has been used recently to detect parental genomes of known origin in plant hybrid cells. For example, total rye DNA probes were used to identify rye chromosomes in the hybrids from *Hordeum chilense* × *Secale africanum* [13] and in Tricolor and Wheat-Rye Migration Line [14, 15]. However, the present study is the first to use the GISH technique to detect the genomes of *J. curcas* and *J. integerrima* in their progeny from different generations. *J. curcas* and *J. integerrima* have been reported to have small chromosome sizes [26], with the length of the 11 *J. curcas* chromosomes that displayed homomorphic pairs ranging from 1.71–1.24 μm [27]; due to their size, it is quite difficult to study these chromosomes. However, we still obtained strong signals of *J. integerrima* in selected hybrids from different generations of the interspecific hybridization. The strongest signal of *J. integerrima* was seen in F_2#36, which was the closest relative of *J. integerrima* among the seven selected hybrids used in our GISH analysis. Interspecific *Jatropha* hybrids share common DNA sequences with parents, but the proportion of common repetitive sequence decreases in subsequent generations. Backcrossing can be used to generate populations that serve as a good source of variability and genetic improvement of *Jatropha* as well as other commercial plants. The interspecific translocation found in this study may be helpful in establishing introgression lines bearing the valuable agronomical traits of *J. integerrima*, such as seed yield, oil content [28], dwarfism [8], and even ornamental quality [7], to create commercial *Jatropha* varieties.

In conclusion, we conducted a cytogenetic analysis using the GISH method to analyze progeny from the interspecific hybridization between *J. curcas* and *J. integerrima*. The results showed that GISH could be used effectively to detect donor genomes in *Jatropha* hybrids from different generations. The reduction of *J. integerrima* donor genome was detected in breeding populations as expected, indicating the efficacy of GISH as a valuable tool for genomic analysis in *Jatropha* interspecific hybrids. The chromosome proportions of *J. curcas* : *J. integerrima* : crossovers in the F_2#36, F_1 of #325 from open pollination of F_1, BC_1F_1#201, BC_1F_1#202, BC_2F_1#524, BC_2F_1#525, and F_1 of #VH1 from open pollination of BC_1 generations were 13:9:1, 20:2:1-2, 17-5:2, 21:1:1-2, 22:0:4, 22:0:3, and 22:0:2, respectively. Currently, interspecific hybridization is an important technique for transferring genes into and between *Jatropha* species. The GISH method for *Jatropha* will be an important tool to monitor the

success of donor genome introgression, opening up the possibility of substantially enhancing the *Jatropha* breeding program.

Acknowledgements: This work was financially supported by the Research and Researcher for Industry (RRI) PhD level and by Ladda Co., Ltd. Mr. Suksan Suttipolpaiboon and Mr. Pichai Maneechote supplied *Jatropha* samples. The Petroleum Authority of Thailand (PTT) funded research on breeding *Jatropha* using interspecific hybridization under the KU-Biodiesel Project, and the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand supported research on *J. curcas*.

REFERENCES

1. Parfit M (2005) Future power: Where will the world get its next energy fix? *Nat Geo* **208**, 2–31.
2. Azam MM, Waris A, Nahar NM (2005) Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. *Biomass Bioenergy* **29**, 293–302.
3. Soontornchainksaeng P, Janjittikul T (2003) Karyology of *Jatropha* (Euphorbiaceae) in Thailand. *Thai For Bull (Bot)* **31**, 105–112.
4. Ram SG, Parthiban KT, Kumar RS, Thiruvengadam V, Paramathma M (2008) Genetic diversity among *Jatropha* species as revealed by RAPD markers. *Genet Resour Crop Evol* **55**, 803–809.
5. Pamidiamarri DVNS, Pandya N, Reddy MP, Radhakrishnan T (2008) Comparative study of interspecific genetic divergence and phylogenic analysis of genus *Jatropha* by RAPD and AFLP. *Mol Biol Rep* **36**, 901–907.
6. Ovando-Medina I, Espinosa-García FJ, Núñez-Farfán JS, Salvador-Figueroa M (2011) State of the art of genetic diversity research in *Jatropha curcas*. *Sci Res Essays* **6**, 1709–1719.
7. Muakrong N, Tanya P, Srinives P (2014) 'Kamphaeng Saen 1', 'Kamphaeng Saen 2', and 'Kamphaeng Saen 3': New ornamental *Jatropha* cultivars derived through an interspecific cross. *HortScience* **49**, 1083–1085.
8. One KT, Muakrong N, Phetcharat C, Tanya P, Srinives P (2014) Inheritance of dwarfness and erect growth habit in progenies of *Jatropha curcas* × *Jatropha integerrima*. *J Amer Soc Hort Sci* **139**, 582–586.
9. Sakthanutpata N, Kuleunga C, Hongtrakul V (2020) Nucleotide sequencing of specific genes in nuclear and plastid genomes of good performance hybrids derived from interspecific hybridization in the genus *Jatropha*. *Agr Nat Resour* **54**, 55–63.
10. Prabakaran AJ, Sujatha M (1999) *Jatropha tanjorensis* Ellis and Saroja, a natural interspecific hybrid occurring in Tamil Nadu, India. *Genet Resour Crop Evol* **46**, 213–218.
11. Wang Y, Zhi H, Li W, Li H (2009) A novel genome of C and the first autotetraploid species in the *Setaria* genus identified by genomic *in situ* hybridization. *Genet Resour crop Evol* **56**, 843–850.
12. Barba-Gonzalez R, Ramanna MS, Visser RGF, Van Tuly JM (2005) Intergenomic recombination in F_1 lily hybrids (Lilium) and its significance for genetic variation in the BC_1 progenies as revealed by GISH and FISH. *Genome* **48**, 884–894.
13. Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) *In situ* localization of parental genomes in a wide hybrid. *Ann Bot* **64**, 315–324.
14. Le HT, Armstrong KC, Miki B (1989) Detection rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. *Plant Mol Biol Rep* **7**, 150–158.
15. Heslop-Harrison JS, Leitch AR, Schwarzacher T, Anamthawat-Jonsson K (1990) Detection and characterization of 1B/1R translocations in hexaploid wheat. *Heredity* **65**, 385–392.
16. Guerra M (2004) Hybridization *in Situ*: Princípos Básicos. In: Guerra M (ed) *FISH: Fonceitos e Aplicações na Citogenética*, Sociedade Brasileira de Genética, Ribeirão Preto, pp 1–32.
17. Melo CA, Silva GS, Souza MM (2015) Establishment of the genomic *in situ* hybridization (GISH) technique for analysis in interspecific hybrids of *Passiflora*. *Genet Mol Res* **14**, 2176–2188.
18. Markova M, Michu E, Vyskot B, Janousek B, Zluvova J (2007) An interspecific hybrid as a tool to study phylogenetic relationships in plants using the GISH technique. *Chomosome Res* **15**, 1051–1059.
19. Czernicka M, Mścichowska A, Klein M, Muras P, Grzebelus E (2010) Paternity determination of interspecific rhododendron hybrids by genomic *in situ* hybridization (GISH). *Genome* **53**, 277–284.
20. Anamthawat-Jónsson K (2019) Hybrid introgression: the outcomes of gene flow in birch. *ScienceAsia* **45**, 203–211.
21. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.
22. Ohmido N, Fukui K (1997) Visual verification of close disposition between a rice a genome-specific DNA sequence (TrsA) and the telomere sequence. *Plant Mol Biol* **35**, 963–968.
23. Ohmido N, Akiyama Y, Fukui K (1998) Physical mapping of unique nucleotide sequence on identified rice chromosomes. *Plant Mol Biol*. **38**, 1041–1052.
24. Basha SD, Sujatha M (2007) Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. *Euphytica* **156**, 375–386.
25. Changjalern M, Srinophakun P, Hongtrakul V (2023) Development of microsatellite, gene-specific and species-specific markers for hybrid detection of plants in *Jatropha* genus. *Agr Nat Resour* **57**, 31–42.
26. Dehgan B, Webster GL (1979) Chromosome number. In: *Morphology and Infrageneric Relationships of the Genus Jatropha (Euphorbiaceae)*, Univ of California Press, Berkeley, pp 29–34.
27. Carvalho CR, Clarindo WR, Rraca MM, Araujo FS, Carels N (2008) Genome size, base composition and karyotype of *Jatropha curcas* L., an important biofuel plant. *Plant Sci* **174**, 613–617.
28. One KT, Muakrong N, Tanya P, velette J, Girard P, Srinives P (2014) Physicochemical properties of seeds and oil from an F_2 population of *Jatropha curcas* × *Jatropha integerrima*. *ScienceAsia* **40**, 428–435.