Introduction

Jatropha (Jatropha curcas L., Euphorbiaceae; \(2n = 2x = 22\); Miller and Webster 1962, Perry 1943) is a highly branching and semi-evergreen tree, and an oil-bearing plant. Jatropha is thought to be a promising plant for biodiesel production because of its higher oil content (40%–60% in seeds), rapid growth, ease of propagation, drought tolerance, and pest resistance. Because of its assumed environmental benefits, more than 106 ha of jatropha plantations have been established in Asia (especially in India and China), Africa, and Latin America (Singh et al. 2014) over the last 10 years, with a prospective seed yield of 4–5 Mg ha\(^{-1}\) yr\(^{-1}\). However, the actual seed yield (0.5–2 Mg ha\(^{-1}\) yr\(^{-1}\)) was considerably lower than expected owing to underperformance under field conditions in various countries (Edrisi et al. 2015). To commercialize jatropha, genetic improvement of oil yield and quality is required.

Assessment using molecular markers such as random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR) markers indicated a modest level of inter-accessional variability in J. curcas (Basha and Sujatha 2007, Tanya et al. 2011). Using 54 SSRs and 120 single-nucleotide polymorphisms, Montes et al. (2014) found that only 7 of 70 J. curcas accessions from Asia, Africa, and Latin America had more than 10% of markers with multiple alleles per locus, suggesting a high level of self-fertilization. Thus, evaluation

Cytological characterization of an interspecific hybrid in Jatropha and its progeny reveals preferential uniparental chromosome transmission and interspecific translocation

Shuto Fukuhara\(^{+1}\), Narathid Muakrong\(^{+1,2}\), Shinji Kikuchi\(^*^{+1}\), Patcharin Tanya\(^{3,4}\), Hidenori Sassa\(^{1}\), Takato Koba\(^{1}\) and Peerasak Srinives\(^{3,4}\)

\(^{1}\) Laboratory of Genetics and Plant Breeding, Graduate School of Horticulture, Chiba University, Matsudo 648, Chiba 271-8510, Japan

\(^{2}\) Faculty of Agriculture, Princess Naradhiwas University, Narathiwat 96110, Thailand

\(^{3}\) Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

\(^{4}\) Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok 10900 Thailand (CASAF, NRU-KU, Thailand)

Genetic variation in Jatropha curcas, a prospective biodiesel plant, is limited, and interspecific hybridization needed for its genetic improvement. Progeny from interspecific crosses between J. curcas and Jatropha integerrima can be used to improve agronomic characters and to increase oil content and yield. However, these hybrids have not been characterized cytologically. The present study was aimed at the analysis of chromosome behavior during meiosis and chromosome composition of S\(_1\) plants derived from an interspecific F\(_1\) hybrid using genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH). Bivalents that formed as a result of interspecific pairing were frequently observed, suggesting the presence of homoeologous chromosomes from the two species. Almost half of microspores were derived from the reduction division; GISH analysis indicated random transmission of the parent chromosomes to microspores. Male fertility measured as pollen staining with acetocarmine was 48.4%. In contrast, GISH analysis of S\(_1\) plants revealed preferential transmission of J. curcas chromosomes. We also found segment exchange between chromosomes of the two species (interspecific translocation) by GISH and FISH analyses. Introgression of J. integerrima chromosome segments into the J. curcas genome would help to improve Jatropha cultivars for mass production.

Key Words: fluorescence in situ hybridization (FISH), genomic in situ hybridization (GISH), interspecific hybrid, interspecific translocation, Jatropha curcas, Jatropha integerrima, preferential transmission.
Cytological characterization of an interspecific hybrid in Jatropha

Plant materials

This study used J. curcas ‘Chai Nat’ (a local Thai cultivar), J. integerrima (a local ornamental dwarf type from Thailand), and an F1 hybrid (plant no. F1-4) from a cross between ‘Chai Nat’ as the female parent and J. integerrima as the male parent. Plants were grown in the jatropha research field of the Department of Agronomy, Kasetsart University, Kamphaeng Saen campus, Thailand. S1 seeds were obtained by self-pollination of F1-4.

GISH and FISH

Genomic DNA of J. curcas and J. integerrima was purified from young leaves without thick veins using the cetyltrimethylammonium bromide (CTAB) method according to Kikuchi et al. (2010). Plasmid DNA containing the Jatropha curcas satellite 1 (JcSat1) sequence (Kikuchi et al. 2010) and 45S rDNA (pTa71; Gerlach and Bedbrook 1979) was used to prepare a FISH probe. The FISH probes (plasmid DNA) were generated by nick translation (Sigma) and the GISH probes (genomic DNA) were prepared by High Prime (Sigma). GISH and FISH were conducted according to the procedures of Wang et al. (2015).

For the sequential FISH and GISH detection, the slides were first processed for the detection of JcSat1 and 45S rDNA (FISH), denatured in 50% formamide/2× SSC for 2 min on a heat block at 80°C, washed, and air-dried. GISH was then conducted as mentioned above.

Acetocarmine staining

Pollen grains were collected at flowering, incubated in 0.5% acetocarmine on a glass slide for at least 20 min, and observed under a bright-field microscope (CX41; Olympus Corp.).

Results

Chromosome number of the interspecific F1 hybrid (F1-4)

The somatic cells of F1-4 contained 22 chromosomes, which came from both parents. No morphological abnormalities were detected in the metaphase chromosomes (data not shown).
Chromosome behavior during meiosis

Meiotic chromosomes of F₁-4 showed normal and abnormal behavior (Fig. 1). In pachytene spreads, well-paired bivalent chromosomes were observed (Fig. 1A). In each pair, chromosome ends had different chromatin structure, i.e., only one end carried heterochromatin (inset in Fig. 1A). Since J. curcas has a tandem repeat, JcSat1, in subtelomeric heterochromatin (Kikuchi et al. 2010), the pachytene chromosomes of F₁-4 might have formed by pairing of homologous chromosomes from both parents. Eleven bivalents, including rod and ring chromosomes, were present in most diakinesis cells (Fig. 1B). In metaphase I, mean chromosome association frequency was 0.88I + 10.56II per cell (Table 1). However, chromosomes in metaphase I frequently overlapped or were associated like multivalent (Fig. 1C). Although local pairing might be involved, we were unable to detect it by using the squash method. Observations of metaphase I and early anaphase I suggest that chromosomes did not show synchronized orientation on the equatorial plate; several chromosomes started moving to each pole earlier than other chromosomes (Fig. 1C). Normal dyads and tetrads with 11 fluorescence foci of centromeric heterochromatin were generated at frequencies of 37.5% (n = 24; remaining 62.5% is abnormal dyad) and 69.2% (n = 78; remaining 30.8% is abnormal tetrad), respectively (Fig. 1D, 1E, Table 2).

Several abnormalities were observed from meiotic anaphase I to the tetrad stage. One or two lagging chromosomes that could not enter daughter cell nuclei were observed (Fig. 1F). The average number of dyad cells with lagging chromosome(s) was 23.5% (Table 2). Clumping of chromosomes was observed in tetrad stages (3.9%; Fig. 1G, Table 2). In addition, 5.1% of the cells carried only three nuclei at the tetrad stage; i.e., they were triads (Fig. 1H, Table 2). Because the three nuclei had 10 to 12 centromeres, which was similar to the haploid chromosome number (Fig. 1H), the triads could not have resulted from the first or second division restitution without equational cell wall formation. Lagging chromosomes in telophase II to tetrad stages were observed at a rate of 21.8% (Table 2). Centromeric foci revealed chromosomally variable gametes (aneuploids), which were generated because of chromosome lagging and unbalanced chromosome segregation; e.g., 9 and 12 foci in a dyad in Fig. 1F and 10 foci in a microspore in Fig. 1I (left

Table 1. Chromosome pairing configurations of an F₁ hybrid between J. curcas and J. integerrima

| Chromosome number (2n) | No. of cells observed | Chromosome pairing configurations |
|------------------------|-----------------------|----------------------------------|
|                        | I                     | II Total | Rod | Ring | III TOXN^a |
| F₁-4                   | 22                    | 16       | 0.875 | 10.56 | 9.56 | 1.00 | 0 | 1.28 |

^a TOXN = Total chiasmata / haploid chromosome number. Possible chiasma numbers of rod and ring bivalents are 1 and 2.

Table 2. Frequencies of meiocytes showing abnormal chromosome behaviors and characteristics in F₁-4

| Lagging chromosomes^b | Un-reduction division^b | Lagging chromosomes^c | Clumping nuclei^d | Triads | Aneuploid microspores^d |
|-----------------------|-------------------------|-----------------------|-------------------|--------|------------------------|
| 23.5% (12/51 cells)   | 62.5% (15/24 cells)     | 21.8% (17/78 cells)   | 3.9% (3/78 cells)  | 5.1% (4/78 cells) | 46.5% (33/71 cells) |

^a Cells with at least one lagging chromosome.

^b Telophase I/dyad cells without expected 11 centromere foci in the daughter cell nuclei.

^c Tetrads including clumping nuclei.

^d Microspores without expected 11 centromere foci in the nucleus.
Cytological characterization of an interspecific hybrid in Jatropha

Breeding Science
Vol. 66 No. 5 BS

We detected 46.5% of aneuploid microspores with 10 to 12 chromosomes (Table 2).

Pollen formation in F₁-4

The frequency of pollen grains stained with acetocarmine was 87.5% ± 2.7% (out of 1127 pollen grains) in J. curcas, 93.8% ± 0.3% (out of 1083) in J. integerrima, and 48.4% ± 6.4% (out of 680) in F₁-4 (Fig. 2). Unstained pollen grains were empty. In addition, the size of the pollen grains of F₁-4 varied (Fig. 2B), suggesting variability in their DNA content.

GISH analysis of meiotic metaphase I in F₁-4 and somatic chromosomes in S₁ plants

GISH analysis allowed us to identify the parental chromosomes in hybrid cells (Fig. 3). Heterochromatic condensation was observed in the proximal regions of all chromosomes derived from both parents (Fig. 3A); GISH signals appeared mainly at the centromeres (Fig. 3B), suggesting the presence of species-specific repeats in these regions. GISH signals in the heterochromatic subtelomeric regions of J. curcas chromosomes corresponded to JcSat1, whereas the subtelomeric regions of J. integerrima lacking heterochromatin showed no prominent GISH signals. GISH analysis also confirmed that bivalents were formed by interspecific pairing between chromosomes of the two species (Fig. 4).

We also examined whether the paired parent chromosomes underwent reduction division. Aneuploid microspores were removed, and 20 microspores with 11 centromeric foci (haploid chromosome number) were used for GISH analysis to determine the composition of each chromosome (Fig. 5A). The average number of J. curcas and J. integerrima chromosomes per microspore were 5.3 and 5.7, respectively, and did not differ significantly from the expected number (5.5 chromosomes from each parent) by chi-square test (P > 0.05); therefore, reduction division occurred in male meiosis.

We also analyzed the chromosome composition of 26 randomly selected S₁ individuals derived from F₁-4 (Figs. 5B, 6A–6C). No aneuploids were observed in any of them, and each S₁ plant had 22 chromosomes in its somatic cells. In contrast to the chromosome composition of microspores (Fig. 5A), all S₁ plants (except No. 327) had more

Fig. 2. Acetocarmine staining of pollen grains in the F₁ hybrid and parental species. (A) In J. integerrima, the cytoplasm of most pollen grains was stained; an unstained grain is marked with an arrowhead. (B) An increase in the proportion of unstained pollen grains (single arrowheads) was observed in the F₁ hybrid. A double arrowhead indicates a pollen grain that was slightly larger than the pollen grains of the J. integerrima in Fig. 2A. Scale bars = 50 μm. (C) Percentage of acetocarmine-stained pollen grains in the F₁ hybrid and the parental species. Data; J. curcas, J. integerrima and F₁-4 are means 87.5% ± 2.7% (n = 1127 pollen grains), 93.8% ± 0.3% (n = 1083 pollen grains), 48.4% ± 6.4% (n = 680 pollen grains), respectively.

Fig. 3. GISH analysis of parental chromosomes in the F₁ hybrid. (A) DAPI staining. (B) GISH probes clearly show 11 chromosomes derived from J. curcas (red) and J. integerrima (green). Scale bar = 2.5 μm.

GISH analysis of meiotic metaphase I in F₁-4 and somatic chromosomes in S₁ plants

GISH analysis allowed us to identify the parental chromosomes in hybrid cells (Fig. 3). Heterochromatic condensation was observed in the proximal regions of all chromosomes derived from both parents (Fig. 3A); GISH signals appeared mainly at the centromeres (Fig. 3B), suggesting the presence of species-specific repeats in these regions. GISH signals in the heterochromatic subtelomeric regions of J. curcas chromosomes corresponded to JcSat1, whereas the subtelomeric regions of J. integerrima lacking heterochromatin showed no prominent GISH signals. GISH analysis also confirmed that bivalents were formed by interspecific pairing between chromosomes of the two species (Fig. 4).

We also examined whether the paired parent chromosomes underwent reduction division. Aneuploid microspores were removed, and 20 microspores with 11 centromeric foci (haploid chromosome number) were used for GISH analysis to determine the composition of each chromosome (Fig. 5A). The average number of J. curcas and J. integerrima chromosomes per microspore were 5.3 and 5.7, respectively, and did not differ significantly from the expected number (5.5 chromosomes from each parent) by chi-square test (P > 0.05); therefore, reduction division occurred in male meiosis.

We also analyzed the chromosome composition of 26 randomly selected S₁ individuals derived from F₁-4 (Figs. 5B, 6A–6C). No aneuploids were observed in any of them, and each S₁ plant had 22 chromosomes in its somatic cells. In contrast to the chromosome composition of microspores (Fig. 5A), all S₁ plants (except No. 327) had more

Fig. 4. GISH analysis of meiotic metaphase I in the F₁ hybrid. (A) DAPI staining. (B) J. integerrima genomic DNA. (C) J. curcas genomic DNA. (D) Merged images shown in A–C. Scale bar = 5 μm.
chromosomes from *J. curcas* (16.04 per cell on average) than from *J. integerrima* (5.96 per cell on average) (Fig. 5B). The average numbers differed significantly from the expected number (11 from each parent) by chi-square test ($P < 0.05$). Plant No. 327 had 9 chromosomes from *J. curcas* and 13 from *J. integerrima* (Figs. 5B, 6A). Thus, preferential transmission of *J. curcas* chromosomes was detected in almost all S₁ plants.

In S₁ plants, we found small *J. curcas* GISH signals at the ends of *J. integerrima* chromosomes (Fig. 6A–6C), suggesting translocation between chromosomes of the two species. Because *J. integerrima* chromosomes did not have clear GISH signals at their ends, such mosaic pattern of two GISH signals could not be clearly observed in *J. curcas* chromosomes. Our FISH analysis revealed that *J. integerrima* does not have JcSat1 (manuscript in preparation). Sequential FISH and GISH analysis of plant No. 387 confirmed that three chromosome pairs exchanged JcSat1 between the two species, because JcSat1 signals were irregularly observed at the ends of three *J. integerrima* chromosomes (Fig. 6D–6E). A pair of chromosomes with translocation was identified as SAT-chromosomes carrying 45S rDNA (Fig. 6F). Translocation of JcSat1 found in their SAT-chromosomes might indicate that these chromosomes were originally homoeologous and exchanged segments by meiotic recombination.

**Discussion**

**Chromosome polymorphism in *J. curcas* and *J. integerrima***

We showed that meiotic chromosomes often formed 11 bivalents in F₁-4. Therefore, the two species had homoeologous chromosome-like. The homoeologous chromosomes showed different distribution of heterochromatin at their ends; however, they could form well-paired pachytene chromosomes. Formation of ring-shaped bivalents (including two chiasmata) (Fig. 1B) suggested strong chromosomal (genomic) affinity. Although the distribution of heterochromatic
regions reflected the different structures of the two species, their overall karyotypes probably indicate no large-scale translocations or chromosomal inversions, as indicated by the absence of multivalent and loop structures at meiosis (Fig. 1A).

Disordered chromosome orientation on the equatorial plate was observed in meiotic metaphase I in F1-4 (Fig. 1C). Several bivalents showed precocious chromosome segregation (Fig. 1C). GISH analysis suggested that it was not premature separation of sister chromatids (Petronczki et al. 2003). In an interspecific Lilium hybrid, half of the bivalents always disjoin prematurely at metaphase I (Lim et al. 2001). The inability of chromosomes to orient on the equatorial plate may be related to inflexible activity of kinetochore and spindle assembly checkpoint. Our observation suggests that the centromeres of J. integerrima were distributed at the polar side, whereas the centromeres of J. curcas tended to be localized on the equatorial plate (Fig. 4). Thus, polymorphism of centromere function may also contribute to the disordered orientation of bivalents in the F1 hybrid.

We also observed preferential transmission of J. curcas chromosomes to S1 plants (Fig. 5B). Because we did not examine an F1 hybrid and its S1 progeny from the reciprocal cross, it is not certain whether preferential transmission is affected by cytoplasmic factors. Disordered chromosome orientation on the equatorial plate during meiotic metaphase I in F1-4 (Fig. 1C) suggests that centromere function is a potential cause of preferential transmission. A meiotic drive for distorted segregation was found in Mimulus hybrids from polymorphism of their centromeres (Fishman and Willis 2005, Fishman and Saunders 2008).

Use of interspecific hybridization for jatropha improvement

We observed variable karyotypes of S1 plants (Figs. 5B, 6). Using EST-SSR primers, One et al. (2014b) found high genetic variability in an F1 population derived from an F1 hybrid of J. curcas × J. integerrima. Dwarfness (a local J. integerrima trait) and erect growth (a J. curcas trait) segregated according to Mendelian inheritance in the F2 population (One et al. 2014c). Genetic maps with 11 linkage groups were constructed on the basis of the interspecific cross (Wang et al. 2011, Wu et al. 2015). Although we observed meiotic errors (Fig. 1, Table 2) and preferential uniparental chromosome transmission (Fig. 5B), they are unlikely to cause severe problems for genetic analysis and construction of genetic maps. Bivalent formation, production of a sufficient number (about half) of normal pollen grains, and generation of S1 plants with the normal chromosome number (2n = 22) may contribute to successful genetic analysis.

The rate of seed formation after self-pollination of the F1 hybrid is low (Muakrong et al. 2014, Parthiban et al. 2009, Sujatha and Prabakaran 2003), and thus it is not easy to advance selection to the next generation. However, backcrossing to produce BC1F1 was successful, although only when J. curcas was used as the female parent (Muakrong et al. 2014). We have screened a dwarf jatropha plant with J. integerrima chromosome among the backcrossing plants. Thus, the backcross could easily be used for forming population and serving as good sources of variability for genetic analysis and genetic improvement of jatropha. Interspecific translocation found in this study might be useful to produce introgression lines with valuable agronomical traits of J. integerrima such as seed yield and oil content (One et al. 2014a, Parthiban et al. 2009), dwarfness and erect growth (One et al. 2014c), woody biomass (Muakrong et al. 2013), and ornamental qualities (Muakrong et al. 2014, Sujatha and Prabakaran 2003), for creation of commercial jatropha varieties.

Acknowledgements

This work was partially supported by JSPS Grant-in-Aid for Young Scientists (A), Grant Number 25711024 to S.K. A part of this work was also supported by the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University Under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand and Thailand’s NSTDA Chair Professor Project (grant no. P-11-00599) to P.T. and P.S.

Literature Cited

Basha, S.D. and M. Sujatha (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.

Dahmen, N., M.T. Schifino-Wittmann and L.A.S. Dias (2009) Chromosome numbers of Jatropha curcas L.: an important agrofuel plant. Crop Breed. Appl. Biotech. 9: 386–389.

Dehgan, B. (1984) Phylogenetic significance of interspecific hybridization in Jatropha (Euphorbiaceae). Syst. Bot. 9: 467–478.

Edrisi, S.A., R.K. Dubey, V. Tripathi, M. Bakshi, P. Srivastava, S. Jamil, H.B. Singh, N. Singh and P.C. Abhilash (2015) Jatropha curcas L.: A crucified plant waiting for resurgence. Renew. Sustain. Energy Rev. 41: 855–862.

Fishman, L. and J.H. Willis (2005) A novel meiotic drive locus almost completely distorts segregation in Mimulus (monkeyflower) hybrids. Genetics 169: 347–353.

Fishman, L. and A. Saunders (2008) Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. Science 322: 1559–1562.

Gerlach, W.L. and J.R. Bedbrook (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. 7: 1869–1885.

Kikuchi, S., H. Tsujimoto, H. Sassa and T. Koba (2010) JcSat1, a novel subtelomeric repeat of Jatropha curcas L. and its use in karyotyping. Chromosome Sci. 13: 11–16.

Lakshminarayana, M. and M. Sujatha (2001) Screening of Jatropha species against the major defoliators of castor (Ricinus communis L.). J. Oilseeds Res. 18: 228–230.

Laosatit, K., P. Tanya, N. Muakrong and P. Srinives (2014) Development of interspecific and intergeneric hybrids among jatropha-related species and verification of the hybrids using EST–SSR
markers. Plant Genet. Resour. C. 12: 58–61.
Lim, K.-B., M.S. Ramanna, J.H. de Jong, E. Jacobsen and J.M. van Tuyl (2001) Indeterminate meiotic restitution (IMR): a novel type of meiotic nuclear restitution mechanism detected in interspecific lily hybrids by GISH. Theor. Appl. Genet. 103: 219–230.
Miller, K.L. and G.L. Webster (1962) Systematic position of Cnidoscolus and Jatropha.Brittonia 14: 174–180.
Montes, J.M., F.T. Technow, M. Martin and K. Becker (2014) Genetic diversity in Jatropha curcas L. assessed with SSR and SNP markers. Diversity 6: 551–566.
Muakrong, N., K.T. One, P. Tanya and P. Srinives (2013) Interspecific jatropha hybrid as a new promising source of woody biomass. Plant Genet. Resour. 12: S17–S20.
Muakrong, N., P. Tanya and P. Srinives (2014) ‘Kamphaeng Saen 1’, ‘Kamphaeng Saen 2’, and ‘Kamphaeng Saen 3’: New ornamental Jatropha cultivars derived through an interspecific cross. HortScience 49: S17–S20.
One, K.T., N. Muakrong, P. Tanya, J. Velette, P. Girard and P. Srinives (2014a) Physicochemical properties of seeds and oil from an F2 population of Jatropha curcas × Jatropha integerrima. Sci. Asia 40: 428–435.
One, K.T., P. Tanya, N. Murakrong, K. Laosatit and P. Srinives (2014b) Phenotypic and genotypic variability of F2 plants derived from Jatropha curcas × integerrima hybrid. Biomass Bioenergy 67: 137–144.
One, K.T., N. Muakrong, C. Phetcharat, P. Tanya and P. Srinives (2014c) Inheritance of dwarfness and erect growth habit in progenies of Jatropha curcas × Jatropha integerrima. J. Am. Soc. Hortic. Sci. 139: 582–586.
Parthiban, K.T., R.S. Kumar, P. Thiagarajan, V. Subbulakshmi, S. Venilla and M.G. Rao (2009) Hybrid progenies in jatropha—a new development. Curr. Sci. 96: 815–823.
Perry, B.A. (1943) Chromosome number and phylogenetic relationships in the Euphorbiaceae. Am. J. Bot. 30: 527–543.
Petronczki, M., M.F. Siomos and K. Nasmyth (2003) Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. Cell 112: 423–440.
Rupert, E.A., B. Dehgan and G.L. Webster (1970) Experimental studies of relationships in the genus Jatropha I. J. curcas × integerrima. Bull. Torrey Bot. Club 97: 321–325.
Schwarzacher, T., A.R. Leitch, M.D. Bennett and J.S. Heslop-Harrison (1989) In situ hybridization of parental genomes in wild hybrid. Ann. Bot. 64: 315–324.
Singh, K., B. Singh, S.K. Verma and D.D. Patra (2014) Jatropha curcas: a ten year story from hope to despair. Renew. Sustain. Energy Rev. 35: 356–360.
Sudheer Pamidiamarri, D.V.N., N. Pandya, M.P. Reddy and T. Radhekrishnan (2009a) Comparative study of interspecific genetic divergence and phylogenetic analysis of genus Jatropha by RAPD and AFLP. Mol. Biol. Rep. 36: 901–907.
Sudheer Pamidiamarri, D.V.N., B. Chattopadhyay and M.P. Reddy (2009b) Genetic divergence and phylogenetic analysis of genus Jatropha based on nuclear ribosomal DNA ITS sequence. Mol. Biol. Rep. 36: 1929–1935.
Sujatha, M. and A.J. Prabakaran (2003) New ornamental Jatropha hybrids though interspecific hybridization. Genet. Resour. Crop Evol. 50: 75–82.
Tanya, P., P. Taeratyon, Y. Hadkam and P. Srinives (2011) Genetic diversity among Jatropha and Jatropha-related species based on ISSR markers. Plant Mol. Biol. Rep. 29: 252–264.
Wang, C.M., P. Liu, C. Yi, K. Gu, F. Sun, L. Li, L.C. Lo, X. Liu, F. Feng, G. Lin et al. (2011) A first generation microsatellite- and SNP-based linkage map of Jatropha. PLoS ONE 6: e23632.
Wang, L., S. Kikuchi, C. Muto, K. Naito, T. Isemura, M. Ishimoto, X. Cheng, A. Kaga and N. Tomooka (2015) Reciprocal translocation identified in Vigna angularis dominates the wild population in East Japan. J. Plant Res. 128: 653–663.
Wu, P., C. Zhou, S. Cheng, Z. Wu, W. Lu, J. Han, Y. Chen, Y. Chen, P. N.N. Wang et al. (2015) Integrated genome sequence and linkage map of physic nut (Jatropha curcas L.), a biodiesel plant. Plant J. 81: 810–821.