ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2014) **12**(S1); S58–S61
doi:10.1017/S1479262114000276

Development of interspecific and intergeneric hybrids among jatropha-related species and verification of the hybrids using EST–SSR markers

Kularb Laosatit[1], Patcharin Tanya[2,3]*, Narathid Muakrong[1] and Peerasak Srinives[2,3]

[1]*Program in Plant Breeding, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand,* [2]*Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand and* [3]*Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand*

Abstract

Jatropha curcas (jatropha) is an important non-edible oilseed crop with potential as a raw material for biofuel production. Although *J. curcas* has 30–35% oil content in its seeds, it has low seed yield (<2 ton/ha) and thus cannot become an economically viable crop. However, jatropha has many related species and genera such as *J. integerrima*, *J. multifida*, *J. podagrica* and *Ricinus communis* that are suitable for interspecific and intergeneric hybridization. The desirable features that can be obtained from these species are high number of inflorescences from *J. integerrima*, large fruit size from *J. multifida*, high oil content from *J. podagrica* and raceme-type inflorescence from *R. communis*. We were initially successful in producing hybrids between *J. curcas* and these related species. Hybridity was confirmed using expressed sequence tag (EST)–simple sequence repeat markers developed from the *J. curcas* EST database.

Keywords: EST–SSRs; genetic relationship; jatropha hybrids; *Jatropha curcas*

Introduction

Jatropha (*Jatropha curcas* L.) is a promising oilseed crop for biofuel production as it is not a food crop, except for some low-phorbol ester varieties from Mexico that can be cooked as snacks (Valdes *et al*., 2013). There are reports indicating that the genetic diversity of *J. curcas* is too low for improving the existing varieties. The available germplasm has low seed yield, low number of inflorescences per year and uneven maturity. Tar *et al*. (2011) made crosses between *J. curcas* from Mexico, Myanmar and Thailand and found high heterosis for yield, yield components and agronomic characteristics. However, hybrid yield was still far too low from an economic standpoint. Fortunately, jatropha has many related species and genera, which allows for interspecific and intergeneric crosses. Bottleplant shrub (*J. podagrica*) is a good donor for high oil yield with its seed oil content being over 50%, spicy jatropha (*J. integerrima*) for profuse flowering year-round and coral plant (*J. multifida*) for large fruit size (Ratha and Paramathma, 2009).

Establishment of genetic relationships through DNA information can be done for genetic improvement of *J. curcas*. Tanya *et al*. (2011) employed inter-simple sequence repeat markers to assess genetic variation among 30 accessions of *J. curcas*, two accessions of

* Corresponding author. E-mail: altanya55@yahoo.com

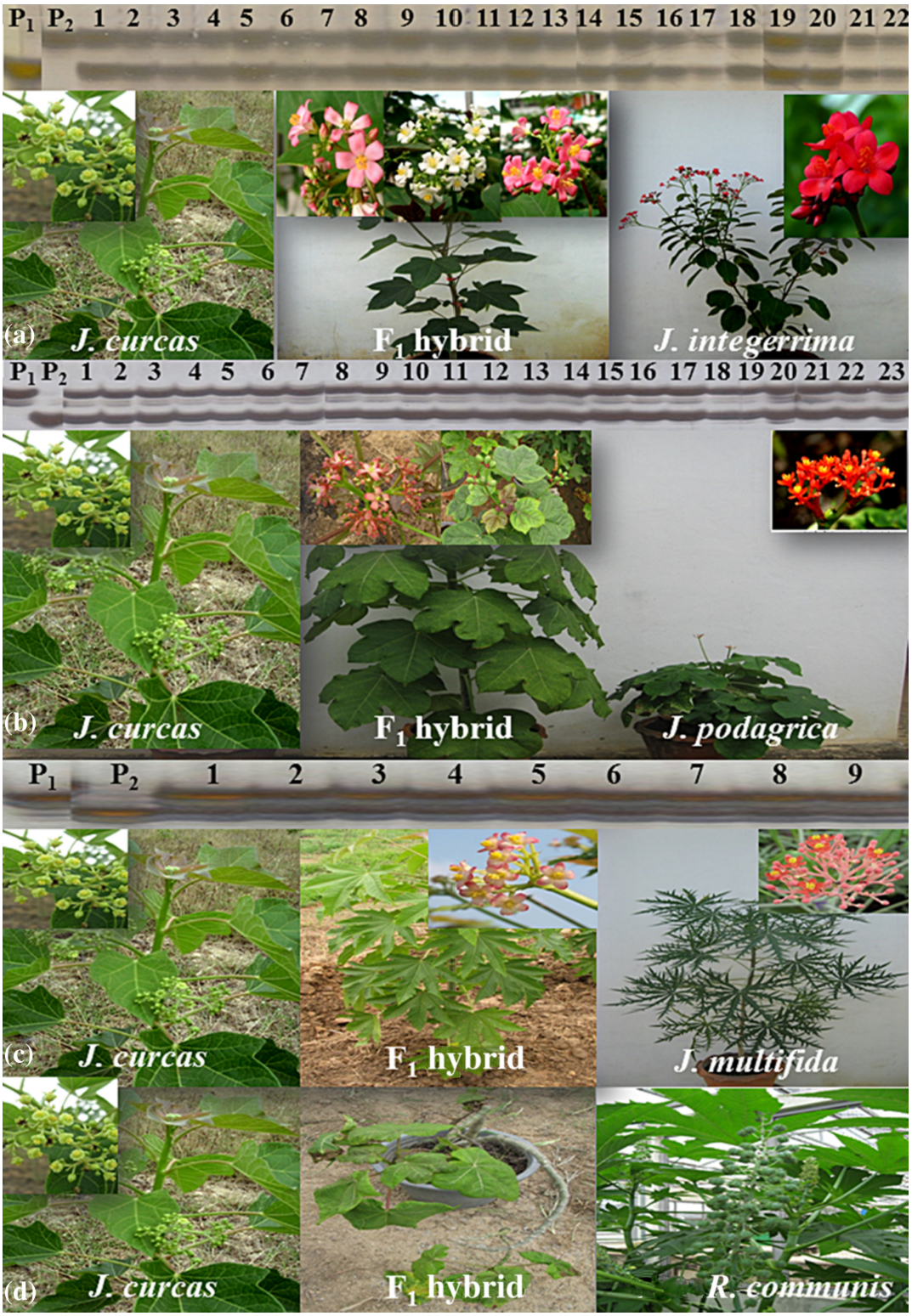

Fig. 1. Expressed sequence tag–simple sequence repeat profile and morphology of (a) *Jatropha curcas* (P_1) and *Jatropha integerrima* (P_2) and their F_1 hybrid assessed by the marker MPN081; (b) *J. curcas* (P_1) and *Jatropha podagrica* (P_2) and their F_1 hybrid assessed by the marker MPN150; (c) *J. curcas* (P_1) and *Jatropha multifida* (P_2) and their F_1 hybrid assessed by the marker MPN150; (d) morphology of the parents *J. curcas* and *Ricinus communis* and their F_1 hybrid plant.

https://doi.org/10.1017/S1479262114000276 Published online by Cambridge University Press

bellyache bush (*J. gossypiifolia*), two accessions of spicy jatropha, two accessions of bottleplant shrub and three accessions of castor bean (*Ricinus communis*). The genetic relationships among species can be used to predict the success of interspecific hybridization. Expressed sequence tag (EST)–simple sequence repeat (SSR) markers are good tools for such a study, owing to their co-dominance, high reproducibility and high polymorphism. Varshney *et al.* (2005) reported high transferability of EST–SSRs across species and genera due to a higher level of conservation found in transcribed sequences compared with the level found in the other genomic regions. In this study, several F_1 plants from crosses between *J. curcas* and *J. integerrima*, *J. multifida*, *J. podagrica* and *R. communis* were obtained. The hybrids were verified using EST–SSR markers developed earlier by our group.

Materials and methods

An accession of *J. curcas* was hybridized with one accession each of *J. integerrima*, *J. podagrica*, *J. multifida* and *R. communis*. In the direct cross, 100 female jatropha flowers were emasculated and hand-pollinated by pollen of each related species. In the reciprocal cross, 100 female flowers of each related species were hand-pollinated by pollen from *J. curcas*. The resulting hybrid seeds were germinated and maintained in the experimental field of the Department of Agronomy, Kasetsart University, Kamphaeng Saen, Thailand. Upon germination, hybridity was confirmed using EST–SSR markers developed by Laosatit *et al.* (2013). The genomic DNA was extracted from young leaves using the protocol of Tanya *et al.* (2011), while the polymerase chain reaction (PCR) and EST–SSR marker analysis were carried out using the protocol of Laosatit *et al.* (2013). These EST–SSR sequences were submitted to the NCBI Probe database with the assigned accession numbers from #PROBEDB_PUID 16586515 to 16586649. The PCR products were run on a 5% denaturing polyacrylamide gel and subsequently silver-stained to check for hybridity.

Results and discussion

We were successful in producing a number of hybrid plants of *J. curcas* with *J. integerrima*, *J. podagrica* and *J. multifida*, but only one plant was obtained with *R. communis*. The hybrid nature was confirmed using both morphology and EST–SSR markers (Fig. 1). Sujatha and Prabakaran (2003) and Basha and Sujatha (2009) reported earlier the success of crossing between *J. curcas* and *J. integerrima*, while Parthiban *et al.* (2009) tried to produce *J. curcas* × *J. integerrima*, *J. multifida* × *J. curcas*, *J. maheshwari* × *J. curcas*, *J. gossypifolia* × *J. curcas* and *J. curcas* × *J. gladulifera* with varying degrees of success. In this study, we were successful in obtaining seeds from *J. podagrica* × *J. curcas* as shown in Table 1. Kumar *et al.* (2009) reported an incompatibility in the cross *J. curcas* × *J. podagrica* due to bulging pollen tubes together with a reverse direction of pollen tube growth. Although the reciprocal cross was successful and six seeds were obtained, all of them aborted. We were successful in producing several mature F_1 seeds from the reciprocal crosses that were able to germinate and grow (Fig. 1(b)). Our direct cross gave immature seeds with no endosperm and a very small embryo that could be observed only under 10 × stereo microscope. However only a single F_1 plant was obtained for *J. curcas* × *R. communis* (Fig. 1(d)). The species-specific characteristics confirmed the true hybrid nature, as observed from flower colour, flower shape and leaf shape. The EST–SSRs developed by Laosatit *et al.* (2013), especially MPN078, MPN091, MPN119, MPN130, MPN134, MPN146, MPN150 and MPN155, clearly identified the hybrid progenies (Fig. 1). Behaviours with regard to flowering, seed setting and breeding of the F_1 plants are being studied.

Table 1. Results of interspecific crossing between *Jatropha curcas* and *Jatropha podagrica* and results obtained from pollinating 100 female flowers in each cross

Crosses[a]	No. of female flowers without fruit set	No. of fruits set but dropped prematurely	No. of mature fruits obtained
$P_1 \times P_3$	59	41	0
$P_3 \times P_1$	87	0	13
$P_2 \times P_3$	63	37	0
$P_3 \times P_2$	92	0	8

[a] P_1 and P_2 are *J. curcas* and P_3 is *J. podagrica*.

Acknowledgements

The senior author thanks the Royal Golden Jubilee PhD programme jointly funded by the Thailand Research Fund and Kasetsart University. The authors thank the Center for Advanced Studies in Agriculture and Food, Institute for Advanced Studies, Kasetsart University, the Chair Professor Project of Thailand's National Science and Technology Development Agency, and the Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok, Thailand.

References

Basha SD and Sujatha M (2009) Genetic analysis of *Jatropha* species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. *Euphytica* 168: 197–214.

Kumar RS, Parthiban KT, Hemalatha P, Kalaiselvi T and Govinda MR (2009) Investigation on cross-compatibility barriers in the biofuel crop *Jatropha curcas* L. with wild *Jatropha* species. *Crop Science* 49: 1667–1674.

Laosatit K, Tanya P, Saensuk C and Srinives P (2013) Development and characterization of EST–SSR markers from *Jatropha curcas* EST database and their transferability across jatropha-related species/genus. *Biologia* 68: 41–48.

Parthiban KT, Kumar RS, Thiyagarajan P, Subbulakshmi V, Vennila S and Rao MG (2009) Hybrid progenies in *Jatropha* – a new development. *Current Science* 96: 815–823.

Ratha KP and Paramathma M (2009) Potentials and *Jatropha* species wealth of India. *Current Science* 97: 1000–1004.

Sujatha M and Prabakaran AJ (2003) New ornamental *Jatropha* hybrids through interspecific hybridization. *Genetic Resources and Crop Evolution* 50: 75–82.

Tanya P, Taeprayoon P, Hadkam Y and Srinives P (2011) Genetic diversity among *Jatropha* and *Jatropha*-related species base on ISSR markers. *Plant Molecular Biology Reporter* 29: 252–264.

Tar MM, Tanya P and Srinives P (2011) Heterosis of agronomic characters in jatropha (*Jatropha curca*s L.). *Kasetsart Journal (Natural Science)* 45: 583–593.

Valdes ROA, Sanchez SO, Perez VA and Caplan J (2013) The Mexican non-toxic *Jatropha curcas* L., food resource or biofuel? *Ethnobotany Research and Applications* 11: 1–7.

Varshney RK, Graner A and Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology* 23: 48–55.

https://doi.org/10.1017/S1479262114000276 Published online by Cambridge University Press