

Full Length Research Paper

Genetic relationships among *Jatropha curcas* L. clones from Panzhihua, China as revealed by RAPD and ISSR

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Although *Jatropha curcas* L. is known as a source of oil-rich seeds as economically viable substitute of diesel, little is currently known about the genetic resource of *J. curcas* in China. In this study, RAPD and ISSR data were utilized to determine the genetic relationships among *J. curcas* clones from Panzhihua, China. Five RAPD and twelve ISSR primers generated reproducible amplification banding pattern of 43 polymorphic bands out of 126 scored accounting, for 34% polymorphism across the clones. The UPGMA cluster analysis indicated two broad groups, one comprising all the clones except the clones from YuanMou, which formed the smaller second group. Congruously, the samples belonging to the same origin grouped into their distinct origin lineages. The overall grouping pattern of clustering was irrelevant to the seeds oil content of these clones. This study provides valid guidelines for collection, conservation and characterization of *J. curcas* genetic resources from Panzhihua and also for further breeding program towards biodiesel production.

Key words: *Jatropha curcas* L., genetic relationship, molecular marker.

INTRODUCTION

Jatropha curcas L. commonly known as purging nut or physic nut, is a perennial deciduous, multipurpose shrub belonging to the family Euphorbiaceae. Primarily *Jatropha* plant was used for its medicinal and ecological values. However, *J. curcas* recently received tremendous attention for its seed oil that can be converted into biodiesel and is considered to be a universally acceptable energy crop (Kumar et al., 2008). *Jatropha* is a native to Mexico and Central American region and later naturalized in many parts of tropic and subtropic regions of the world, where it is grown as a hedge crop to protect gardens and fields from animals and for traditional use (Heller, 1996). *J. curcas* was first introduced in China about three centuries ago, but there were no records of the source origin or where it was first planted (Shen et al., 2010). The species has since been cultivated in Guizhou, Hainan, Sichuan and Yunnan. It is estimated that there are at least two

million hectares under cultivation in China for production of oil-seed (Fairless, 2007). Among these places, Panzhihua area in Sichuan is one of the most important areas for distribution and feedstock of *J. curcas* (Wu et al., 2010).

However, the crop is characterized by variable and unpredictable yield for reasons, which have not been identified which limit the large-scale cultivation and warrant the need for genetic improvement of the species (Kaushik et al., 2007). Despite the potential as a source of vegetable oil for the replacement of petroleum and the interest that is being shown in the large-scale plantation systems of *J. curcas* in cultivated areas of China, the genetic structure of local varieties remains poorly characterized and breeding programs for the selection of improved varieties are scanty in this species (Ye et al., 2009). Accurate identification of species and varieties, and knowledge of their genetic relationship are essential information for effective selection, breeding, genetic resource management programs, variety control, protection and registration, and for the handling of planting and breeding stock (Rahman et al., 2002).

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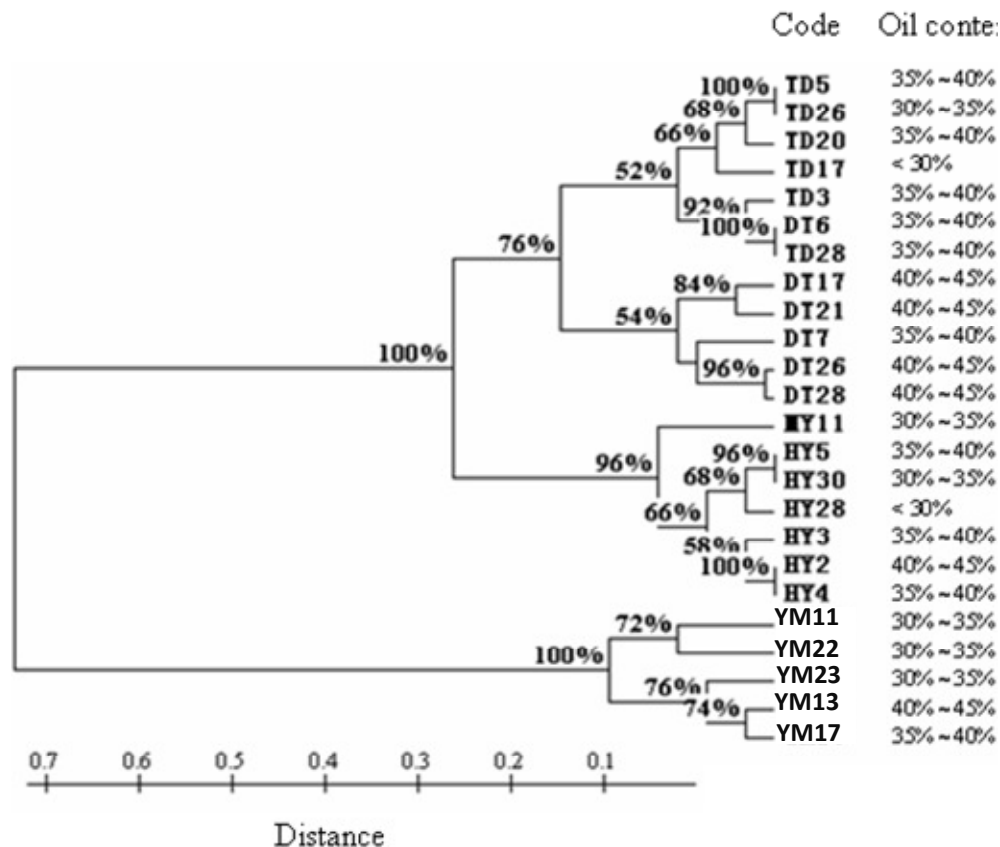


Figure 1. Dendrogram (UPGMA) representing genetic relationships of *J. curcas* clones based on RAPD and ISSR data.

Establishing genetic distances through DNA fingerprinting methods and the information generated can be used for genetic improvement of the species. In *Jatropha*, taxonomic classification and genetic relationships were based on leaf epidermal morphology, petiolar anatomy (Dehgan et al., 1980, 1982). Morphological studies and other traits are insufficient by themselves as taxonomic evidences. Molecular markers reveal more quickly and accurately genetic difference far exceeding those obtainable using morphological or biochemical methods without obscuration of environment. Nuclear DNA analysis represent an important tool for phylogenetic and diversity analysis of plants. RAPD markers cover the entire genome revealing length polymorphisms in coding or non-coding and repeated or single copy sequences while ISSR markers generate polymorphism from sequences between two microsatellite primer sites (Williams et al., 1990; Zietkiewicz et al., 1994).

The aim of the present study was to assess genetic relationships among *J. curcas* clones from Panzhihua, China using molecular markers. Two types of molecular markers RAPD, ISSR were applied. We also evaluated the grouping pattern of clustering associated with seed oil content of these clones.

MATERIALS AND METHODS

Plant materials and DNA extraction

The 24 *J. curcas* clones with different seed-oil content from Panzhihua, China were selected, including 6 clones from TongDe, 6 clones from Datong, 5 clones from HanYu, 5 clones from YuanMou and 1 clone from MiYi. Details of the codes and seed-oil content are given in Figure 1. For each plant, about 100 mg of young leaves were used for DNA extraction with modified protocol of Pamidimarri et al. (2008). DNA concentration was determined electrophoretically versus known amount of λ DNA as standards. For PCR, DNA samples were adjusted to a concentration of 25 ng μl^{-1} .

RAPD analysis

74 RAPD primers (Operon Technologies, Alameda, CA, USA) were used for amplification of DNA according to the method of Williams et al. (1990). PCR amplification was carried out in a 25 μl reaction mixture containing reaction buffer, 150 μM of dNTP (Promega), 0.3 μM of each primer, 1.5 U of Taq polymerase (TaKaRa) and 25–50 ng of genomic DNA. PCR amplification was carried out in Bio-Rad C1000 thermal cycler with an initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 36°C for 60 s and extension at 72°C for 1.5 min with a final extension at 72°C for 5 min. The amplified PCR products were resolved by electrophoresis on 1.5% agarose (Spain) gel in 0.5 \times TBE buffer by electrophoresis at 100 V for 2.5 h and visualized with GelRed™ (Biotium) staining. Every PCR reaction was repeated

twice to check reproducibility of bands and a negative control (no DNA) was used in all reactions to avoid erroneous interpretations.

ISSR analysis

A total of 69 ISSR primers (UBC primer set No. 9, University of British Columbia, Canada) were used for the analysis. PCR amplification was performed in a 20 µl reaction volume, containing 2.0 µl of the 10×reaction buffer (TaKaRa, Dalian, Mg²⁺ free), 1.8 mM MgCl₂ buffer, 150 µM dNTP (Promega), 0.25 µM primer, 1.0 U Taq polymerase (TaKaRa, Dalian) and 25 ng of genomic DNA. The mixture was overlaid with mineral oil and subjected to PCR on a Bio-Rad C1000 thermal cycler programmed for an initial step of 5 min at 95°C, followed by 40 cycles of 1 min at 94°C, 1 min at annealing temperature, and 2 min at 72°C, with a final extension step of 10 min at 72°C. PCR products were analyzed on 1.8% agarose gels and stained with GelRed™ (Biotium). The gel images were recorded and the band sizes were quantified using a Gel Doc 2000 system (Bio-RAD). Each PCR reaction was performed at least twice to ensure the reproducibility.

Data analysis

For the RAPD and ISSR analysis, the fragment size scored ranged from 200 to 2000 bp. Weak bands were excluded from final data analysis. Since both markers were interpreted as dominant markers, the bands were scored as diallelic characters either as 1 (present) or 0 (absent). A pair-wise similarity matrix was calculated using the simple matching coefficient. This similarity matrix was employed to construct a dendrogram by UPGMA, using the TREECON 1.3b software to illustrate the genetic relationships among the species.

RESULTS AND DISCUSSION

Out of the 74 primers employed to detect genetic differences among the samples, 35 detected at least one RAPD marker, 29 showed amplification without detection of polymorphisms, while 10 gave a weak or no amplification. Of the 69 ISSR primers tested, 18 primers resulted in ISSR bands. For both markers, only those bands showed clear amplification and polymorphisms were scored to estimate the genetic relationships of *J. curcas* clones. Eventually, a total of 126 bands from 5 RAPD and 12 ISSR primers were scored. Of these, 43 were polymorphic resulting in a polymorphism of 34%. There were no specific-bands amplified exclusively for each clone.

A similarity matrix was calculated based on RAPD and ISSR data, and a dendrogram was constructed (Figure 1) to reveal the genetic relationships among the *J. curcas* clones. The UPGMA-based dendrogram showed that the 24 clones could be classified into two broad groups. In addition, the UPGMA dendrogram separated the first group into three subgroups: (1) a cluster formed by the clones mainly from TongDe (TD5, TD26, TD20, TD17, TD3, TD28 and DT6) and (2) a cluster containing clones from DaTong (DT17, DT21, DT7, DT26, DT28) and (3) a cluster consisting of clones mainly from HanYu (HY5, HY30, HY28, HY3, HY2, HY4 and MY11). The second,

smaller group consisted of 5 clones (YM11, YM13, YM17, YM22 and 23) from YuanMou. Congruously, the samples belonging to the same origin grouped into their distinct origin lineages, with the exception of the clone DT6 grouped in TongDe subgroup and MY11 grouped in HanYu subgroup.

Our results demonstrated that the *J. curcas* clones studied from the same place have close affinity and cluster together. The greatest genetic distance was found between clones from YM with other subgroups. This result revealed that clones from YuanMou, located geographically furthest apart from the other clones, were also genetically most distant. The average polymorphism obtained from this study was low, 34%, which is in the range of 26 ~ 39.3% from the previous ISSR and RAPD analysis from *J. curcas* (Basha et al., 2009), but still higher than the 14.75 and 26.99% from the previous AFLP analysis by Sun et al. (2008) and Shen et al. (2010) for other *J. curcas* materials in China.

However, high polymorphism in *J. curcas* populations in China based on ISSR analysis has been reported. Nine populations from 5 different provinces in China were examined by He et al. (2007) and a 97% polymorphism among these materials was reported. Another 11 populations from 3 provinces in China were examined, and 69% polymorphism was reported by Ou et al. (2009). Panzhihua is only located in the Southwest Sichuan province, China and is famous for its development of *J. curcas* cultivated program. As the pedigree data are not available on the clones analyzed, the low polymorphism indicates the higher probability of origin of all these clones from the same source and eventually distribution to difference parts of Panzhihua area.

As percentage oil content data were available for all the clones analyzed, an attempt was made to understand the correlation between different groups/clusters with percentage oil content. It reported that no specific trend between clusters based on molecular markers and clusters based on seed-oil content and seed weight (Tatikonda et al., 2009). In the present study, any specific trend was not observed between clusters with percentage oil content. However, in the same group or subgroup, some clones with same seed oil content level clustered together, that is DT17 and DT21, DT26 and DT28 with 40~45% oil content, TD3 and TD28 with 35~40% oil content, YM11 and YM22 with 30~35% oil content, probably indicating their similar oil content might result from close genetical origin.

In summary, the present study provided a certain number of reliable and reproducible fingerprint profiles for the 24 clones of *J. curcas* collected from Panzhihua, China. It suggested that the selection of local Chinese material for genetic improvement program must be done with due consideration to the low genetic diversity found in this and other studies about *J. curcas* (Shen et al., 2010). Availability of specific fragments present in different clones together with genetic similarity would be very

useful for improvement of the species through conventional breeding methodologies, as well as molecular breeding approaches such as marker assisted selection.

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REFERENCES

- Basha SD, Francis G, Makkar HPS, Bechker K, Sujatha M (2009). A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. *Plant Sci.*, 176: 812-823.
- Basha SD, Sujatha M (2009). Genetic analysis of *Jatropha* species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. *Euphytica*, 168(2): 197-214.
- Dehgan B (1980). Application of epidermal morphology to taxonomic delimitations in the genus *Jatropha* L. (Euphorbiaceae). *Bot. J. Linnean Soc.*, 80: 257-278.
- Dehgan B (1982) Comparative anatomy of the petiole and infrageneric relationships in *Jatropha* (Euphorbiaceae). *Amer. J. Bot.*, 69: 1283-1295.
- Fairless D (2007). The little shrub that could-maybe. *Nature*, 449: 652-655.
- He W, Guo L, Wang L, Yang W, Tang L, Cheng F (2007) ISSR analysis of genetic diversity of *Jatropha curcas* L. *Chinese J. Appl. Environ. Biol.*, 13(4): 466-470.
- Heller J (1996). Physic nut-*Jatropha curcas* L., in *Promoting the Conservation and Use of Underutilized and Neglected Crops*. International Plant Genetic Resources Institute, Rome, Italy, <http://www.ipgri.cgiar.org/publications/pdf/161.pdf>.
- Kaushik N, Kumar K, Kumar S, Kaushik N, Roy S (2007). Genetic variability and divergence studies in seed traits and oil content of *Jatropha* (*Jatropha curcas* L.) accessions. *Biomass and Bioenergy*, 31: 497-502.
- Kumar N, Pamidimarri SDVN, Kaur M, Boricha G, Reddy MP (2008). Effect of NaCl on growth, ion accumulation, protein, proline contents and antioxidant enzymes activity in callus cultures of *Jatropha curcas*. *Biologia*, 63(3): 378-382.
- Ou WJ, Wang WQ, Li KM (2009). Molecular genetic diversity analysis of 120 accessions of *Jatropha curcas* L. germplasm. *Chinese J. Trop. Crops*, 30(3): 284-292
- Pamidimarri DVNS., Meenakshi, Sarker R, Boricha G, Reddy MP (2008). A simplified method for extraction of high quality genomic DNA from *Jatropha curcas* for genetic diversity and molecular marker studies. *Indian J. Biotechnol.*, 8: 187-192.
- Rahman MH, Rajora OP (2002). Microsatellite DNA fingerprinting, differentiation, and genetic relationships of clones, cultivars, and varieties of six poplar species from three sections of the genus *Populus*. *Genome*, 45: 1083-1094.
- Shen JL, Jia XN, Ni HQ, Sun PG, Niu SH, Chen XY (2010). AFLP analysis of genetic diversity of *Jatropha curcas* grown in Hainan, China. *Trees*, 24: 455-462
- Sun QB, Li LF, Li Y, Wu GJ, Ge XJ (2008) SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. *Crop Sci.*, 48: 1865-1871.
- Tatikonda L, Wani SP, Kannan S, Beerelli N, Sreedevi TK, Hoisington DA, Devi P, Varshney RK (2009) AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant. *Plant Sci.*, 176: 505-513.
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.*, 18: 6531-6535.
- Wu WG, Huang JK, Deng XZ (2010) Potential land for plantation of *Jatropha curcas* as feedstocks for biodiesel in China. *Sci. China (Earth Sciences)*, 53(1): 120-127.
- Ye M, Li CY, Francis G, Makkar HPS (2009). Current situation and prospects of *Jatropha curcas* as a multipurpose tree in China. *Agrofor. Syst.*, 76: 487-497.
- Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.