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SHORT COMMUNICATION

Genetic diversity of *Jatropha curcas* L. in Senegal compared with exotic accessions based on microsatellite markers

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Abstract Significant efforts have been undertaken in West Africa to increase biofuel production with the expectation to alleviate the dependency on fossil energies and to reduce rural poverty by diversifying cultivated crops. In this context, *Jatropha curcas* L., a shrub belonging to Euphorbiaceae family, has gained great interest because of its oil which can be converted to biodiesel. It is also highly adaptable to marginal soils due to its drought-tolerant characteristics. Characterisation of *J. curcas* germplasm in Senegal could be an important input for its better management and in identifying genotypes that could be used in breeding program. Genetic diversity of 103 accessions including 82 accessions from different agro ecological zones in Senegal and 21 exotic accessions was assessed through 33 microsatellite markers. All the markers gave amplifications at the expected band size. Only one microsatellite marker, JCT17, was polymorphic showing 3 alleles and allows distinguishing 2 accessions from Burkina Faso. The surprisingly low level of genetic variation might be because introduction of *J. curcas* in Senegal seems to have been done from one or a few origins and the species has not regained genetic diversity since then due to vegetative propagation. Cultivation of *J. curcas* at large scale may face to vulnerability to pests and require many cautions. They are necessity to widen the genetic base of *J. curcas* in Senegal via new introductions from its centre of origin.

Keywords Genetic diversity · *Jatropha curcas* · Microsatellite marker · Senegal

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Introduction

Jatropha curcas L. is a small tree or large shrub which can reach height up to 5 m and has high potential for greening and eco-rehabilitation of wastelands (Heller 1996; Wani et al. 2006). Belonging to family Euphorbiaceae it is believed to be a native species of Mexico,

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has been introduced in Africa and Asia and cultivated worldwide (Heller 1996; Dias et al. 2012). *J. curcas* is known to exhibit drought-tolerant characteristics, similar to other stem-succulent oil producing species such as *Ricinus communis* (Simbo et al. 2013). In the last decade, owing to its multiple uses and its drought ability, the species has received attention of both researchers and policy makers in Sahelian regions as a source of biodiesel. Indeed, *J. curcas'* oil has been identified as an efficient substitute that can be used as fuel for diesel engine (Gübitz et al. 1999; Villena-Denton 2008). In addition, it is expected that the cultivation of *J. curcas* will reduce poverty especially among women by stimulating economic activities in rural areas. Because of these advantages, the government of Senegal has planed to produced 3,210,000 T of *J. curcas* seed per year (Dia et al. 2010). However, the development of *J. curcas* as a biodiesel crop is confronted by low yield that is also highly variable from year-to-year, making effective planning difficult (Surwenshi et al. 2011; Terren et al. 2012).

Collection and characterization of germplasm around the world are encouraged in order to identify genotypes that could be used in breeding for high yielding seeds and high oil content varieties (Ovando-Medina et al. 2011). Previous characterization of *J. curcas* germplasm in Senegal has focused on phenotypic aspect. To the best of our knowledge, only one molecular characterisation of 6 accessions of *J. curcas* through random amplified polymorphic DNA (RAPD) markers has been reported (Ndoye et al. 2013). Heller (1996) reported significant differences in the development of 11 provenances of *J. curcas* in multi-location field trials in Senegal and Cape Verde. Leye et al. (2009) noted variability in seedling growth of 7 provenances of *J. curcas* inoculated with mycorrhizal fungi. Ouattara et al. (2013) showed significant differences in seed traits (100 seeds weight, seed length) of *J. curcas* collected in Senegal. These results showed high phenotypic variations but the determinism of such variations is unknown especially in a species that has been introduced in Senegal and propagated mainly by cutting (Ndoye et al. 2013). Molecular markers used in assessment of genetic variation in *J. curcas* germplasm (Basha and Sujatha 2007; Sun et al. 2008; Pamidimarri et al. 2009; Tatikonda et al. 2009; Cai et al. 2010; Shen et al. 2010; Machua et al. 2011; Pecina-Quintero et al. 2011; Khurana-Kaul et al. 2012; Rafii et al. 2012; Shen et al. 2012) showed a very low genetic diversity in *J. curcas*. So, one can ask if high phenotypic variation of *J. curcas* in Senegal is as a result of a large genetic base or of plasticity? This question is of great importance for future *J. curcas* germplasm collection and preservation in Senegal and for breeding programs.

Microsatellites or simple sequence repeat (SSR) markers are known as an appropriate tool to investigate the genetic diversity in many species because of its multi-allelic nature, reproducibility, codominant inheritance, high abundance and extensive genome coverage (Gupta and Varshney 2000). In India, microsatellite markers have been used to assess genetic diversity of *J. curcas* (Basha et al. 2009; Pamidimarri et al. 2009). In this study, 33 microsatellite markers have been used to characterize 103 accessions of *J. curcas*, of which 82 accessions from different geographical regions within Senegal and 21 exotic accessions.

Materials and methods

Plant material

Mature fruits of *J. curcas* were collected in 82 sites from different ecological zones Senegal with different rainfall patterns and type of soil (Fig. 1). For each site, one accession was collected. For each of the 82 accessions, fruits were collected from ten trees (500 g per tree), separated by at least 10 m. The fruits of each tree were labeled separately. All the collection sites were geo-referenced through a global position system (GPS). Arcview 3.2 was used for point mapping of the sites. Ten seeds of ten different trees (one seed per tree) of each Senegalese accession and 21 exotic accessions were sown in pots in the experimental station of CERAAS, Regional Centre for Studies on the Improvement of Plant Adaptation to Drought (Table 1). Accessions from Burkina Faso, Benin and Mali were collected by scientific associates. The others exotics accessions were available in *J. curcas* collection at ENSA (National high school of agriculture, Senegal).

DNA extraction

DNA was extracted from young fresh leaves of each seedling separately (one seedling per accession) following the standard CTAB method with minor

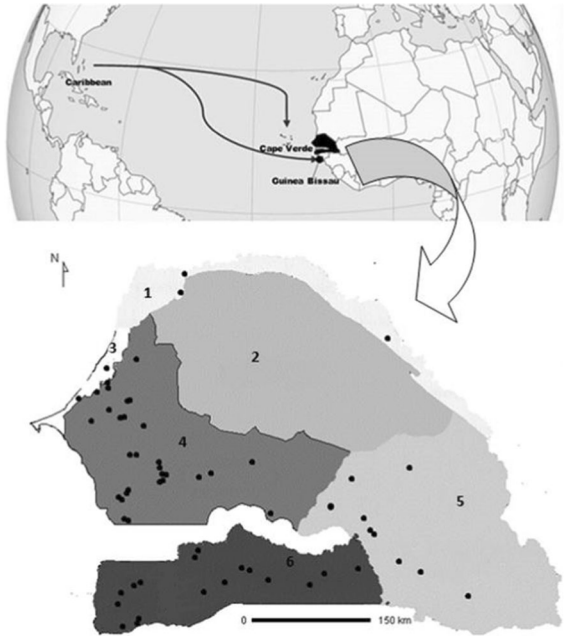

Fig. 1 Mapping of the collection sites of *J. curcas* L. accessions from Senegal and diffusion of *J. curcas* from Caribbean according to Heller (1996). The colors represent different ecological zones of Senegal (1 Senegal river zone; 2 Sylvopastoral zone; 3 Niaye zone; 4 Groundnut basin; 5 Eastern Senegal; 6 Casamance). Some sites are very close and it was not possible to represent them separately

Table 1 Geographical origins of the 103 *J. curcas* accessions

Continent	Country	Number of accessions
Africa	Senegal	82
	Burkina Faso	10
	Benin	4
	Mali	1
	RD Congo	1
	Mozambique	1
	Tanzania	1
Asia	Cambodge	1
	India	1
America	Guatemala	1
Total		103

modifications (Bi et al. 1996). About one hundred milligrams of fresh leaves of each seedling were ground separately to a fine powder, then homogenized in 750 µl of extraction buffer (2 % (p/v) CTAB, 20 mM EDTA, 4 % (p/v) PVP, 2 M NaCl, 100 mM Tris–HCl pH 8.0 and 5 % (v/v) β-mercaptoethanol) and incubated at 65 °C for 1 h. During incubation, from time to time, the microtubes were gently mixed by inverting. After the 1 h, 750 µl of chloroform isoamyl alcohol (24:1 v/v) was added in each microtube under fume hood. The microtubes were gently mixed for 5 min by inverting and spun for 20 min at 13,000 rpm at 20 °C. The acqueous phase was transferred carefully into clean microtubes. Then the DNA was precipitated with a cold solution of isopropanol (¾ of the recovered volume of acqueous phase). The microtubes were gently inverted to be sure mixing is complete before incubating at −20 °C for 1 h. After, microtubes were spun for 20 min at 4 °C and the supernatant was removed. The pellet DNA was washed with a washing buffer (76 % ethanol and 10 mM ammonium acetate) by mixing gently for 30 min. Then, the microtubes were spun for 20 min at 4 °C. The supernatant was removed and the pellet DNA was air dried at room temperature by leaving microtubes open. The pellet DNA was re-suspended in 100 µl of 1× TE (pH 8.0). The solution was treated with RNase A (100 µg/ml), incubated at 37 °C for 30 min before storing at 4 °C overnight. The following day, DNA concentration was determined by running the samples on a 0.8 % agarose gel based on the intensities of bands by comparison with the Lambda DNA marker as standard and diluted in 1× TE to a concentration of 5 ng/µl.

Microsatellite primers

Primer pairs specific to 33 microsatellite containing sequences of *J. curcas* were used. They were selected considering their high level of polymorphism reported by different authors (Table 2). The forward of each of the 33 primer pairs used (Table 2) was designed with a 5′-end M13 tail (5′-CACGACGTTGTAAAACGAC-3′) and PCR products were separated on sequencer (Licor 4300 DNA Analyzer). The designed primers were synthesized from SBS Genetech (Beijing, China).

PCR reactions were set up in a total volume of 10 µl, consisting of 25 ng of template DNA, 1× PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 500 µM $MgCl_2$, 200 µM of mix dNTPs, 0.2 µM of each of the forward and reverse primers and 1 U of Taq DNA polymerase (SBS Genetech, China). In the second study, 0.1 µM of the M13 primer-fluorescent dye IR700 or IR800 (MWG, Germany) were added to the reagents. The touchdown PCR program used was as described by Fonceka et al. (2009).

Table 2 Origin and associated information for the 33 microsatellites. Primers Jcps1 and jcms30 were excluded in the second study

Reference	Primer code	Repeat Motif	Expected size (bp)	Number of allele reported
Basha et al. (2009)	JcSSR-18	$(TA)_3(GT)_{18}$	387–397	3
	JcSSR-21	$(C)_7(A)5(CA)_9$	245–324	3
	JcSSR-26	$(CA)_{18}$	193–234	4
	jcSSR-28	$(CA)_{17}$	Multilocus	3
Zubieta et al. (2009)	JcSSR1	$(AG)_{15}$	168–187	4
	JcSSR2	$(CT)_{13}$	123–138	4
	JcSSR3	$(GA)_{24}$	164–195	6
	JcSSR4	$(AG)_{20}$	178–198	6
	JcSSR6	$(TG)_{23}$	170–182	4
Ambrosi et al. (2010)	JcSSR7	$(TG)_{18}$	148–202	3
	JcSSR8	$(AC)_{17}$	154–182	4
	JcSSR10	$(CT)_{16}$	178–184	5
Pamidimarri et al. (2009)	jcds10	$(TG)_6CACGCA(TG)_4$	108–122	4
	jcds24	$(CA)_5(TA)_8(CA)_4\ldots(TA)_3GA(TA)_4$	204–206	11
	jcds41	$(CA)_6(TA)_2$	102–114	5
	jcds58	$(GT)_4(GA)_5$	104–112	3
	jcds66	$(CT)_2(GT)_3ATTGCA(AT)_4$	216–228	3
	jcps6	$(AT)_3G(TA)_3\ldots(CT)_3\ldots(GT)_5CT(GT)_3$	288–305	4
	jcps9	$(TG)_{12}(GA)_{22}$	140–165	4
	jcps20	$(CA)_2\ldots(CA)_4$	224–260	9
	jcps21	$(CA)_7$	189–200	3
	jcms21	$(GT)_5T(TG)_2$	15–88	3
Phumichai et al. (2011)	JCT15	$(A)_{22}\ldots(CT)_{10}$	–	3
	JCT17	$(GA)_6\ldots(GA)_{11}(GT)_{21}$	–	4
	JCT27	$(CT)_{17}$	–	3
Na-ek et al. (2011)	JCT158	$(CT)_7$	–	3
	JCT249	$(TC)_4\ldots(TC)_{14}$	–	3
Kumar et al. (2011)	JEMO13	$(AGAGGC)_4$	208–230	4
	JEMO65	$(AG)_7$	187–221	3
	JEMO99	$(TA)_5$	100–150	3
	JEM100	$(TA)_6$	132–154	4
	JMDB04	$(AT)_7$	104–128	3
	JMDB57	$(AAT)_8$	118–187	3

Revelation on sequencer Licor 4300 DNA Analyzer (USA)

IR700 or IR800-labeled PCR products were diluted 12-fold in 1× blue-urea, subjected to electrophoresis in a 5.5 % polyacrylamide gel and then sized by the IR fluorescence scanning system of the sequencer (Licor 4300 DNA Analyzer).

Results

All the 33 primer pairs used gave good amplification in the expected band size and all the individuals were homozygote. Only one primer (JCT17) was polymorphic and revealed three alleles, others 32 microsatellite primer pairs yielded a monomorphic product across the 103 *J. curcas* accessions studied (Fig. 2).

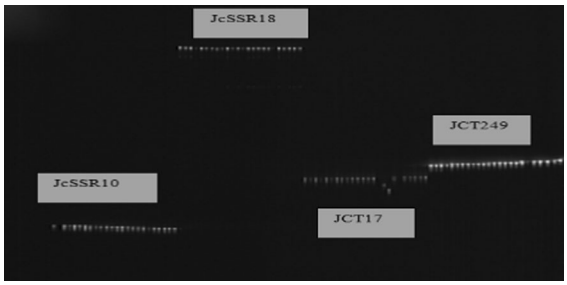

Fig. 2 Molecular analysis of *J. curcas* germplasm through microsatellite primers. PCR products were analyzed on sequencer Licor 4300 DNA Analyzer

Although JCT17 was polymorphic, it failed to distinguish accessions from different ecological zones of Senegal. All the Senegalese accessions showed the similar banding pattern with all the microsatellite primers including JCT17. The primer JCT17 grouped together all the accessions independently of their origins except 2 accessions from Burkina Faso that showed each one specific allele. There was no overall partitioning of genetic diversity that corresponded to geographic origin.

Discussion

The 33 microsatellite primer pairs used failed to distinguish the 82 accessions from Senegal. Despite of large diffusion of *J. curcas* in different ecological zones in Senegal and early high phenotypic variation reports (Heller 1996; Ouattara et al. 2013), genetic diversity is very low. That reflects an initial introduction with a low genetic base and assumes a vegetative propagation as the main way of multiplication of *J. curcas* as reported by Ndoye et al. (2013). The ability of *J. curcas* to grow in different ecological zones in Senegal seems to be based on its ecological plasticity than genetic diversity as reported by Johnson et al. (2011). Because of the multilocation trials of *J. curcas* in Senegal and Cape Verde (Heller 1996) and the possible exchange of plant material due to the proximity of Senegal to Cape Verde and Guinea Bissau, hypothesis of a narrow genetic basis in *J. curcas* introduced in Africa by Portuguese seafarers is not unreasonable. The low genetic diversity recorded in the accessions from other countries of Africa confirms this hypothesis (Ovando-Medina et al. 2011). Our result revealed a high degree of similarity among the accessions from America, Asia and Africa. All the 33 microsatellite primers used revealed a single allele except JCT17 which revealed 3 alleles. Polymorphism observed with the marker JCT17 in accessions from Burkina Faso may be due to recent introductions or the result of somatic mutations accumulated since introduction of *J. curcas* in this country (Ovando-Medina et al. 2011). Low genetic diversity and lack of genetic substructing according to geographic origin can be explained by the fact that *J. curcas* is an introduced species and has been multiplied mainly by cutting to build live fence. Because of vegetative propagation of *J. curcas*; exchange of *J. curcas* cuttings between parents and population drift in the past (Traoré 1994) make the possibility that all the accessions of Senegal being derived from the same or restricted origins. Ndoye et al. (2013) reported 42.68 % of polymorphism using five RAPD markers on six accessions of Senegal. These results were not confirmed in this study. This highlighted the problem of the accuracy of RAPD markers. False positive and false negative bands with RAPD markers have been reported (Lamboy 1994; Pomper et al. 1998).

The introduction history of *J. curcas* propagation in Senegal seems to be similar to that in China and India where narrow genetic diversity has been reported. Basha et al. (2009) reported that 17 microsatellite primers used on 42 accessions of *J. curcas* from different geographic regions of India failed to reveal polymorphism. In addition, the authors reported that *J. curcas* accessions from different countries of the world (from Africa, Asia and America) failed to be distinguished except accessions from Mexico and El Salvador. Studying the genetic relationships of 58 accessions of *J. curcas* from China, Sun et al. (2008) highlighted a very low genetic diversity; among the 17 microsatellite primers used in their study, only 1 primer was polymorphic with 2 alleles. In the same study AFLP analysis revealed 14.3 % of polymorphism. Using RAPD, AFLP and combinatorial tubulin based polymorphism; Popluechai et al. (2009) reported a narrow genetic variation among 38 accessions of *J. curcas* from 13 countries on 3 continents. All these results seem to demonstrate a very low genetic divergence among accessions in introduction areas. These results support the fact that the cultivated forms of *J. curcas* across the world have derived from one or a few clones of *J. curcas* as it has been reported in the case of rubber (Carron et al. 1989). These authors reported a constricted genetic base in rubber worldwide and concluded that the plantations of

rubber in the world have been derived from a limited number of trees.

The explanation given for the exceptionally low levels of variation in several species is that they went through severe genetic bottlenecks during their evolutionary history and have not regained genetic diversity via mutation since that time (Godt and Hamrick 1996). That seems to be the case of *J. curcas* which has been introduced in Africa via Portuguese seafarers. Despite long time introduction, *J. curcas* did not regain genetic diversity. Anthropogenic and environmental factors in generating genetic variability seems to be missing in case of *J. curcas* probably due to the fact that it is not a crop and is a well-surviving plant that likely acquired adaptive genomic characters before its global distribution (Popluechai et al. 2009).

The low genetic base of *J. curcas* in Senegal could reduce the possibilities of its improvement through intra-specific breeding using local accessions. Moreover, its cultivation at large scale might face to vulnerability to pests. Terren et al. (2012) reported severe attacks of fungus that killed about 65 % of *J. curcas* plants, in 3 years, in a plantation of six hectares in Northern Senegal.

To increase genetic base of *J. curcas* especially aiming to improve agronomic traits suggests exploring the diversity in countries of origin. In Senegal, the expectation of national biofuel program in producing biodiesel from *J. curcas'* oil is based on high yielding genotypes that yields were estimated to 10 t ha^{-1}. To our knowledge, no genotype can produce under semi arid conditions in Senegal as much. So, significant efforts have to be done exploring different methods in crop improvement to develop genotypes high yielding and oil content of *J. curcas* to make its cultivation a profitable business.

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