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Establishment of new strategies to quantify and increase the variability in the Brazilian Jatropha genotypes

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ABSTRACT

The genetic diversity of Brazilian Jatropha (*Jatropha curcas*) germplasm collection (BJGC), which consists of 192 accessions, has been previously reported based on the evaluation of a limited number of RAPD and SSR markers. In addition, accessions from other countries (Guatemala and Mexico) were introduced to this collection without prior information on their relation with the existing collection. Thus the objective of this study were to develop and validate a panel for high-density genome-wide molecular markers for Jatropha; to compare the diversity of the germplasm collected in Brazil and the materials introduced and/or generated by crossing selected genotypes; and to determine whether the germplasm introduction strategies and the controlled crossings were efficient in generating/increasing the genetic variability available to the breeding program. This study reported an evaluation on the existence and on the structure of the genetic diversity of a core collection of 92 accessions by using DArT and SNP markers. The genotyping-by-sequencing approach allowed genotyping 747 polymorphic SNP and 4007 DArTs. The pairwise genetic dissimilarity was estimated according to the Jaccard's coefficient, and clustered by the UPGMA and Tocher's clustering methods The genetic diversity distribution was assessed by the analysis of molecular variance (AMOVA). The mean dissimilarity between accessions was low (0.165), and it confirmed that the diversity of the BJGC is very limited. Cluster analysis demonstrated that the introduced genotypes were divergent, and they formed a separate group, indicating that new introductions such as these will be important to promote future efforts on genetic breeding. Accordingly, data from SNP also confirmed the development of a significant amount of genetic variability within families (65%), which probably resulted from the use of the Mexican accession that was found to be considerably divergent when compared with the Brazilian accessions. These results indicate that breeders should focus on two main strategies to generate variability in the BJGC: introduction of new accessions from other countries and crossings between potential genotypes.

1. Introduction

Jatropha (*Jatropha curcas* L.) is a perennial shrub of the family Euphorbiaceae. Although it is speculated that the species is native to Central America (Abbink et al., 1998), Jatropha is currently dispersed worldwide. Jatropha is a diploid species with 22 small chromosomes (1.71–1.24 µm), of which five are metacentric (1, 2, 5, 6 and 11), and six are submetacentric (3, 4, 7, 8, 9 and 10). Its genome is considered small (C = 416 Mb), when compared with other species in the family Euphorbiaceae. Its adjusted base composition is AT = 61.3% and GC = 38.7% (Carvalho et al., 2008). Jatropha has unisexual, insect-pollinated monoeceious flowers, and shows protandry. However, hermaphroditic, self-pollinated flowers may occasionally occur (Kumar and Sharma, 2008). Jatropha has spread around the world due to its robustness, easy propagation, resistance to drought, high oil content, low seed cost, fast growth, easy adaptation to a wide range of agro-climatic conditions, bushy nature, and to the multiple uses of its different parts (Achten et al., 2010). Jatropha has increasingly stood out due to its huge potential as raw material for biofuel production demonstrated in studies (Junqueira et al., 2016; Peixoto et al., 2016). In Brazil, for example, Jatropha has been considered as a potential culture to be used by the National Program for Biodiesel Production and Use released by the Brazilian government (Durães et al., 2011; Teodoro et al., 2017), and also for the production of drop-in jet biofuels (Road map – FAPESP). A single Jatropha plant (non-improved genetic material) can yield more than 2.5 kg of seeds, with oil content ranging

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between 30 and 40% (Bhering et al., 2013; Sunil et al., 2008; Rao et al., 2008). This means that a single plant can produce 0.75–1.0 kg of oil. For instance, if 1.250 plants can be grown in one hectare (spaced 4 × 2 m apart), Jatropha can produce from 937 to 1250 kg of oil per hectare. To put in perspective, soybean, the most planted oil seed crop in Brazil, produces on average 500 kg of oil per hectare. Therefore, even at lower production estimates, Jatropha plants can yield twice as much as soybean. Added to this, Jatropha is a perenial crop, which means that it can be commercially explored for a long period.

Jatropha's origin is very controversial, and no scientific evidences have yet confirmed its center of origin and/or species diversity. According to reports, Jatropha can be found in greater proportion in intertropical regions (Heller, 1996), especially in countries from South America, Central America, Africa and Asia. Despite being present in all of these regions, the strongest evidence lies on the fact that the species is originated in Central America (Heller, 1996). History reports that from the Caribbean, where the species was used by the Mayas, Jatropha was probably dispersed by Portuguese vessels from Cape Verde and Guinea-Bissau islands to other African and Asian countries (Heller, 1996). Around 1783, it was introduced to Cape Verde archipelago, subsequently reaching Africa and India. Later, the species was spread throughout all tropical regions. The introduction of the species in Cape Verde islands was attributed to the interest of the Portuguese people in taking advantage of the unsuitable land in the archipelago, which had low fertility soil and could be barely used for less hardy crops. It is likely that, close to that time, Jatropha had also been introduced to Brazil, where its oil was used for rural and urban public lighting in the state of Rio de Janeiro (Saturnino et al., 2005).

Although the interest in Jatropha has recently increased, and several information on the genetic diversity of germplasm collections is available in the world (Peixoto et al., 2017; Montes et al., 2014; Durães et al., 2011), it is necessary more studies about Brazilian accessions and new accessions that have been introduced in Brazilian germplasm collection. A germplasm bank, known as Brazilian Jatropha germplasm collection (BJGC), with about 200 Jatropha accessions and over 2000 unique genotypes was established in Brazil in order to support future breeding efforts (Bhering et al., 2015; Laviola et al., 2010; Rosado et al., 2010). The BJGC is one of the largest collections in the world in terms of absolute numbers of Jatropha genotypes, and it represents most of the Jatropha genetic variability in Brazil (Bhering et al., 2015; Rosado et al., 2010). Its initial characterization was performed by means of phenotypic (Laviola et al., 2010) and molecular data (Rosado et al., 2010). This characterization indicated limited genetic diversity, and the presence of duplications within the collection, which is a possible reflection of the common ancestry for these accessions and of the drift effects and intensive selection of the currently cultivated material since its introduction to Brazil (Rosado et al., 2010). These results highlighted the urgent need for increasing the representation of this germplasm collection by incorporating new subsamples brought mainly from the species' center of origin, in order to ensure the genetic diversity required to perform a robust breeding program for such culture.

The research carried out by Rosado et al. (2010) was the first comprehensive assessment on the genetic diversity of *J. curcas* germplasm cultivated in Brazil using molecular markers. However, only 23 of the 381 tested RAPD markers were polymorphic (6.2%) in all the 192 accessions. Although the 23 markers had sufficient resolution to estimate genetic diversity of the Jatropha accessions panel, even for a relatively small genome (~400 Mbp, 717 cM, 11 linkage groups), this amount of markers is too small to precisely estimate the accessions' pairwise relationships. This fact will assist the decision-making on the removal/addition of new accessions, and the creation of a core collection and of a base population for plant breeding (Durães et al., 2011). The urgency of such analysis is further strengthened by Alves et al. (2013), who indicated that the molecular markers used by Rosado et al. (2010) to assess the germplasm bank diversity do not sample the genomic regions related to the genetic control of the traits of interest. As a result, the diversity sampled by molecular markers differs from that sampled by the evaluation of quantitative traits. In this regard, Laviola et al. (2010), Bhering et al. (2012), Junqueira et al. (2016) and Peixoto et al. (2016) demonstrated that the existing phenotypic diversity can assist in Jatropha breeding procedures, even though the molecular diversity is limited. Another point that indicates the importance of performing new studies on diversity is that after 2010, new accessions from several international sources, including Mexico and Guatemala (the probable center of origin and diversification of the species), were introduced to the Brazilian Jatropha germplasm collection (BJGC) without any prior information on their relation with accessions previously collected in Brazil.

Genome coverage can be achieved by genotyping the material of interest, by using newer platforms based on the genotyping-by-sequencing strategy (Rowan et al., 2017; Davey et al., 2011). Besides having higher resolution, the latest genotyping-by-sequencing methods, such as GBS (Ipek et al., 2017; Elshire et al., 2011), RAD-Seq (Marrano et al., 2017; Baird et al., 2008), and DArT-Seq (Hahn and Würschum, 2014; Sansaloni et al., 2011), are faster than the genotyping methods based on electrophoresis, and are able to genotype a greater amount of loci at a time (i.e., high performance technologies). Moreover, in most cases, they do not require prior knowledge on the genome polymorphic regions to be genotyped. This last feature makes such methods directly applicable to species with very little available genomic information, such as Jatropha. Although a reference genome has been recently published (Sato et al., 2011), and large number of SNP has been discovered (Wang et al., 2011, Gupta et al., 2012), the genotyping of germplasm collection and breeding populations still require investments in chip/arrays assembly.

The original genotyping method via DarT platform (which is based on reducing genome complexity by using restriction enzymes (Mace et al., 2008), cloning fragments from the reduced complexity genomic representation, and then printing the microarray chip) has been developed for rice (*Oryza sativa*) (Jaccoud et al., 2001). However, it was subsequently applied to many other plant species, such as barley (*Hordeum vulgare*) (Poland et al., 2012), potato (*Solanum bulbocastanum*) (Lorizzo et al., 2014), Banana (*Musa* sp) (Sardos et al., 2016), oil seed lesquerella (*Physaria fendleri*) (Von Mark et al., 2013), wheat (*Triticum aestivum*) (Orabi et al., 2014), and eucalyptus (*Eucalyptus* sp.) (Sansaloni et al., 2011). More recently, the technology has been improved to use next generations of Illumina sequencing platforms (HiSeq) (Huq et al., 2016), which developed the technology known as DArT-Seq (Zou et al., 2014). In this case, the genomic representations are sequenced and the discovery of DArT markers (also called Silicodarts or PAVs – Presence/Absence Variants) occurs *in silico*, via bioinformatics. Besides the DArT markers (in which polymorphism is sampled in terms of presence/absence of fragment within the sequenced representation), this new technology enables the simultaneous genotyping of SNP (which are called based on the mapping of sequences that are nearly identical to the reference genome). Thus, the DArT-Seq platform allows increasing the genotyping resolution when compared with the original DArT Platform (Sansaloni et al., 2011). Furthermore, the DArT-Seq platform provides a high multiplexing level (obtained both by the combined use of restriction enzymes and by the use of specific tags (barcodes)), consequently allowing the simultaneous genotyping of thousands of loci for hundreds of samples by sequencing run.

Based on the aforementioned, the current study aimed: (i) to develop and validate a panel for high-density genome-wide molecular markers (DArTs and SNPs) for Jatropha; (ii) to compare the diversity of the germplasm collected in Brazil and the materials introduced and/or generated by crossing selected genotypes; (iii) to determine whether the germplasm introduction strategies and the controlled crossings were efficient in generating/increasing the genetic variability available to the breeding program.