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Short communication

Classification of *Jatropha curcas* L. genotypes into germplasm groups associated with the presence of phorbol esters by means of seed characteristics

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ABSTRACT

Jatropha curcas L. (jatropha) is a perennial plant with a high potential for sustainable production of food, feed and biofuels in the tropics and subtropics. Breeding is at an early stage and easily assessable seed traits could be used for germplasm classification and management. The main goal of this study was to investigate the potential of five seed traits to classify genotypes into germplasm groups associated with the presence of phorbol esters. The seed traits were evaluated in a panel of 31 genotypes (12 self-fertilized and 19 cross-fertilized). Within the set of self-fertilized genotypes, all traits showed clear contrasting frequencies between the germplasm groups. In the set of cross-fertilized seeds, paternal effects were present for specific genotype combinations. We conclude that classification of genotypes into germplasm groups associated with the presence of phorbol esters by means of seed traits is promising but needs further investigation with a larger set of genotypes, plants per genotype and environments. These results are of importance for management and characterization of jatropha germplasm.

1. Introduction

Jatropha curcas L. (jatropha) is a perennial plant, which was promoted to be exploited in biodiesel production (Contran et al., 2013). The plant has a high potential for greening of wastelands in the tropics and subtropics and the seeds have a high oil concentration with excellent quality for conversion into biodiesel (Becker et al., 2013). The protein remaining after oil extraction can be used for production of animal feed or channeled into human nutrition (Vera-Castillo et al., 2014).

In jatropha, the presence of toxic phorbol esters represents a crucial trait that determines handling and utilization of jatropha products (Montes et al., 2013; King et al., 2013). There exist two jatropha germplasm groups characterized by the absence and presence of phorbol esters (Montes et al., 2014). These germplasm groups need to be considered in breeding programs to develop improved cultivars for both market segments, *i.e.*, food and fuels.

The standard method to detect phorbol esters by means of HPLC is time-intensive, destructive and costly. Utilization of visually assessable seed traits for classification of genotypes into the germplasm groups characterized by the absence and presence of phorbol esters could be a useful tool in jatropha breeding programs. It might allow fast, non-destructive and low cost characterization and classification of germplasm.

Our specific objectives were to (i) assess the relative frequencies of seed traits between germplasm groups characterized by absence and presence of phorbol esters, (ii) investigate the effect of self- and cross-fertilization on the seed traits, and (iii) examine the potential of seed traits for classification of genotypes into germplasm groups characterized by the absence and presence of phorbol esters.

2. Materials and methods

Nine genotypes from the germplasm group A (phorbol esters absent) and six genotypes from the germplasm group B (phorbol esters present) were investigated (Table 1). All genotypes comprised a high level of homozygosis as shown previously (Montes et al., 2014). The controlled self- and cross-pollinations resulted in a total of 31 genotypes (12 self-fertilized and 19 cross-fertilized). For 28 genotypes, the seeds originated from a single plant per genotype. For the remaining three genotypes, seeds originated from four different plants. A total of 40 plants and 20 randomly drawn seeds per plant comprised the final panel of 800 seeds analyzed.

Five visually assessable seed traits following either a binary or an ordinal scale were monitored (Figs. 1 and 2). We observed the caruncle, which is a type of elaiosome at the top of the seed, as well

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Table 1
Geographical origin, phorbol ester presence and germplasm group of the genotypes investigated.

Genotype	World region	Country	Phorbol esters	Group
JS01	Africa	Gambia	Present	B
JS02	Africa	Chad	Present	B
JS03	Africa	Chad	Present	B
JS04	Africa	Cameroon	Present	B
JS05	South America	Brazil	Present	B
JS06	North America	Mexico	Absent	A
JS07	North America	Mexico	Absent	A
JS08	North America	Mexico	Absent	A
JS09	North America	Mexico	Absent	A
JS10	North America	Mexico	Absent	A
JS11	North America	Mexico	Absent	A
JS12	North America	Mexico	Absent	A
JS13	North America	Mexico	Absent	A
JS14	North America	Mexico	Absent	A
JS15	Africa	Madagascar	Present	B

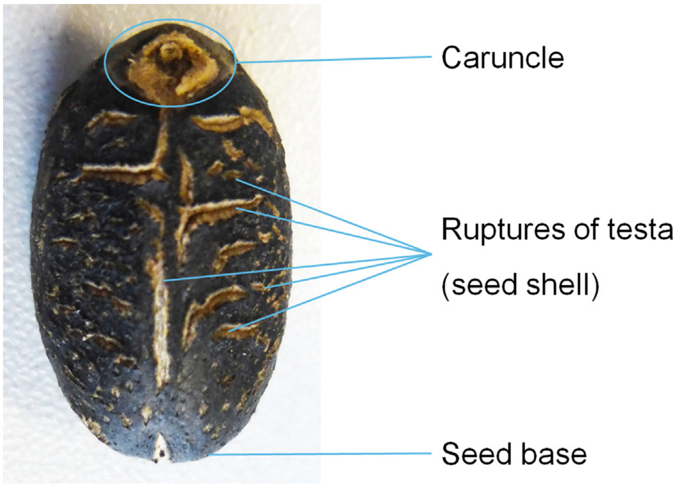

Fig. 1. Description of caruncle, ruptures of testa and seed base in seeds of *Jatropha curcas* L.

as different characters of the seed shell. Caruncle color was classified into red-purple or orange-brown. Color of ruptures in testa surface was recorded as bright or dark. Abundance of such ruptures was assessed with categories low, medium and high. Seed base shape was grouped into the categories round, flat or double bulged. Then, seeds were cracked manually with flat-nosed pliers to separate kernel and testa. On the inner side of the testa, presence of a silver shimmer was recorded as present or absent.

Descriptive statistics in the form of frequency tables per genotype and germplasm group were elaborated. We used canonical variate analysis (CVA) to assess the power of multi-trait classification (Gardner et al., 2006). Statistical analyses were all performed with GenStat 16.1 (VSN International Ltd., 2013).

3. Results

Within the panel of self-fertilized genotypes, each germplasm group predominantly exhibited a distinct phenotype for all traits (Table 2). The canonical variate analysis (CVA) revealed that most of the variance of the data set (>96%) was represented by the first canonical variate (CV1). Vector loadings showed that the traits silver shimmer inside testa and caruncle color had the highest effect on CV1. Separation of parental genotypes from group A was possible along the axis of CV1 (Fig. 3).

4. Discussion

The utilization of visually assessable seed traits for germplasm classification could be an important tool in jatropha breeding. Because the standard HPLC method for detection of phorbol esters is expensive and not always available to breeders, an alternative method based on seed traits would be useful. In addition, safety is enhanced when seeds that belong to the germplasm group containing phorbol esters can easily be identified in the whole production chain from field to post-harvest and storage.

In this study, we found two seed traits (caruncle color and silver shimmer inside testa) with high power to classify genotypes into germplasm groups characterized by the absence and presence of phorbol esters. In addition, the combination with additional seed traits such us seed weight and oil content might increase the precision of the classification system (Montes et al., 2015). This preliminary study needs to be extended with a larger set of genotypes, plants per genotypes, environments and seed traits. A precise classification system needs to be calibrated and validated.

Visual assessment of seed traits could be affected by the physical and psychological conditions of the operator (tiredness, vision, stress and operating conditions) and result in subjective non-reproducible data collection and miss-classification. To overcome this difficulty and apply the system to a larger extend, image analysis could be used instead of visual assessment. An automatic system based on image analysis will increase objectivity, data reproducibility and classification certainty.

The number of seeds to be assessed per plant is an important factor in determining the precision and practicability of the classification system based on seed traits. The 20 seeds per plant analyzed in this study yielded a good differentiation pattern between the germplasm groups. The optimal definition of the number of seeds to be analyzed per plant will require the assessment of calibration and validation models based on different number of seeds measured per plant. This might also be achieved by simulation approaches.

The seed traits investigated in this study develop from tissue of maternal origin. Therefore, the effect of cross-pollination in seed traits should be absent or extremely low. In general, the canonical analysis corroborates that hypothesis. However, the group of crosses B × A showed a large change in the relative frequencies of the seed traits. We concluded that, in these particular cases, paternal effects affected the expression of seed traits.

Compliance with ethical standards

The authors declare that they have no conflict of interest.