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Chemical composition and ultrastructure of the foliar cuticular wax of two Brazilian cultivars of castor bean (*Ricinus communis* L.)

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ABSTRACT

The main goal of this study is to provide an overview of the composition and ultrastructure of the leaf cuticular wax from two Brazilian cultivars of castor bean (*Ricinus communis* L.), one of them drought tolerant. The cuticular wax constituents were identified by capillary gas chromatography-mass spectrometry (GC/MS), and the micromorphological characteristics of the wax were revealed by scanning electron microscopy (SEM). The cuticular wax content varied significantly between the cultivars (88.1–139.4 $\mu g\,cm^{-2}$). *n*-Alkanes were the main constituent class identified (ranging from 65.1 to 71.7% of total wax) with nonacosane as main compound. Triacontanoic acid and triacontanol were the major compounds found for *n*-fatty acid and *n*-primary alcohol classes, respectively. Lupeol, α-amyrin, β-amyrin and betulin triterpenes were found in both cultivars, but these compounds can be quantitatively differentiated from cultivars. SEM revealed that the cuticles of both cultivars are covered with a thick cuticular layer striated and are devoid of wax crystals on both surfaces.

1. Introduction

Castor bean (*Ricinus communis* L.) is an oilseed crop that has the ability to adapt to different environments and is therefore cultivated in several countries (Severino et al., 2012). Castor bean oil has many different chemical properties that allow its application in the chemical and pharmaceutical industries, and this plant is a renewable resource and a promising crop for biofuel production (Beltrão et al., 2004; Sausen and Rosa, 2010). Due to its tolerance to drought stress, the cultivation of *R. communis* is possible in less favourable areas of Brazil and elsewhere in the world, such as semi-arid regions, and the plant also produces excellent results in other regions of the country (Babita et al., 2010; Severino et al., 2012).

Several agricultural cultivars of economic importance have been released by EMBRAPA (Brazilian Agricultural Research Corporation), the mission of which is to provide feasible solutions for the sustainable development of Brazilian's agriculture. Breeding studies aimed at developing and identifying *R. communis* cultivars that are more productive and tolerant to environmental stresses, particularly conditions of water stress, have been performed by EMBRAPA. Nonetheless, investigations concerning the morphological and/or physiological adaptations involved in drought tolerance remain scarce (Sausen and Rosa, 2010; Babita et al., 2010).

The various castor bean cultivars developed in Brazil exhibit different performance. For example, Costa et al. (2006) found divergence among different castor bean accessions and cultivars based on the days to flowering, number of racemes per plant, pistillate plant height, potential yield, and seed oil content. Bahia et al. (2008) also found phenetic divergence in five castor bean cultivars with regard to adaptive traits, yield components, and productivity. Significant differences in the ricin content of the seeds were observed in twenty accessions of castor bean (Baldoni et al., 2011).

Several studies have demonstrated that the cuticular wax profile (content and composition) may be important for improving drought tolerance in such economically interesting species as sorghum *Sorghum bicolor* (L.) Moench (Premachandra et al., 1992), cotton *Gossypium hirsutum* L. (Bondada et al., 1996), peanut *Arachis hypogaea* L. (Samdur et al., 2003), sesame *Sesamum indicum* L. (Kim et al., 2007a), soybean *Glycine max* (L.) Merr. (Kim et al., 2007b), barley *Hordeum vulgare* L. (Gonzalez and Ayerbe, 2010),

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Table 1
Phenotypic characteristics of the two cultivars of *Ricinus communis* L. released by Brazilian Agricultural Research Corporation (EMBRAPA).

Characteristics[a]	Cultivars	
	BRS Energia	BRS Nordestina
Release	2007	1998
Main quality	precocity	drought tolerance
Life cycle (days)	120	250
Seed productivity (kg/ha)	1800	1500
Plant height (cm)	140	190
Length of raceme (cm)	80	30
Number of raceme per plant	8	30
Flowering (days)	30	45
Number of fruits per raceme	100	60
Weight of 100 seeds (g)	50–55	65–72
Oil content (%)	48	48
Length of leaves (cm)	45–55	55–65
Stem color	green with wax	green with wax
Seeds color	beige and brown	black
Raceme and fruits form	conical and indehiscent	conical and semi-dehiscent
Method of harvest	hand	mechanized or hand
Ideal precipitation	≥500 mm	≥500 mm

[a] Andrade et al. (2010), Milani et al. (2007).

and sunflower *Helianthus annuus* L. (Franchini et al., 2010). Cuticular wax is a complex mixture of aliphatic compounds with long chain lengths, including fatty acids, aldehydes, primary and secondary alcohols, ketones, and alkanes. Various cyclic classes, such as triterpenoids, flavonoids, and tocopherols, can also be found in some species (Kollatukudy, 1996; Jetter et al., 2006). Waxes play a crucial role in drought tolerance, reducing water loss and thus promoting more efficient water use by the plant in natural conditions (Figueiredo et al., 2012, 2015). In addition to their critical function in preventing desiccation, cuticular waxes are also believed to play a role in defences against pathogens and protection against UV and frost damage (Kollatukudy, 1996; Seo and Park, 2011; Ni et al., 2012).

Although many of the cultivars released by EMBRAPA have a high productivity and tolerance to pathogens and hydric stress, studies that address the chemical analysis of cuticular lipids of these cultivars are scarce (Souza et al., 2010). Silva et al. (2016), recently have analysed under in vitro conditions, the wax composition of two cultivars of *R. communis* released by EMBRAPA in response to water stress. According to these authors, cuticular wax content increased when both cultivars were subjected to water stress in vitro. Plants also demonstrated quantitative changes in some wax compounds.

Due to its xerophytic characteristics, castor bean has great economic potential in the northeast region of Brazil (Azevedo and Lima, 2001). Because cuticular lipids act as a non-cellular membranous structure that protects the plant from environmental stress, our goals were to identify the main wax constituents and to analyse the ultrastructure of the leaves from two mature cultivars of *R. communis* with different drought-tolerance characteristics. Due to the difficulty of distinguishing between epicuticular wax (lipids deposited on the outer surface of the cutin) and intracuticular wax (lipids embedded within the cutin polymer matrix) when organic solvent extraction is performed, we adopted the term 'cuticular wax' in this study.

2. Materials and methods

2.1. Plant material and growth conditions

The seeds of two *R. communis* cultivars (BRS Energia and BRS Nordestina) were obtained from Embrapa Algodão, Campina Grande, Paraiba State, Brazil. BRS Energia is a precocious cultivar released at 2007 with a life cycle of 120 days and a productivity of 1800 kg seed per ha, whereas BRS Nordestina, release at 1998, has a longer life cycle, is more tolerant to drought, with productivity of 1500 kg seed per ha. Other phenotypic differences between the cultivars are shown in Table 1.

BRS Energia and BRS Nordestina plants were grown from seeds in plastic bags containing 9 kg of washed sand, and the plants were irrigated daily to field capacity using Hoagland's solution. The plants were maintained in a greenhouse at the Department of Chemistry, Federal Rural University of Pernambuco. The average temperature and relative humidity were 29.6 °C and 64.8%, respectively. After six months of cultivation, mature and fully expanded intact leaves were collected for chemical analyses.

2.2. Chemicals and standard compounds

All the solvents used were analytical-grade reagents or were purified according to standard procedures. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), *n*-alkane standard solution (C21-C40), and *n*-primary alcohol (C20-C32) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Wax content

After six months of cultivation, sixteen mature and fully expanded intact leaves were randomly collected from six plants of each cultivars. The mean leaf area was calculated from sixteen leaves using the software ImageJ 1.46r (Rasband, 2012). Cuticular waxes of each cultivar were extracted from sixteen intact leaves by immersion in dichloromethane (twice for 30-s each). The solvent was eliminated using a rotary evaporator and under N_2 flux (Souza et al., 2010). The extracts were weighed and wax content was determined gravimetrically and expressed in $\mu g\,cm^{-2}$.

2.4. Wax composition analysis (GC/MS)

Prior to the analysis, a defined amount of *n*-tetracosane was added as an internal standard and the wax mixtures were treated with BSTFA in pyridine (30 min at 70 °C) to transform all the hydroxyl-containing compounds into the corresponding trimethylsilyl (TMSi) derivatives. The quantitative analysis was recorded using a gas chromatograph (Shimadzu GC-17A, Kyoto, Japan) equipped with a flame ionisation detector and a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The GC oven temperature was maintained at 150 °C for 3 min, increased by