Scientia Horticulturae 201 (2016) 238–246

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Structure and composition of the wax of the date palm, *Phoenix dactylifera* L., from the septentrional Sahara

Rahma Khelil[a], Emilie Jardé[b], Francisco Cabello-Hurtado[c], Aminata Ould-el-Hadj Khelil[a], Marie-Andrée Esnault[c,*]

[a] Laboratoire de Protection des Ecosystèmes en Zones Arides et Semi-Arides, Université Kasdi Merbah, BP 511, 30000 Ouargla, Algeria
[b] UMR Géosciences, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes cedex, France
[c] UMR Ecobio 6553, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes cedex, France

ARTICLE INFO

Article history:
Received 5 January 2016
Accepted 2 February 2016
Available online 11 February 2016

Keywords:
Wax
Rodlet
Ultrastructure
n-Alkane
Triterpenoid
Date palm
Septentrional Sahara

ABSTRACT

The date palm, *Phoenix dactylifera* L. is an important economic species in arid regions of the septentrional Sahara. As this species survives under drastic conditions, the waxes of the leaf, as a strategy in response to stress, were the focus of these studies. From a structural point of view, the cuticle is overlaid with crystalloid waxes corresponding to epicuticular wax. The majority of these waxes were polygonal rodlets forming rings around the stomata, corresponding to the 'Strelitzia Type'. The effects of intense erosion were observed on the oldest leaves. From a chemical aspect, the wax primarily comprised *n*-alkanes (65.0% of the total wax), the most abundant of which is *n*-hentriacontane (24.3% of the *n*-alkanes). In the polar fraction, the triterpenoids represented the highest percent (19.6% of the total wax). The widely distributed ursolic acid and betulin were the most important triterpenoids identified, and the two other compounds were sterols. Betulin is an uncommon triterpenoid. According to previous studies, we hypothesized that the two main classes, *n*-alkanes and triterpenoids, are involved in formation of the rodlet wax structure. Fatty acids, aliphatic alcohols and ketones were also identified. A homologous series of aliphatics were described.

1. Introduction

The date palm, *Phoenix dactylifera* L. (Arecaceae, monocotyledon) is widely cultivated in arid and semiarid regions of the Middle East and North Africa. The earliest records of date palm cultivation date from approximately 7000 years in the lower Mesopotamian basin (Zohary et al., 2012; Yannopoulos et al., 2015). In traditional Saharan oases, the date palm is a keystone species, from an economical or social point of view, based on the traditional uses of the products and by-products of this plant. In the oasis agrosystems, the date palm grove hosts a wide array of other crops important for local production. *P. dactylifera* is also an important species for the implementation of economic investment plans while managing water resources in order to develop a sustainable agriculture (Valipour, 2015a,b; Valipour et al., 2015d). The development of traditional practices is one of the pathways that could reduce poverty while maintaining economic activity in North Africa (Valipour, 2015c).

On an ecological level, this species survives the drastic conditions of the desert when enough irrigation occurs. High temperature, intense light, UV, salt and sand erosion are the main stresses encountered. In a broad sense, the extremely xeric conditions of this environment lead to the development of morphological, physiological and molecular strategies for the survival of these plants. Thus, the formation of soils through the accumulation and dissolution of organic materials is reduced. The soil comprises sand or stony grounds with extremely low water storage capacity and is often salty. Among various strategies, the massive deposition of wax layers covering the epidermal cells prevents the dehydration of tissues and reflects solar radiation (Shepherd and Griffiths, 2006; Kim et al., 2007; Koch et al., 2009a). Thus, the evaporation of water through the cuticle is reduced, and only occurs through the stomata, which are often reduced in number, and protected at the bottom of a pit by the epicuticular waxes to reduce air turbulence above the guard cells and therefore prevent sweating. The cuticle is essential for maintaining the physiological integrity of the plant and incorporates other numerous functions of major importance for plant life. Indeed, this structure is also involved in the transport of lipophilic compounds, as a barrier limiting the loss of ions and

* Corresponding author.
E-mail addresses: rkhelil2002@yahoo.fr (R. Khelil), emilie.jarde@univ-rennes1.fr (E. Jardé), francisco.cabello@univ-rennes1.fr (F. Cabello-Hurtado), aminatakhelil@yahoo.fr (A. Ould-el-Hadj Khelil), marie-andree.esnault@univ-rennes1.fr (M.-A. Esnault).

http://dx.doi.org/10.1016/j.scienta.2016.02.012
0304-4238/

as a general protection against biotic or abiotic stresses. Other general properties have also been attributed to the cuticle, such as the reduction of surface temperature (Müller and Riederer, 2005; Koch et al., 2009a; Bernard and Joubès, 2013).

The cuticle is a film comprising polymeric lipids, the polyester cutins, and soluble waxes localized to the outer surface of the cells of the epidermis (Jeffree, 2006). Compounds, such as polysaccharides or phenolics might also be present in the cuticle. The incorporated waxes are called intracuticular waxes. On the surface, crystals of varying shapes visible under scanning electron microscopy are epicuticular waxes. The cuticle covers all parts of the plant and can be reduced to a thin film of less than 0.5 μm or a microscopic film 100 μm thick (Koch et al., 2009a). The cuticle is occasionally visible as a glaucous or bluish wax covering leaves or fruits. Quantitatively, the waxes might only cover 1 mg per cm^2 on the leaves of plants in temperate environments, while the palm waxes of *Copernicia prunifera* (Mill.) H.E. Moore (*C. cerifera* (Arruda) Macedo), a species of the arid Brazilian region, possesses several mg per cm^2 waxes that can be mechanically extracted (Jeffree, 2006). The waxes comprise a mixture whose composition varies depending on the species, organ, developmental stage and environmental constraint. These waxes primarily contain aliphatic hydrocarbon chains and derivatives (alkanes, alcohols, aldehydes, acids, etc.), predominantly comprising alkanes (Kim et al., 2007; Koch and Ensikat, 2008; Koch et al., 2009b). The chain lengths are predominantly 20 and 40 carbon atoms, with some monoester corresponding to chains up to 60 carbon atoms (Domínguez and Heredia, 1998; Koch et al., 2009b). Among the derivatives, primary and secondary alcohols, ketones, fatty acids, aldehydes and some aromatic compounds, such as triterpenoids (Koch et al., 2009b), are often present.

The waxes are at the interface between the plant and the environment. Wax accumulates in response to environmental factors, such as light or wind. Considering the economic importance of these plants and the objective of further studies on the response of this species to environmental constraints, the aim of the present study was to determine the composition of the waxes of date palms. Indeed, even if there is a variability in the amount of each wax compound according to the environmental conditions, the composition is markedly stable (Paroul et al., 2009). In addition, the physiology of the date palm has never been studied and deserves further clarification. To this end, in the present study, we examined the structure and the chemical composition of these waxes. The epicuticular and intracuticular waxes were not separated for this first study even if the short time used for extraction preferentially targeted the epicuticular wax (Buschhaus and Jetter, 2011).

2. Materials and methods

2.1. Materials

The palms were collected in Algeria in the Wilaya of Ouargla, in the septentrional Sahara. All samples were collected in the same week to avoid potential variations in the chemical composition of the waxes, as reported for other species. For the wax extraction, the palm leaves of three different plants were harvested in a palm grove (Hassi Ben Abdallah) in the surrounding area of Ouargla and three other in Hassi Messaoud, located 80 km from Ouargla. Ouargla is in the 'Deglet Nour' region, containing many traditional palm groves. In Hassi Messaoud, the date palms are urban plantations cultivated along the street. The leaves were collected from the 'Deglet Nour' cultivar. For scanning electron microscopy (SEM), we prepared the palm using a freeze-drying method to dehydrate the leaves after simple air-drying according to Pathan et al. (2008). Indeed, the lamina of the leaves had low water content (40–50%) and dried out easily and pretreatment at the critical point removed a part of the

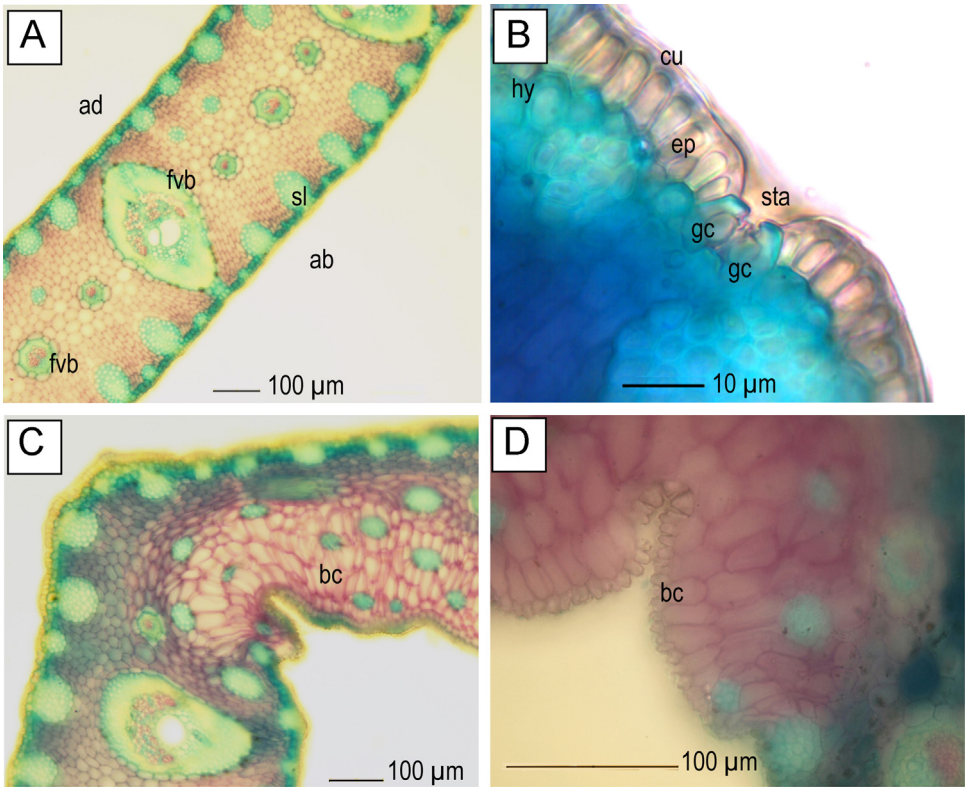

Fig. 1. Transverse section of date palm leaf. (A) Fibro-vascular bundles and mass of the sclerenchyma cells; (B) detail of the epidermis area with stomata; (C and D) bulliform cells in the midrib. ad, adaxial side; ab, abaxial side; bc, bulliform cell; cu, cuticle; ep, epidermis; gc, guard cell; hy, hypodermis; fvb, fibro-vascular bundle; sl, sclerenchyma; sta, stomatal aperture.