Available online at www.sciencedirect.com
ScienceDirect
Scientia Horticulturae 114 (2007) 298–301
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Short communication

Biochemical investigations on peroxidase contents of male and female inflorescences of date palm (*Phoenix dactylifera* L.)

N. Qacif [a], M. Baaziz [a,*], K. Bendiab [b]

[a] *Laboratory of Biochemistry and Plant Biotechnology, Cadi Ayyad University, Faculty of Sciences Semlalia, P.O. Box 2390, 40000 Marrakech, Morocco*

[b] *Department of Biology, Cadi Ayyad University, Faculty of Sciences and Technology, Guéliz, P.O. Box 549, 40000 Marrakech, Morocco*

Received 20 May 2007; received in revised form 26 July 2007; accepted 27 July 2007

Abstract

Peroxidases (EC 1.11.1.7) were extracted from male and female inflorescences of date palm (*Phoenix dactylifera* L.). Enzyme extraction by a progressive solubility method gave rise to soluble peroxidases (S) and ionically wall-bound peroxidases (I). When expressed in units g^{-1} fresh weight, peroxidase was present in inflorescence rachillae essentially as soluble fraction, where it represents 94% and 89% of the total activity (S + I) in male and female rachillae, respectively. In flowers, a tendency to increase of peroxidases I was observed, where this fraction, represents 51% and 78% of the total activity in female and male flowers, respectively. Low peroxidase activity was found in male flowers. When subjected to polyacrylamide gel electrophoresis, both S and I enzyme fractions exhibited each one, two forms based on their electric charges that occurred in basic and acidic gel media. Acidic peroxidase forms, resolved on basic gels, are more represented than basic forms, well separated on acidic gels. Acidic soluble peroxidases showed two migration zones on 6–15% gradient polyacrylamide gels. Acidic bound peroxidases are characterized by fast isoforms. Basic peroxidases, separated on 11% polyacrylamide gels and represented in fractions I, exhibited two major isoforms within three to five bands. Differences between female and male inflorescences are more marked on the basis of acidic peroxidases. Female inflorescences showed two major acidic isoperoxidases in rachillae with R_f 0.32 and 0.35 in enzyme fractions S and I, while male material showed one major band of R_f 0.35. Evaluation of peroxidases as sex markers in young date palm not yet at their reproductive stage is discussed in this study.

Keywords: Palms; Flowers; Peroxidase isoenzymes; Electrophoresis; Markers; Male and female trees

1. Introduction

The date palm (*Phoenix dactylifera* L.) is a perennial dioecious Monocotyledon, which is grown in arid regions in the Middle East, North Africa and to a small extent in California. This plant is slow flowering and fruiting. So, distinguishing male trees from female ones is not possible before up to approximately 5 years of culture on soil. When propagated by seeds, about half of the progeny would be males and the other half female trees. The propagation method traditionally used to maintain the genetic integrity of date palm cultivars corresponds to separating and independently establishing the offshoots produced by an individual tree (Saaidi, 1979). The attempts to increase fruit production include the improvement of marginal palm groves, derived from seedlings and, thus, contain high percentage of male trees when compared with inner groves of high date production (males do not exceed 2%).

Peroxidases (EC 1.11.1.7) are phenol oxidizing enzymes widely used as markers in the plant kingdom, due to their high polymorphism (Obinger et al., 1996). As studied in leaves, date palm contains highly active peroxidases (Baaziz, 1989; Majourhat et al., 2002; Azeqour et al., 2002). However, no published studies have been carried out on date palm inflorescences as plant material. Since plant peroxidases are involved in many functions such as growth, vegetative development, resistance against biotic and abiotic stresses (Obinger et al., 1996; Gonzalez-Verdejo et al., 2006; Mc Innis et al., 2006), the exact role of these enzymes is not yet elucidated in date palm.

Our previous study carried out on date palm calli, showed that peroxidases were represented essentially by two important active fractions, which were soluble and ionically wall-bound enzymes (Baaziz et al., 1994). This study had not been extended to inflorescences of adult date palms. For many plants such as

* Corresponding author.
E-mail address: baaziz@ucam.ac.ma (M. Baaziz).

0304-4238/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2007.07.015

Zinnia elegans, the ionically bound peroxidases were suggested to be involved, through their basic isoforms, in lignification process (Sato et al., 1995; Lopez-Serrano et al., 2004).

In this work the peroxidase activities were studied using male and female inflorescences of date palms of the Marrakech palm grove, which is characterized by 54% of male trees. The objective of this work was to evaluate the diversity of inflorescence peroxidases in date palm and to show possible qualitative differences and/or similarities of these enzymes obtained from female and male date palm trees. The possible use of peroxidases as sex markers in date palm has been discussed starting from the hypothesis that these markers are likely more expressed in reproductive organs of the plant. Seeking them in leaves of young plants by using appropriate techniques could lead to identify plant sex at early stages.

2. Materials and methods

2.1. Plant material

Inflorescences of date palm (*P. dactylifera* L.) were collected from 30 date palms (15 females and 15 males) belonging to the Marrakech (Morocco) palm grove (31°39′ latitude North, 8°00′ longitude West, 800 ha). All trees belong to 'khalt' material (unknown cultivar) planted near the Faculty of Science, Marrakech. Inflorescences were separated into two lots with respect of the plant sex. Flowers and rachillae of each inflorescence (Fig. 1) were labelled and stored at −20 °C until use.

2.2. Extraction of peroxidases

Peroxidases were extracted from date palm flowers and inflorescence rachillae as described in Baaziz et al. (1994).

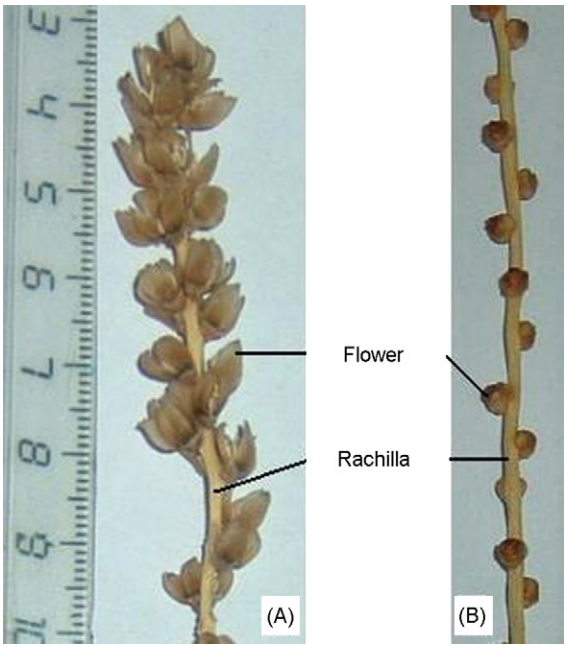

Fig. 1. Parts of male (A) and female (B) inflorescences obtained from date palm (*Phoenix dactylifera* L.).

Plant materials (0.25 g) from each inflorescence were ground separately in a cold mortar in 1.5 ml 5 mM Tris–HCl buffer (pH 7.2) containing 0.25 M sucrose and 1 mM $MgCl_2$. After centrifugation (7 min at 9000 × *g*), the supernatant collected corresponded to the soluble enzyme fraction (S). The pellet was washed three times in 1.5 ml of 5 mM Tris–HCl buffer (pH 7.2) containing 1% (v/v) Triton X-100 and the residue was incubated 30 min in 1.5 ml of 5 mM Tris–HCl (pH 7.2) and 1 M NaCl. The supernatant obtained after centrifugation corresponded to the ionically wall-bound enzyme fraction (I). High ionic strengths (1 M NaCl or 1 M KCl) have been used to obtain cell-wall-associated peroxidases in many plants such as *Picea abies* (Otter and Polle, 1994) and *Zinnia elegans* (Sato et al., 1995). For the electrophoresis, four bulks of male and female extracts were prepared separately by mixing equal volumes of each kind of enzyme fraction (S, I) derived from different individuals. Guaiacol and hydrogen peroxide were used as substrates for spectrophotometric quantification of peroxidase (Baaziz, 1989). One unit of peroxide activity corresponds to the enzyme quantity that gives a change of 0.1 in absorbance at 470 nm.

2.3. Electrophoresis and staining gels for enzyme activity

Acidic and basic isoforms of peroxidase were separated at +4 °C on polyacrylamide gels for both the soluble and the ionically wall-bound enzyme fractions. Acidic isoperoxidases were separated by electrophoresis as described by Baaziz et al. (1994) and Majourhat et al. (2002), using 6–15% gradient polyacrylamide gels prepared in 0.37 M Tris–HCl buffer (pH 8.8) and 0.1% SDS. Stacking gels (5% polyacrylamide) contained 0.125 M Tris–HCl buffer (pH 6.8) and 0.1% SDS. The electrode buffer (pH 8.3) contained 0.025 M Tris, 0.19 M glycine and 0.1% SDS. Samples were loaded on gels without heating. In this condition, SDS does not alter peroxidase activity (Ludwig-Müller et al., 1994; Baaziz et al., 1994). Electrophoresis was carried out at 35 mA per gel for about 6 h. Basic isoperoxidases were separated at low pH, on 11% polyacrylamide gels containing 0.06 M KOH and 0.375 M acetic acid (final pH 4.3). Stacking gels (5% polyacrylamide) were prepared in 0.06 M KOH and 0.062 M acetic acid (final pH 6.8). The electrode buffer (pH 4.5) corresponded to 0.35 M β-alanine and 0.14 M acetic acid. The voltage was fixed at 125 V. The separation was allowed to proceed about 6 h until the pyronin Y red dye front reached the bottom of the gel. Enzyme samples of 40 μl (separation by volume) or 25 μg of proteins (separation by mass) were loaded before running electrophoresis.

Gels were stained for peroxidase isoforms as described previously (Baaziz et al., 1994) using benzidine and hydrogen peroxide as substrates. Isoperoxidases corresponds to visible colored stains resulted from the enzyme-substrate reaction.

3. Results

When expressed as units g^{-1} fresh weight, peroxidase was present in inflorescence rachillae essentially in soluble fraction,