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Somatic embryogenesis and analysis of peroxidases in *Phoenix dactylifera* L.

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Abstract

To determine some physiological parameters implicated in somatic embryogenesis in date palm (*Phoenix dactylifera* L.), peroxidases have been studied. Activated charcoal commonly used in date palm tissue culture as an essential antibrowning factor decreased cellular protein contents and peroxidase activities. During the first months of culture, the conventionally used medium (100 mg dm^{-3} of 2,4-dichlorophenoxyacetic acid, 3 g dm^{-3} charcoal) reduces 2 to 3 and 4 to 6 times protein contents and peroxidase activities, respectively, in comparison with the same one containing only 5 mg dm^{-3} of 2,4-D and with or without 150 mg dm^{-3} charcoal. In addition, the standard procedure decreased the embryogenic potential which is positively related to the intra- and extracellular (excreted into culture medium) peroxidase activities. In medium with embryogenic calli, extracellular peroxidase activity was three times as high as the activity determined in the same medium with non-embryogenic calli. There were two basic isoforms and four to five acidic bands characterizing the embryogenic calli. It can be suggested that peroxidases play a key role in somatic embryogenesis of date palm and the charcoal used at 3 g dm^{-3} constitute a perturbating factor for this process.

Key words: benzylaminopurine, charcoal, date palm, 2,4-dichlorophenoxyacetic acid, 2-isopentyl-adenosine

Introduction

In date palm (*Phoenix dactylifera* L.), very little is known about the specific physiological parameters that affect somatic embryogenesis and/or tissue browning which is one of factors limiting embryogenic potential.

Ubiquitous in plants, peroxidases are considered as a good marker of some physiological processes. In tobacco (Thorpe *et al.* 1978) or *Kalmia latifolia* (Kevers

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and Gaspar 1992), it has been described that increased peroxidase activity accompanies shoot and root formation from callus. The profile of peroxidase activity is considered as a good marker for rhizogenesis (Moncousin 1991, Gaspar *et al.* 1992). Somatic embryogenesis is also characterized by an increase in peroxidase activity (Kochba *et al.* 1977, El Hadrami *et al.* 1989, Zhou *et al.* 1992). Nevertheless, in our system the tissue browning is also accompanied by an increase in peroxidase activity (El Hadrami *et al.* 1992). Similar results have been described in *Hevea brasiliensis* (El Hadrami and d'Auzac 1992, Housti *et al.* 1991) where tissue browning is positively related to peroxidase activity. In an attempt to differentiate between these two phenomena and to determine the degree of implication of peroxidase in somatic embryogenesis this investigation has been conducted considering changes in peroxidase activity and its isoenzyme patterns.

Materials and methods

In vitro culture of explants derived from young offshoots of two date palm embryogenic cultivars Iklane (IKL) and Jihel (JHL) was initiated on MS medium supplemented with 100 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D), 3 mg dm^{-3} 2-isopentenyladenosine (2IP) and 3 g dm^{-3} activated charcoal (MD1). In order to minimize somaclonal variability attributed to somatic embryogenesis this control medium has been lowered in growth regulators and activated charcoal. MD3 medium contained only 5 mg dm^{-3} 2,4-D, 5 mg dm^{-3} benzylaminopurine (BAP) or 2IP with or without charcoal (150 mg dm^{-3}). In these media, the first embryogenic calli were obtained in JHL or IKL after 6 - 8 months of culture. Embryogenic (white, friable and nodular) and non-embryogenic (non organized) calli used for this work were obtained in the same media. The calli are transferred to a fresh medium every 6 weeks.

To determine peroxidase activity and isoenzyme patterns, crude enzyme has been extracted with cold Tris-maleate buffer 0.1 M, pH 6.5 (1 g (f.m.) per 20 cm^3). The enzyme activity has been evaluated spectrophotometrically (*Varian DNS 200* UV visible spectrophotometer) at 470 nm as previously described (El Hadrami and d'Auzac 1992) using guaiacol as substrate. Polyacrylamination of peroxidase isoenzyme pattern was determined according to Davis (1964). Protein contents were determined according to the method of Bradford (1976).

Results and discussion

In date palm, the initiation of embryogenic calli takes several months. MD3 medium characterized by a low level of plant growth regulators has been found to be more efficient than the MD1 (El Hadrami and Baaziz 1993).

In comparison with the MD1, MD3 with and without activated charcoal gave higher cellular protein contents and peroxidase activities expressed as nkat g^{-1}(f.m.) or nkat mg^{-1}(protein) (Fig. 1). Addition of activated charcoal to the culture medium

Table 1. Effect of activated charcoal (150 mg dm^{-3}) on protein content and peroxidase activity during cultivation date palm cv. Iklane. Each value is the mean of three analysis (SE = 5 - 6%).

	MD3 without charcoal			MD3 with charcoal		
	100 d	130 d	180 d	100 d	130 d	180 d
Proteins [mg g^{-1}(f.m.)]	18.39	5.78	5.18	12.36	3.88	4.62
Peroxidase activities [nkat g^{-1}(f.m.)]	66500	17800	8350	33000	9000	2800
Peroxidase activities [nkat g^{-1}(protein)]	3616	3080	1612	2670	2320	606

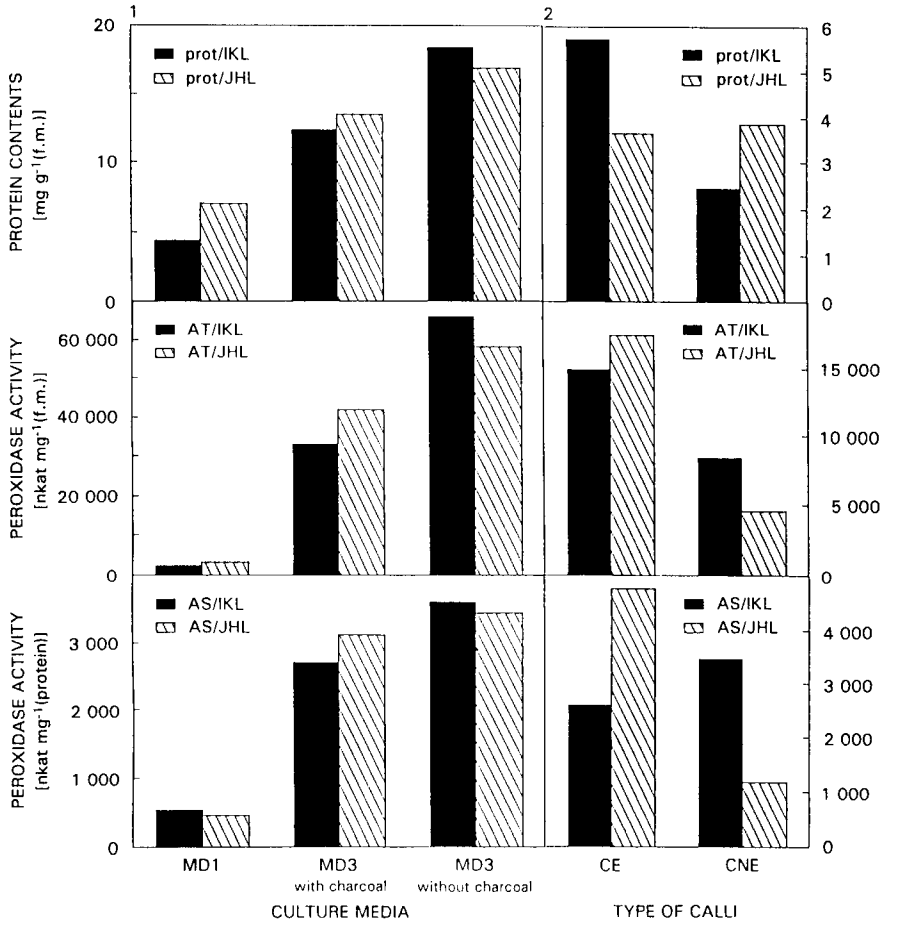

Fig. 1. Influence of culture medium on cellular protein content and peroxidase activity in date palm cv. Jihel (JHL) and cv. Iklane (IKL) after 100 d of culture. Each value is represented by the mean of three analysis (SE = 4 - 6 %). AT - peroxidase activity [nkat g^{-1} (f.m.)] AS - peroxidase activity [nkat mg^{-1} (protein)].

Fig. 2. Comparison of peroxidase activities in embryogenic (CE) and non-embryogenic (CNE) calli of cv. IKL at 300 d of culture. Mean of three analyses (SE = 5 - 7 %).

decreased the protein content and peroxidase activity in IKL (Table 1). These results could be explained by the effect of charcoal on plant growth regulator balance. Charcoal used at 3 g dm^{-3} may be one of the factors limiting the embryogenic capacity in date palm tissue. A lower peroxidase activity in MD1 medium might cause the alterations in endogenous auxin/cytokinin balance and the embryogenic potential was consequently lowered. In addition, it is well known that peroxidases are implicated in cell wall rigidification (Kolattukudy *et al.* 1992), and it has been shown that the cell wall of the embryogenic tissue is thicker than the cell wall of the parenchymatous one (Lu and Vasil 1985). This is in accordance with the positive correlation found between peroxidase activity and somatic embryogenesis in date palm (Fig. 2) and in *Hevea brasiliensis* (El Hadrami *et al.* 1989, El Hadrami and d'Auzac 1992). Among ten *Hevea* clones, the most embryogenic one shows the highest peroxidase activity. Similar results were found between peroxidases and embryogenic potential in *Citrus* (Kohlenbach 1978), *Daucus carota* (Anderson *et al.* 1986, Cordewener *et al.* 1991, De Jong *et al.* 1992), *Lactuca sativa* (Zhou *et al.* 1992), and recently in *Medicago sativa* (Gazaryan *et al.* 1993, Cvikrová *et al.* 1993). In addition, it has been shown that the carrot embryogenic cells excreted into the growth medium some glycosylated proteins which were not produced by non-embryogenic cells (Cordewener *et al.* 1992, De Jong *et al.* 1992). In this work, the positive correlation was not shown for the cv. IKL when the peroxidase activity is expressed on protein unit basis. This result is in relation with the low protein content in IKL non-embryogenic calli compared with embryogenic one obtained in the same medium (Fig. 2). These results suggest that peroxidases participate in the cellular differentiation.

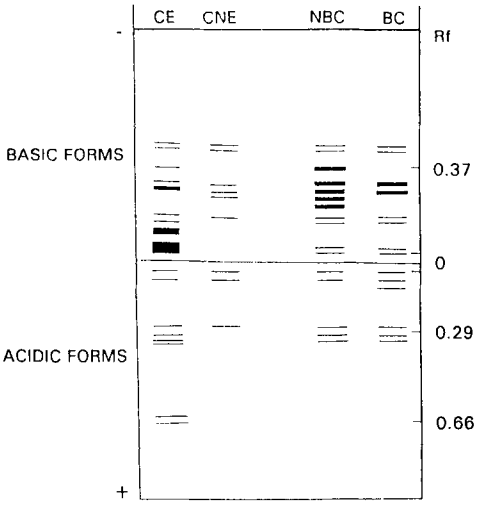

Fig. 3. Peroxidase isoforms detected in the soluble enzyme fraction of embryogenic calli (CE), non-embryogenic calli (CNE), browned (BC) and non browned tissue (NBC) of cv. IKL obtained on medium MD3 with 150 mg dm^{-3} charcoal after 300 d of culture.

Qualitative and quantitative differences in peroxidase isoenzyme patterns were noted between the embryogenic and non-embryogenic calli (Fig. 3). The embryogenic tissue is characterized by two basic isoforms of Rf 0.04 and 0.10. These isoforms were absent in non-embryogenic calli. In addition, the isoforms of Rf 0.04, 0.19, 0.27 and 0.45 were more abundant in the embryogenic calli. This result confirms the higher peroxidase activity in embryogenic tissue than in non-embryogenic calli (Fig. 2). The same results were found when extracellular peroxidases excreted into the culture medium were evaluated (Table 2). Peroxidase activity was three times higher in the medium with embryogenic calli (Table 2). The culture medium of date palm embryogenic calli shows similar extracellular protein contents in comparison to the same culture medium of non-embryogenic calli.

Table 2. Comparison of protein contents and activity of peroxidase excreted into the culture medium in date palm cv. Iklane embryogenic and non-embryogenic calli after 300 d of culture.

	Non-embryogenic calli	Embryogenic calli
Proteins [mg g^{-1} (f.m.)]	8.49 ± 0.42	8.77 ± 0.44
Peroxidase activity [nkat mg^{-1}(f.m.)]	173 ± 9	527 ± 26

Considering the acidic peroxidases, only three bands were present in non-embryogenic calli. The bands of Rf 0.08 and 0.29, common with the embryogenic calli, were less abundant. Four to five acidic bands of Rf 0.30 to 0.66 are characteristic of date palm embryogenic tissue. In these experiments, the date palm embryogenic callus preferentially contained basic isoenzymes while in *Medicago sativa* embryogenic cells the number of acidic isoenzymes was higher than that of the cationic forms (Gazaryan *et al.* 1993).

Browned and non browned calli compared in this work were not embryogenic. The non browned calli could be characterized by two basic isoperoxidases of Rf 0.21 and 0.37 which are absent in the browned tissue. In addition, the bands of Rf 0.21, 0.28, 0.30 and 0.37 were more abundant when tissue browning was absent. Using the acidic forms, it is not very easy to distinguish between these two types of calli. Tissue browning could be related to the disappearance of some basic isoperoxidases and to the synthesis of some acidic forms (Rf 0.13 in this experiment).

In conclusion, the acquisition of embryogenic competence is accompanied not only by an increase in peroxidase activity but also by the synthesis and/or the regulation of specific isoforms. Thus, peroxidases constitute a potential marker for somatic embryogenesis in date palm. The mechanisms how the identified isoenzymes could participate in the process of differentiation remain not clear. The interaction of certain factors implicated in somatic embryogenesis may be involved in the stimulation and activation of embryogenic genes leading to the enhancement of peroxidase activity and isoenzyme patterns. Further studies are needed to confirm the degree of peroxidase implication in somatic embryogenesis in our system.

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