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Anatomical and biochemical changes during adventitious rooting of apple rootstocks MM 106 cultured *in vitro*

Sélima Naija [a], Nadhra Elloumi [b], Najoua Jbir [a], Saida Ammar [a], Claire Kevers [c,*]

[a] *Laboratory of Plant Biology and Biotechnology, Faculty of Sciences of Tunis Campus Universitaire, 1060 Tunis, Tunisia*
[b] *Laboratory of Biotechnology, National Institute of Agronomic Research of Tunisia, rue Hedi-Karray, 2049 Ariana, Tunisia*
[c] *Plant Molecular Biology and Biotechnology, Plant Biology Institute B22, University of Liège–Sart Tilman, B-4000 Liège, Belgium*

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Abstract

Adventitious rooting in microcuttings of *Malus* rootstocks MM106 was studied as regards their histological and biochemical aspects. Microcuttings from shoots raised in Murashige and Skoog's (1962) medium were transferred into a rooting medium containing IBA in the dark, then fixed 0, 3, 5, 7 and 10 days after. Some cambial zone and adjacent phloem cells became dense cytoplasm, nuclei with prominent nucleoli and the first cell divisions were observed at day 3. Meristemoids became individualized, consisting of densely staining cells (with enlarged nucleoli) formed outside the xylem by day 5. Identifiable root primordia with a conical shape and several cell layers were present at day 7. Roots with organized tissue system emerged from the stem 10 days after the root induction treatment. From these histological observations, it can be established that the rooting induction stage ended before day 3. The initiation stage, with the first histological modifications to the formation of meristemoids, would correspond to the transient increase of our biochemical marker (peroxidase activity) until day 5. The best rooting percentage obtained with cultures in the presence of auxin during 5 days confirms this hypothesis. The expression of rooting can then take place. ***To cite this article: S. Naija et al., C. R. Biologies 331 (2008).***

Résumé

Changements anatomiques et biochimiques durant l'enracinement adventif du porte-greffe MM 106 de pommier cultivé *in vitro*. Des observations histologiques et des analyses biochimiques ont permis de suivre l'enracinement adventif de microboutures du porte-greffe MM106 de pommier. Des microboutures sont transférées sur un milieu d'enracinement contenant de l'acide indolbutyrique à l'obscurité, puis fixées après 0, 3, 5, 7 et 10 jours. Les cellules de la zone cambiale, près du phloème, présentent un cytoplasme dense, avec des nucléoles apparents, et les premières divisions cellulaires sont observées dès le 3^{e} jour. Des méristémoïdes individualisés, constitués de cellules denses (avec de larges nucléoles), sont formés à l'extérieur du xylème dès le 5^{e} jour. Au 7^{e} jour, les primordia racinaires sont identifiables par leur forme conique. Les racines émergent de la tige après 10 jours. Ces observations histologiques permettent d'établir que la phase d'induction racinaire se termine avant le 3^{e} jour. La phase d'initiation, caractérisée par les premières modifications histologiques conduisant à la formation des méristémoides, correspond à l'augmen-

Abbreviations: BAP, 6-benzylaminopurine; GA3, gibberellic acid; IBA, 3-indol butyric acid; MS, Murashige and Skoog (1962); NRM, rooting medium without growth regulator; RM, rooting medium.

* Corresponding author.
E-mail address: C.Kevers@ulg.ac.be (C. Kevers).

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tation de l'activité peroxydasique jusqu'au 5e jour. Le meilleur pourcentage d'enracinement obtenu sur un milieu contenant une auxine durant cinq jours confirme cette hypothèse. Le développement des racines peut alors avoir lieu. ***Pour citer cet article : S. Naija et al., C. R. Biologies 331 (2008).***

Keywords: Adventitious roots; Anatomical study; Biochemical marker; Histology; Malus rootstocks; Peroxidase activity; Rooting

Mots-clés : Anatomie ; Activité peroxydasique ; Enracinement ; Histologie ; Marqueur biochimique ; Porte-greffe de pommier ; Racines adventives

1. Introduction

Rooting of microshoots is critical in plant micropropagation systems of woody plants [1–3]. Many researchers have demonstrated that the initiation of adventitious roots *in vitro* in excised shoots of the apple rootstocks is related to several factors [4–7]. The most important factors in rooting induction and initiation are concentration of auxin and treatment duration [8–11]. To control the rhizogenesis steps, it may be indispensable to know the signals and the specific internal mechanisms [12]. Rooting was at first considered as a single-phase process, but there are several successive reports where adventitious rooting was shown to depend on a series of interdependent phases (induction, initiation and expression) [13–16].

Knowledge of biochemical and anatomical events associated with root induction and expression is useful, as it will permit the improvement of rooting procedures. Various studies on adventitious root formation have shown the role of markers played by peroxidase in rooting of plants cultured *in vitro* [17–21]. Hausman [22] and Kevers [23] have also reported the role of auxins in relation to the peroxidase activity of various plant species. Auderset et al. [24] suggested a correlation between ontogenic stages and evolution of biochemical markers during *in vitro* rooting of *Malus domestica*.

In the present paper, histological events leading to *in vitro* root formation in apple rootstocks and evolution of peroxidase activity during *in vitro* rooting of shoots were examined in correlation with the different stages of rooting and the necessary duration of auxin presence.

2. Materials and methods

2.1. Plant material, culture conditions and rooting procedure

Starting material for *in vitro* culture of rootstocks of apple tree MM106 was supplied by the laboratory of *in vitro* culture of 'Mabrouka Company', located at Mornag city, Tunisia.

The proliferating shoots were subcultured every four weeks on Murashige and Skoog [25] medium supplemented with 0.4 $mg\,l^{-1}$ BAP, 0.1 $mg\,l^{-1}$ IBA, and 0.2 $mg\,l^{-1}$ GA_3. These shoots (3–4 cm) were individualized and used later for rooting experiments.

The rooting basal medium (RM) was a half-strength Murashige and Skoog [25] medium supplemented with 1 $mg\,l^{-1}$ IBA, 0.4 $mg\,l^{-1}$ thiamine–HCl. The same medium without growth regulator (NRM) was used as control for non-rooting. All media were supplemented with 30 $g\,l^{-1}$ of sucrose and pH adjusted to 5.7–5.8 with KOH or HCl 0.1 M prior to the addition of agar (5 $g\,l^{-1}$) and subsequently autoclaved for 30 min at 120 °C. For rooting experiments, shoots were cultured in 500-ml 'Le Parfait' glass jars containing 100 ml of medium. Cultures were placed in the dark on RM. Fifteen shoots for *in vitro* root development were used, and the experiments were repeated six times.

With a view to studying the effect of the duration of dark periods on RM, the shoots were transferred after different times (0 to 10 days) into glass jars with auxin-free basal medium (NRM) in the light and maintained in a growth chamber at 26 °C with a 16-h photoperiod and a light intensity of 40 $\mu E\,m^{-2}\,s^{-1}$. Rooting percentages were recorded four weeks after the auxin treatment started.

2.2. Histology

At days 0, 3, 5, 7 and 10 after treatment, bottom 4–7-mm sections of the stem were fixed in formaldehyde/ethanol/acetic acid (FAA) 10:85:5 (v/v/v), dehydrated in alcohol series between 50 and 100%, transferred into gradual alcohol–xylol series and finally embedded in paraffin. Seven-micrometre-thick transverse sections were cut with a rotatory microtome (Leica, RM2125 RT) and stained with haematoxylin and safranin.

2.3. Determination of peroxidase activity

Shoots were collected and their basal part was stored at −80 °C until extraction. Samples (100 mg) were