

# New Strategies for In Vitro Rooting and Plantlet Acclimatization of the 'Paradox' (*Juglans regia* × *Juglans hindsii*) Rootstock

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## Abstract

On the acclimatization of micropropagated *Juglans* plantlets, values fluctuating between 0 and 80% of plant loss can be found in literature, but most studies prefer to ignore the problem. This research focuses on the *Juglans* hybrid 'Paradox' (*Juglans regia* × *Juglans hindsii*), exploiting new methodologies to minimize the failure rates on the acclimatization of in vitro rooted plantlets. Data for in vitro rooting and acclimatization are presented. Rockwool cubes, peat pressed pellets and Jiffy Preformas<sup>®</sup> were tested and compared with the traditional vermiculite moistened with the DKW nutrient solution added with Phytigel<sup>™</sup>, commonly used on *Juglans* micropropagation protocols. These new approaches allowed the transfer of in vitro rooted plants to acclimatization with a balled-root protection, instead of the traditional bare-root procedure, significantly reducing the post-transplant stress. Information concerning plantlet/mycorrhiza association during acclimatization is also presented.

## INTRODUCTION

Most of the research initially carried out on walnut micropropagation targeted the selection of nutrient media, balance of growth regulators and control of explant oxidation reactions, for the different culture stages.

Regarding nutrient media composition, although the first studies have preferably used the MS (Murashige and Skoog) basal media (Chalupa, 1981), currently, after the reports of Driver and Kuniyuki (1984), it is generally accepted that the most appropriate culture media for *Juglans* is the DKW (Driver and Kuniyuki Medium), initially developed for multiplication of the 'Paradox' hybrid (*Juglans regia* × *Juglans hindsii*).

Concerning the use of growth regulators, the available results indicate that for the installation and multiplication phases, gibberellins are dispensable and cytokinins have their optimum at 1.0 mg L<sup>-1</sup> BAP (6-benzylaminopurine) (Driver et Kuniyuki, 1984; Penuela et al., 1988). Auxins have been introduced in the walnut multiplication process in order to promote the elongation of the shoots, but, the published results present some contradictions. While Barge (1981) found that a concentration of 0.1-0.3 mg L<sup>-1</sup> NAA (1-naphthaleneacetic acid) promoted elongation of shoots, Rodriguez (1982) states that concentrations higher than 0.1 mg L<sup>-1</sup> IBA (indole-3-butyric acid), inhibit both, axillary bud breaking and shoot elongation. Driver and Kuniyuki (1984), in their studies about in vitro culture of the 'Paradox' rootstock, pointed to 0.01 mg L<sup>-1</sup> μM IBA as the optimal auxin concentration.

To overcome the oxidation reactions, that usually arise during the early stages of culture, the literature refers to several tests carried out with anti-oxidants, but, the systematic explant transfer into fresh culture media, which may need to be daily in the initial installation, seems to be the most efficient solution (Leslie and McGranahan, 1992).

More recent reports on the in vitro culture of walnut, have developed the subject of rooting and acclimatization. The need of an induction medium with high concentration of auxins, where the explants spend 7-12 days in the dark, followed by a transfer into a rooting expression medium without growth regulators, seems to be consensual.

Ripetti et al. (1994), proposed root induction on MS medium with 3 mg L<sup>-1</sup> IBA, solidified with agar and, as expression media, the DKW formulation mixed with Gelrite +