Scientia Horticulturae 222 (2017) 169–174

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Improvement of plant recovery from avocado zygotic embryos by desiccation under high relative humidity conditions

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ARTICLE INFO

Keywords:
Avocado
Desiccation
Embryo rescue
Maturation
Germination
Persea americana
RITA®

ABSTRACT

Due to the high fruit abscission characterizing avocado, protocols for immature embryo rescue are an important tool in breeding programs based on hybridization of elite genotypes. The objective of this investigation was to evaluate the effect of *in vitro* desiccation under high relative humidity conditions on immature avocado embryo germination. The duration of the desiccation treatment significantly affected embryo germination, with optimum results achieved after a 14-day treatment (66.7% in desiccated embryos *versus* 15% in control, non-treated embryos). Other traits, such as recovery of complete plants and quality of the obtained plants, were also improved following desiccation. Trying to reproduce the final stages of avocado zygotic embryogenesis, the effects of desiccation after an *in vitro* maturation treatment was also evaluated. Results obtained revealed a significant effect of maturation stage, desiccation and the interaction between both factors; *e.g.*, while desiccation significantly improved germination after *in vivo* maturation, a slight decline was observed in *in vitro* matured embryos, probably due to differences in embryo water status. However, recovery of complete plants and length of the structures formed were only significantly affected by maturation stage. Nevertheless, in embryos directly coming from trees, *in vitro* drying significantly improved rescue in all cases, independently of embryo developmental stage. Therefore, desiccation under high relative humidity conditions can be considered a valuable tool to be used in protocols for avocado embryo rescue. However, its use in conjunction with *in vitro* maturation treatments is not recommended.

1. Introduction

Avocado is an evergreen tree cultivated for its nutritious fruits. World production has doubled in the last fifteen years to 4,717,102 t produced in 516,484 ha (FAOSTAT, 2013). Avocado cultivation extends throughout regions with tropical, subtropical and temperate climates including Mexico, Dominican Republic, Colombia, Peru and Indonesia (FAOSTAT, 2013).

Avocado breeding programs based on hybridization of selected genotypes have been reported in multiple countries such as USA, Australia, South Africa, Mexico and Israel (Lahav and Lavi, 2009). However, the avocado is characterized by excessive fruit abscission (Garner and Lovatt, 2016), with peak abscission rates ranging from 50 to 280 immature fruits per day (Garner and Lovatt, 2008). Premature abortion of the developing embryos results in low fruit set (< 0.1%) (Whiley and Schaffer, 1994), which provokes a dramatic reduction of the viable hybrid progeny, significantly reducing the efficiency of avocado breeding programs (Sánchez-Romero et al., 2007).

Previous studies carried out in avocado revealed that, under standard conditions, acceptable germination rates were only obtained at advanced developmental stages (Perán-Quesada et al., 2005). Therefore, development of protocols for immature embryo rescue is becoming an important tool in avocado breeding programs. *In vitro* culture of immature embryos has been used as a rescue technique as it facilitates conversion of these embryos into plants (Raghavan, 2003). In most cases, development of *in vitro* rescue protocols has been focused on the optimisation of the germination process (Sánchez-Romero et al., 2007; Skene and Barlass, 1983). However, embryo germination is significantly influenced by events occurring during maturation and desiccation phases (Bewley and Black, 1994).

The desiccation stage is characterized by an important loss of water which causes seed metabolism to decrease in preparation for a quiescent period and subsequent germination (Kermode and Finch-Savage, 2002). This step occurs between two metabolically different phases. The anabolic phase is characterized by the synthesis and accumulation of reserve products (maturation), while in the catabolic these storage substances are metabolized in order to support development of the new plant (germination).

Abbreviations: DAP, days after pollination; LSD, least significant difference; RH, relative humidity

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http://dx.doi.org/10.1016/j.scienta.2017.05.018
Received 23 January 2017; Received in revised form 3 May 2017; Accepted 4 May 2017
Available online 15 May 2017

During development, orthodox seeds acquire desiccation tolerance and, therefore, during drying, moisture content can decline to very low levels. For most of these seeds, desiccation is considered a potent developmental switch signalling the cessation of seed development and their entry into the germinative programme upon cellular rehydration (Bewley et al., 2013). Although recalcitrant seeds, such as avocado, lack or do not express the various processes and mechanisms typifying the acquisition of tolerance (Berjak and Pammenter, 2013) and therefore are sensitive to desiccation, an important loss of water at the end of the maturation period has also been described (Sánchez-Romero et al., 2002).

Previous investigations carried out in immature avocado embryos revealed that including an *in vitro* maturation phase prior to induction of germination greatly improves the efficiency of plant recovery as well as the quality of the resulting plants (Márquez-Martín et al., 2009).

The objective of the present investigation was to study the effect of desiccation on the efficiency of avocado embryo rescue. Following the model of *in vivo* avocado embryo development (Perán-Quesada et al., 2005; Sánchez-Romero et al., 2002), we studied the effect of sequential application of maturation and desiccation *in vitro* treatments on subsequent embryo germination.

2. Material and methods

2.1. Plant material and culture conditions

Avocado (*Persea americana* Mill.) fruits, cv. 'Hass', were harvested at random from open pollinated trees growing in a monovarietal orchard at IHSM La Mayora (Algarrobo Costa, Spain). After harvesting, fruits were surface sterilized by immersion for 10 min in a 0.5% (v/v) sodium hypochlorite solution containing 20 drops l^{-1} of Tween 20, followed by three rinses in sterile distilled water. Sterilized fruits were cut lengthwise under aseptic conditions and immature zygotic embryos carefully excised.

In all media preparations, the pH was adjusted to 5.74 before autoclaving for 7 min. Subsequently, 25 ml were dispensed into 25 mm × 150 mm test tubes (Bellco Glass Inc., NJ, USA) or 50 ml into 85 mm × 80 mm cylindrical glass jars. Finally, media were sterilized by autoclaving at 121 °C and 0.1 MPa for 15–20 min. RITA® vessels were sterilized by autoclaving for 20 min at the same conditions the day before desiccation treatments were initiated.

All cultures were incubated in a growth chamber at 25 ± 1 °C. Maturation and desiccation treatments were carried out in darkness, while germination was induced under a 16 h light photoperiod, provided by Grolux lamps (Sylvania, Germany) (40 µmol m^{-2} s^{-1}).

2.2. Effect of desiccation treatment duration

According to George (1996), atmospheres with specific relative humidities (RH) can be achieved in confined spaces containing saturated solutions of different salts. In the present investigation, a 97.5% RH was attained by adding 150 ml of a saturated K_2SO_4 solution in the lower compartment of a RITA® temporary immersion system (Cirad, Saint-Mathieu-de-Tréviers, France) and cancelling outside air exchange and solution movement to the upper compartment. Once isolated, zygotic embryos were quickly placed into the bottom half of a sterile 90 × 25 mm Petri dish divided into three sections, located in the upper part of the vessel (Fig. 1).

For testing the effect of desiccation treatment duration, zygotic embryos collected 106 days after pollination (DAP) averaging 19 mm in length, were desiccated as indicated above for 7, 14 and 21 days. Control treatment consisted of embryos directly coming from trees, without having undergone any desiccation treatment.

Variations in fresh and dry weight were determined throughout the desiccation process in a minimum of 6 embryos per treatment. Dry weight was measured after heating zygotic embryos at 75 °C for 48 h and the water content calculated on a fresh weight basis (Pâques and Boxus, 1987).

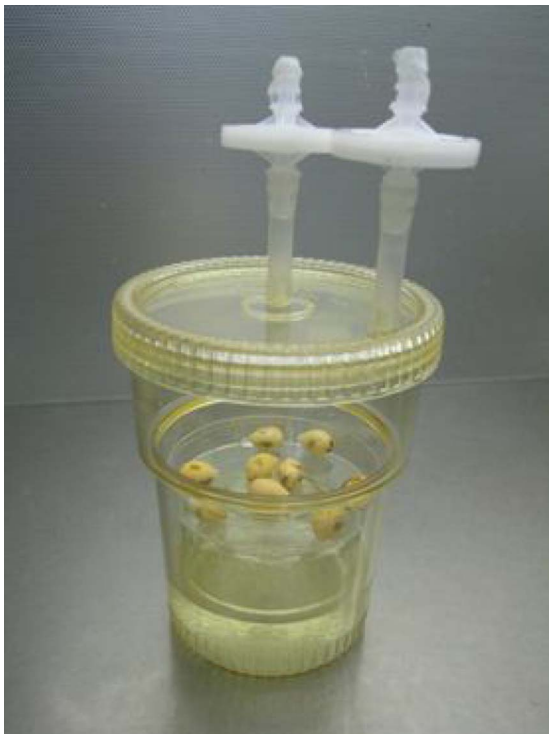

Fig. 1. Immature avocado embryos during desiccation under 97.5% RH into a RITA® system.

To evaluate the effect of desiccation on embryo conversion, embryo germination was induced by partial removal of the cotyledons and culture on M1 medium (Skene and Barlass, 1983; Perán-Quesada et al., 2005). M1 medium consisted of half strength MS formulation supplemented with 2.22 µM benzyladenine and gelled with 1.7 g l^{-1} Gelrite (G-1910, Sigma Chemical Co., St. Louis, MO, USA). Germination phase was carried out during 15 weeks with recultures onto fresh medium at 5-week intervals.

2.3. Effect of desiccation after an in vitro maturation treatment

Trying to reproduce the last phases of zygotic embryogenesis in avocado (Perán-Quesada et al., 2005; Sánchez-Romero et al., 2002), the effects of desiccation after an *in vitro* maturation treatment were tested. In order to elucidate the effect of *in vitro* maturation on subsequent response to desiccation, two controls were included in this experiment: non-matured (control) and *in vivo* matured embryos.

Very immature embryos, collected 65 DAP with 7 mm average length, were used in this experiment. Control embryos were taken directly from the tree at the beginning of the experiment. For *in vitro* maturation, embryos were cultured on B5m medium (consisting of major salts of the B5 formulation (Gamborg et al., 1968), MS minor salts and vitamins (Murashige and Skoog, 1962) and 88 mM sucrose) supplemented with the Jensen's amino acids (Jensen, 1977), extra 88 mM sucrose and 6 g l^{-1} agar (A-1296, Sigma Chemical Co., St. Louis, MO, USA) (Márquez-Martín et al., 2009). Maturation was carried out during 12 weeks with reculture onto fresh medium after 6 weeks. For *in vivo* maturation, avocado embryos remained growing in the trees during the same time period. Thus, 12 weeks after experiment initiation (139 DAP), zygotic embryos averaging 25 mm long were collected and subjected to the same subsequent treatments than those *in vitro* matured.

Half of control, *in vivo* and *in vitro* matured embryos were induced to germinate in M1 medium. The rest of embryos were subjected to a