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In Vitro Embryo Culture of Some Sweet Cherry Genotypes

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Abstract

Due to a very low germination percentage of some sweet cherry's hybrids seeds in the breeding process, a new research using embryo rescue method was started. The goal of this work was to establish an efficient propagation protocol using the embryo culture in vitro. The sweet cherry seeds comes from eight hybrid genotypes as follows: 13.3.M (Giant Red x Early Red), 13.4.M (Giant Red x New Star), 13.8.M (New Star x Kordia), 13.9.M (New Star x Van), 13.11.M (New Star x Burlat), 13.12.M (New Star x Early Red), 13.18.M (Van x New Star), 13.19.M (Van x Early Red). The biological material was sterilised using alcohol 96% (w/v) and $Ca(OCl)_2$ in different concentrations. Four culture mediums with macro and microelements were tested (based on Lee & Fossard and Murashige & Skoog) reacting differently according to the genotype. The LF medium (V1) offers superior nutrition for biological material in terms of macro and microelements and vitamins, comparing to V3 culture medium, constituted as MS medium. The germination period in vitro conditions indicate V1 medium with the lowest infection rate 26.3%. The most vigorous plants obtained in vitro were registered on LF medium.

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Keywords: sweet cherry; hybrids; embryo culture; germination; in vitro culture medium

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1. Introduction

In vitro embryo culture is utilised in fruit growing to obtain viable plants from some varieties with low germination of seeds, those who comes from distant hybridisations or crossings between early varieties (Stanys V., 1998; Dulic J. et al., 2016) especially in stone fruits (Turkey H.B., 1933, 1934; Balla et al., 1996). To solve this problem, often is required the embryo rescue method in aseptic culture medium conditions (Sanders et al., 1963; Raghavan, 1980; Bridgen, 1994).

Modern solutions are always taken into account in order to efficient propagate valuable biological material (Standardi A., 2012). The plant regeneration through embryo culture is in some author's opinion also influenced by environmental conditions of seed production (Chiriacula M. et al., 1985).

But, a decisive factor remains *in vitro* culture medium. More mediums were tested including Murashige & Skoog (1962), Gamborg's (1968) B5 (Rizzo et al., 1998) White and M&S modified (Stanys V., 1998). The evolution of seed germination and plant regeneration differed depending on their components, formulation and of course experienced genotypes.

In this work, we proposed to establish an optimal culture medium for rescue the sweet cherry hybrids by embryo culture technique as a solution for hybrid seeds germination.

2. Materials and methods

The biological material was represented by sweet cherry seeds of eight hybrid combinations as follows: Giant Red x Early Red (13.3.M), Giant Red x New Star (13.4.M), New Star x Kordia (13.8.M), New Star x Van (13.9.M), (13.11.M) New Star x Burlat, 13.12.M (New Star x Early Red), 13.18.M (Van x New Star), 13.19.M (Van x Early Red). They have been obtained in 2013 by cross pollination in the experimental sweet cherry orchard of Istrita, Buzau Nursery of the University of Agronomic Sciences and Veterinary Medicine (UASVM), Bucharest. At the ripening time, the seeds were collected from hybrid fruits and stored in the Breeding Laboratory of UASVM Bucharest. All the biological material was preserved according to Cociu V and Oprea S (1989) method. Precisely, the sweet cherry hybrid seeds were rinsed, disinfected with 0.1% Derosal solution for 10-15 minute, placed in polyethylene bags together with a suitable amount of sand humidified with sterile water. Stone bags were kept in refrigerated temperatures of 4°C.

In March 2014, all the seed were sown individually, in peat substrate, using two liter pots. The most part of the seeds had germinate (92.25%) and start growing, later being planted in the field. The rest of ungerminated seeds (7.75%) were extracted from pots and sent on the 22th of January, 2015 to Micropropagation Laboratory of the Research Institute of Fruit Growing Pitesti Maracineni for embryo culture.

2.1. Culture mediums

Four variants of culture mediums have been set up based on Lee Fossard (1977) and Murashige & Skoog (1962) (Table 1).

Table 1. Culture medium variants used in embryo culture for sweet cherry hybrid seeds

Components	V1	V2	V3	V4
Macroelements	L F (1977)	L F (1977)	MS (1962)	MS (1962)
Microelements	L F (1977)	L F (1977)	MS (1962)	MS (1962)
Vitamins	L F (1977)	L F (1977)	MS (1962)	MS (1962)
Chelates (NaFeEDTA)	32 mg/l	32 mg/l	32 mg/l	32 mg/l
Phytohormons:				
-IBA	5 mg/l	-	5 mg/l	-
-BAP	20 mg/l	-	20 mg/l	-
Sucrose	20 g/ l	20 g/ l	20 g/ l	20 g/ l
Agar	6 g/l	6 g/l	6 g/l	6 g/l