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The *in vitro* Establishment of Walnut (*J. regia* L.) by Using two Alternative Gelling Agents first Results

Rodica GOTEA[1)], Mohammad Ali SHEIKH BEIG GOHARIZZI[2)], Ionuţ GOTEA[1)], Mohammad ZIAEE[3)], Mina FARSI[3)], Radu E. SESTRAS[1)], Kourosh VAHDATI[3*)]

[1)] University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5 Manastur Str., Cluj-Napoca 400372, Romania; rodica@gotea.ro.
[2)] University of Moscow, Russia
[3)] University of Tehran, College of Abouraihan, Iran; kvahdati@ut.ac.ir (*corresponding author)

Abstract. The in vitro establishment of three Romanian walnut cultivars ('Mihaela', 'Muscelean' and 'Jupâneşti') from microcuttings was studied by using two alternative gelling agents (cassava flour and tapioca) and DKW basal medium. The agar concentration was established at 8 g/L and the concentrations for the other two gelling agents where established at 150 g/L for cassava flour and at 15 g/L for tapioca. The best growth was recorded for 'Jupâneşti' cultivar on the DKW basal medium with BAP (1 mg/L) and solidified with tapioca (15 g/L).

Keywords: *Manihot esculenta*, agar, micropropagation

INTRODUCTION

The common walnut (*Juglans regia* L.) is a special species because of his economic, cultural and food qualities. One of the component of the culture medium is the gelling agent. In almost all studies about in vitro micropropagation of walnut are reported the same problems that can apear in the case of the gelling agent: inhibiting action and vitrification (or hyperhydricity).

In this research, we have made an experiment with two alternative gelling agents, cassava flour and tapioca compared with an ordinary agar that can be used in walnut micropropagation.

Cassava flour comes from the roots of *Manihot esculenta* Crantz sp. and has a high content of starch (bigger than 90%) (Onuweme, 1982) and tapioca is made from partially gelatinized cassava starch by wet heating.

The most common available shape for tapioca is in spherical „pearls", where size range from 1mm to 8 mm, with the 2-3 mm beign the most common. Cassava plant is one of the most important food crops in the humid tropics, being particularly suited to conditions of low nutrient availability. Also, cassava plant gives a carbohydrate production >40% than rice and >25% than maize (Nyerhovwo, 2004).

Cassava flour acts as an additional carbon source and adds other ionic supplements (35% carbohydrates, 1% mineral matter) to the medium (Onuweme, 1982).

According to other researchers (Maliro and Lameck, 2004), cassava flour as a gelling agent in media for plant tissue cultures has a valuable potential. The proximate composition of tapioca (cassava starch) is: humidity 1.1%; dry matter: carbohydrates 95.6%, proteins (arginine, tryptophan, cystine and amino acids) 1.5%, lipids 0.3%, crude fiber 0.7%, ash 0.3%, zero cyanide).

MATERIALS AND METHODS

The initial explants (uninodal microcuttings with a length between 1 and 1.2 cm) were obtained from the young walnut seedlings (2-3 years) of three cultivars. The microcuttings were sterilized by washing very well with an ordinary detergent and water followed by the washing with alcohol for 5-10 minutes, 5% hypochlorite for 20 minutes and distillated water.

For analysing the establishment rate of walnut microcuttings, 9 treatments (3 cultivars with three different gelling agents) were compared in a factorial design as a completely randomized design. Thereby, the factor A was represented by three different cultivars ('Mihaela', 'Muscelean' and 'Jupâneşti' and the factor B was represented by three different gelling agents (8 g/L agar, 150 g/L cassava flour and 15 g/L tapioca).

Each treatment included four replications with each replication containing four explants. For all treatments was used DKW as basal medium with 0.1 mg/l IBA, 1 mg/l BAP and sucrose 3%. The pH of culture medium was adjusted to 5.5 before adding the gelling agent and autoclaving. Microcuttings were kept at 25±2°2.1 g/l PhytagelC under a 16 h photoperiod and light intensity of 40-60 µmol m-1sec-1 supplied by cool white Phylips fluorescent lamp (Vahdati *et al*., 2004).

The cultures were maintained four weeks on the same medium and the data were analyzed using analysis of variance followed by Duncan's mean comparisons test. After four weeks the shoots were removed from microcuttings and inoculated on fresh culture medium.

RESULTS AND DISCUSSIONS

Regarding the height growth, where recorded significantly differences between all tree cultivars. Compared with the two others cultivars, the smallest growth was recorded for the 'Mihaela' cultivar. The biggest height growth was recorded for 'Jupâneşti' cultivar (Tab. 1).

Tab. 1

The height of walnut shoots (cm) after 4 weeks of inoculation under the influence of three cultivars and three gelling agents

Cultivar/Geling agent	Agar	Cassava flour	Tapioca	Cultivar average
'Jupanesti'	1.01 cdg	0.74 e	1.42 a	1.06 A
'Mihaela'	0.9 de	0.47 f	1.18 bc	0.85 B
'Muscelean'	1.25 ab	0.43 f	1.2 bc	0.96 C
Gelling agent average	1.10 M	0.50 N	1.30 O	-

Note: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$). LSD5% cvs 0.12; LSD5% gelling agents 0.12; LSD5% interaction cvs x gelling agents 0.21-0.24.

The culture media has influenced significantly the height growth for all experimental variants. The smallest growth was recorded for the Cassava flour and the highest growth for Tapioca medium.

The smallest growth was obtained for the 'Muscelean'/Cassava flour combination and for the 'Mihaela'/Cassava flour combinations. All other combinations cultivar/culture media had height growth significantly superior to the above mentioned combinations.

After four weeks, the culture medium gellified with cassava flour has presented signs of degradation. This problem was also observed by Maliro and Grace (2004). To improve the stability of the culture medium is recommended by the researchers to mix the cassava flour with another agar (Maliro and Grace 2004; Daud *et al*., 2011). Probably, a combination between tapioca and Phytagel will improve the results obtained.

The culture medium gellified with tapioca pearls has presented a good stability after four weeks.

Endale and Santhyanarayana (2001) have obtained a good increase in size of potato microtubers on MS basal medium solidified with tapioca. According to the two authors mentioned above, this increase of potato microtubers was possibly because of improving the organic and inorganic composition of medium while maintaining a good osmotic concentration.

Anoop and Chauhan (2011) have developed a low cost protocol for in vitro culture of potato. They found that, the shoot height and the node number increased on medium with a concentration level of tapioca lower that 15 g/L. The level concentration of tapioca higher than 18 g/L inhibited the development of potato shoots and node number.

The best response of starches media gelled could also be due to the absence of inhibitors, which have been reported to be in agar (Debergh, 1983).

The alternative gelling agents can be a very good opportunity in terms of economic efficiency and effectiveness of the culture medium.

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