Formation of axillary shoots of *Castanea sativa* Mill. in vitro

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ABSTRACT. – The work deals with the effects of two culture media on the formation of axillary shoots of spanish chestnut (*Castanea sativa* Mill.) in vitro culture. Data presented indicate that the composition of culture medium is important for induction of axillary shoots. The mean number of axillary shoots on the S_2 medium was 2.95 per explant. By containing calcium to S_1 medium the number of produced shoots increased significantly (4.28 per explant) and formation of vitrified shoots decreased and drying of tip was suppressed. In the WPM_1 medium containing calcium, and callus formation and the elongation growth of shoots was suppressed. The mean number of axillary shoots on the WPM_1 medium was 4.43 per explant in comparison with WPM_2 medium which was 2.66 per explant.

KEY WORDS - Chestnut, regeneration, medium composition.

INTRODUCTION

Spanish chestnut (*Castanea sativa* Mill.) is not only a fruit tree, but it also occupies an important place in forest stands composition. In the beginning of the 20th century a root system disease caused by fungi from the genus *Phytophtora* attacked the trees. This disease has always been responsible for their getting dry. At present in western and central Europe and as well as in America chestnut blight caused by the fungus *Cryphonectria parasitica* (Murr.) Barr (formerly *Endothia parasitica* (Murr.) And. have been spread and together with excessive felling are causes of this decline.

In vitro methods and biological control are perspective methods for protection and reintroduction of chestnut on its original locality (Serres *et al.* 1990, Kamenicka 1992, Juhasova *et al.* 1993, Nuss 1993). These methods create possibilities not only to save these plants but also reintroduce them into original locality. But more efficient application of these methods is dependent on a number of factors including of culture media. Other factors in the developmental stage of explants, also characterized by the presence of endogenous plant growth regulators in the period of establishment of the culture, play an important function. In the cultivation of edible chestnut explants we often meet vitrification and drying of tips which lower the effectiveness of axillary shoots production (Rypak and Kamenicka 1986).

Positive results in vitro progagation have been reached with *Castanea sativa* Mill., *C. dentata* (Marsh) Borkh. and their hybrids *C. sativa x C. crenata* (Chevre *et al.* 1983, Sanchez and Vieitez 1991).

The aim of the present work is to examine effects of culture media on formation axillary shoot of spanish chestnut (*Castanea sativa* Mill.) in vitro.

Material and Methods

In our experiments for initial explants we used shoots from 70 year old trees. After removing leaves, (April) shoots were disinfected with 0.1-0.2% $HgCl_2$ plus 10 drops of detergent. Tween 20 (0.03-0.05%) per liter from 3 to 4 min. Disinfected shoots were soaked (30 sec.) in etanole and rinsed with distilled water 3 or 4 times. In a laminar flow hood shoots were rinsed with redistilled water (2-3 times) and cut into 3-4 nodal segments. The explants were cultured on basal and modified media according to Lloyd and McCown (1981) marked as «WPM» and Standardi and Catalano (1985) marked as «S». The media were supplemented with 0.3 mg. l^{-1} benzyladenine (BA) and 0.1 mg. l^{-1} naphtaleneacetic acid (NAA), sucrose (20 g. l^{-1}) and agar (6.5 g. l^{-1}). Modified media WPM_1 and S_1 were supplemented with 120 mg. l^{-1} calcium (Ca^{2+}). Culture vessels (100 ml in volume) contained 25 ml of medium. All cultures were kept under 16 hr light (35-40 µmol. m^{-2} s^{-1}) 8 hr dark photocycle at 20-24 °C in airconditioned boxes. The medium acidity was adjusted to pH 5.4-5.6 (1N NaOH) before autoclaving.

After 6-8 weeks the shoot cultures were transfered on to media with a similar composition as for culture initiation. The results were evaluated by the statistic programme STATGRAPHICS 05. The significance of the differences were evaluated using Student's t-test (Table 1, 2, 3).

Results and discussion

Effects of the basal media WPM_2 and S_2 on the number and length of differentiated axillary shoots are not statistically significant (Table 1). The mean number of axillary shoots on WPM_2 medium was 2.667 per explant and S_2 on medium was 2.954 per explant (Fig. 1). Biomass production of the growing plants on the S_1 medium was higher in comparison with another tested media (Table 2). On the S_2 medium 44.5% of sound and 55.5% of vitrified shoots have been differenciated. Vitrified shoots were unable to be continued multiplication or rooting (Fig. 2). In accordance with the literature data it has been documented that the tip necrosis is related to the lowered translocation ability of some culture medium components, especially of calcium into leaves (McCown and Sellmer 1987).

On the culture media supplemented with calcium (WPM_1 and S_1) a high percentage of axillary shoots without dryed tip and doubled multiplication effect was produced (Fig. 3a, 3b). Higher shoot multiplication coefficient on WPM_1 and S_1 media were also confirmed by differences between the fresh and dry mass (Table 2). On the WPM_1 medium growth of callus and slowed down growth elongation of shoots was suppressed.

The S_1 medium stimulated both the growth elongation of shoots and callus formation of them (Table 3).

TABLE 1
EFFECTS OF CULTURE MEDIA ON THE NUMBER AND LENGTH OF AXILLARY SHOOTS OF *CASTANEA SATIVA* MILL.

Media	Mean number of shoots/ explant ± SE	Mean length of shoots/ explant ± SE (mm)
WPM_1	4.437 ± 0.267	8.344 ± 0.669
WPM_2	2.667 ± 0.306	20.442 ± 0.910
S_1	4.286 ± 0.170	19.140 ± 0.688
S_2	2.954 ± 0.169	21.174 ± 0.324

TABLE 2
FRESH (FW) AND DRY (DW) MASS OF THE DIFFERENT PARTS OF THE EXPLANT *CASTANEA SATIVA* MILL.

Media	Fresh mass shoots/explant ± SE	Dry mass shoots/explant ± SE	Fresh/dry mass ratio
WPM_1	0.137 ± 0.011	0.017 ± 0.001	8.05
WPM_2	0.163 ± 0.010	0.028 ± 0.002	5.82
S_1	0.348 ± 0.018	0.036 ± 0.002	9.66
S_2	0.237 ± 0.017	0.044 ± 0.003	5.38

TABLE 3
GROWTH OF CALLUS OF *CASTANEA SATIVA* MILL. SHOOT CULTURE

Media	Fresh mass callus ± SE	Dry mass callus ± SE	Fresh/dry mass ratio
WPM_1	–	–	–
WPM_2	0.474 ± 0,031	0.602 ± 0.003	7.64
S_1	0.511 ± 0.027	0.059 ± 0.003	8.66
S_2	0.212 ± 0.017	0.031 ± 0.002	6.84

We suppose that the effect of calcium was manifested in the influence on cell structure, but especially on functions of mitochondria which are the energetic and metabolic centre of cells (Bobak 1991). Roodyn (1967) indicated that the change of physical conditions led to a change in the contents of Ca, Mg and Na ions in mitochondria. Many authors demostrated extraordinary importance of calcium ions in metabolism (Barlow and Sangent 1975. Esau and Charvat 1978). Calcium might play an important function especially in maintainance meristematic activity of explants (Bornman 1983) and in the translocation of auxins from plant tissue to culture medium (Stonier and Yang Hsin-Mei 1971, Declerk and Korban 1994). Function of culture media components are also important in the regulation of the in vitro morphogenesis. The results confirmed that it is possible to control correlations between cultivation conditions and morphogenetic response of plant cells to in vitro conditions by means of culture media.

Fig. 1 – The formation of axillary shoots of *Castanea sativa* Mill. on S_2 medium in vitro.

Fig. 2 – Vitrified shoots of *Castanea sativa* Mill. with hyperhydrated leaves.

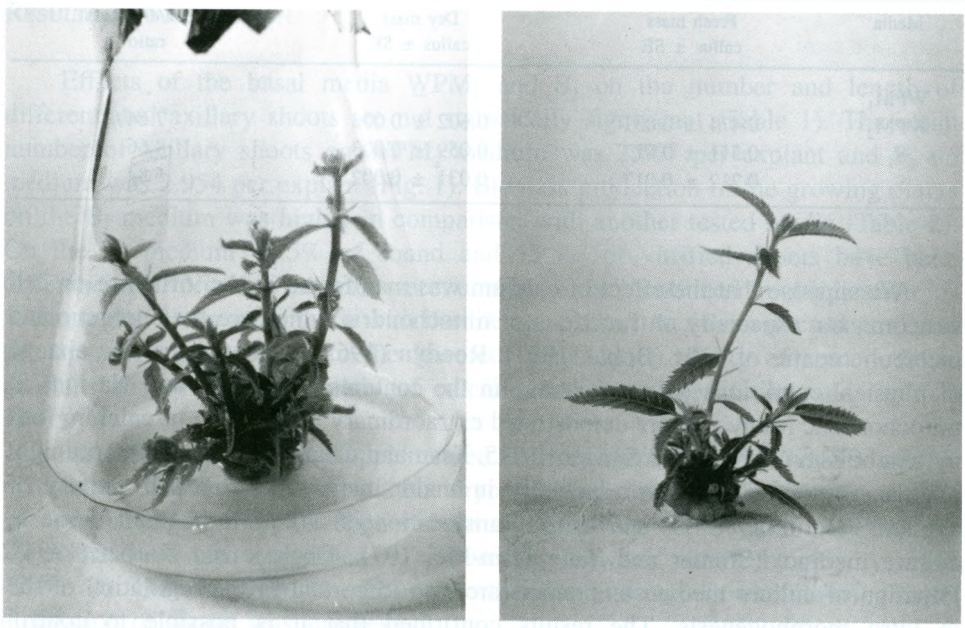

Fig. 3 – Effects of WPM_1 medium (a) and S_1 medium (b) on formation of axillary shoots of *Castanea sativa* Mill.

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