THE USE OF CORN STARCH ON IN VITRO PROPAGATION OF PLUM

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Abstract

Research in the field of in vitro cultivation of plants is designed in two aspects: to reduce costs and to take the highest coefficient of survival plants per explant. The using of corn starch instead of agar as a gelling agent and cheap alternative for plum cv. *Shengjine* was investigated on in vitro propagation media. Explants from plum single node and shoot apicals were subcultured onto MS/2 macro-elements and MS/2 microelements medium. Three levels of corn starch as gelling agent at concentration of 6 g/l (similar to agar concentration), 50 and 70 g/l respectively and an agar mediun of 6g/l were evaluated using apical shoot explants of plum cv. *Shëngjine*. Shoot tip explants were planted singly into test tubes containing ten millilitres each of multiplication medium gelled in the above concentrations and placed on shelves under 14 h photo period supplied by white flurescent tubes. Temperature was maintained at 24±2°C. Corn starch at above concentrations maintained gel integrity throughout the culturing period 42 days. The highest number of shoots/explants after 6 weeks (2,4 and 2,55) was achieved in medium with 50 g/l corn starch and in the medium with 6 g/l Agar. Corn starch had no significant effect in the number of shoots per explant in the height of shoots compared with Agar. While the number, the length and the thickness of the root was better in Agar medium.

Key words: plum, agar, corn starch, gelling, indol butiric aciod, explant

1. Introduction

The objective of this study was evaluate the potential of corn starch as an alternative low-cost gelling agent in culture medium for plum cv. *Shengjine* on in vitro micro-propagation using shoot apical cuttings. Establishment of plant micro-propagation laboratories and the propagation of the plant by in vitro culture must be based on cost effectiveness. Agar, as solidifying agent, due to its stability, high clarity, nontoxic nature and resistance to its metabolism, is commonly used in the plant tissue culture [4]. Although, some researchers have reported that more important is the need to avoid the inhibitory symptoms in cultures caused by toxic substances contained in agar [6] or to enhance shoot growth in species recalcitrant on media gelled in standard gelling agents [9]. Also, agar represents one of the most expensive and commonly used media components, contributing in about 70% of the total production cost [7]. Plant starches are suitable for gelling tissue culture media, comparing favorably with the more conventional gelling agent such as agar [3].

In lieu of agar, many plant based alternatives have been proposed as Corn starch and Cassava starch [9, 2]. Smykalova found that the growth of proliferated shoots of *Humulus lupulus* on corn starch medium was better than on agar one [8]. It has been demonstrated that growth and differentiation of plant cell cultures were increased when media were gelled with corn starch instead of agar [1]. In this research it is tried to find a suitable substrate instead of agar, because of high price of pure agar.

2. Materials and Methods

Single-node and shoot apical explants (5-7mm) produced from tender shoots, born in 1-year branches or in new spring growths of 10- years-old plum trees cv. *Shengjine* were prepared aseptically, disinfected for 3 times x 1.5 min in a 0.1% solution of $HgCl_2$. Single- node and shoot apical explants were grown in 25x150 mm culture tubes with 10 ml of the medium. The medium was consisted of MS with macro-elements and micro-elements reduced at half. The medium supplemented with cystine 0.5 mg/l, pantotheneic acid calcium 10 mg/l, thiamine HCl 0.5mg/l, glycine 2 mg/l, myoinositol 100mg/l, GA_3 0.5mg/l, IAA 0.1 mg/l and sacharose 30 mg/l. Benzyladenine (BAP) at concentration of 2 mg/l was tested in the initiation and multiplication stages. Medium was solidified with 6, 50 or 70 g/l of corn starch. Time of heating was the same for the three mentioned concentrations and equal to 30 min. Medium with 6 g/l of agar was used as a control. The pH was adjusted to 5.6 prior to autoclaving for 20

minutes 121°C. After sterilization 4 random samples with 50 explants from each type of media were individually homogenized. The obtained results were submitted to an analysis of variance and the means were compared using the separation by Duncan's multiple range test.

3. Results and discussion

The optimal corn starch concentration for multiplication (50 g/l) was determined in a test with benzyl adenine at 2 mg/l and in combination with corn starch, as gelling agent at concentration of 6, 50 and 70 mg/l respectively (Table 1).

The results presented in Table 1 show that the use of corn starch at concentrations 50 g/l gives results similar to that of agar at concentration 6 g/l. The shoot number per explants, 6 weeks after planting, was 2.55 to 6 g/l agar and 2, 4 to 50 g/l corn starch. The starch concentration at 6 g/l did not coagulate well the substrate, in temperature 50 ^{o}C and duration of heat 30 minutes. Besides this, the first week of planting water was liberated from the medium in quantities as much as half of the medium's volume. While, the use of 70 g/l of starch reduced the number of shoots (2.15) and their length as well (1.92). This happened because the medium became too rigid. These results coincide with those of other researchers [5, 8], who have used MS gelled with 1g /l agar and 40, 50. 60 g/l commercial starch, where the highest number of shoots per explants (6.8) was achieved in medium with 50 or 60 g/l of potato or corn starch +1 g/l of agar.

In this case the corn starch can be used in place of agar, which is very expensive and constitutes 70% of the cost of in vitro propagation. The mean number of roots per shoot and also mean length of them were higher at medium gelled with 6 g/l agar, 2,45 root/ per shoot. On the other hand the number of roots per shoot at medium gelled with 50 g/l and 70 g/l corn starch was 2.192 and 1.38 respectively. Moreover, the length of roots in the medium gelled with agar was higher than those gelled with corn starch (Table 2)

However, it is shown by visual observations variability in the degree of robustness between roots derived by starch and they derived by agar-gelled medium, as it is illustrated on Fig. 2. In this figure, it is shown that the roots derived from agar, as gelling agent, are thicker and longer, than those derived from the substrate clotted with amidon. The number of shoots and their length did not differ for the two media (Figure 1)

Table 1: Number and length of the shoots per explants in the medium with agar and corn starch and 2 ppm BAP.

Solidified medium	Shoot number	Shoot length
Agar 6gr/l	2,55a	2,45 a
Starch 6gr/l	-	-
Starch 50 gr/l	2,4a	2,54a
Starch 70 gr/l	2,15b+*	1.92 b+*

* Separation by Duncan's multiple range test, at P< 0.05 (+Mean of four replications)

Table 2: Number and length of the roots per explants in the medium with agar and corn starch and 1ppm IBA.

Variant /repetition	P1		P2		P3		P4		Mean	
Solidified medium	Root number	Root length	Root number	Root number	Root length	Root number	Root length	Root number	Root number	Root Length
Agar 6mg/l	2.6	2.46	2.4	2.7	2.4	2.44	2.4	2.68	2.45 a+*	2.57a+*
Starch 30mg/l	-	-	-	-	-	-	-	-	-	-
Starch 50mg/l	2,2	2,24	2,1	1,95	2,35	2.15	2,12	1,86	2,192b	2,05b
Starch 70mg/l	1,3	1,8	1,6	1.43	1,4	1,5	1,23	1,2	1.38c	1,48 c

* Separation by Duncan's multiple range test, at P< 0.05. (+ Mean of four replications).

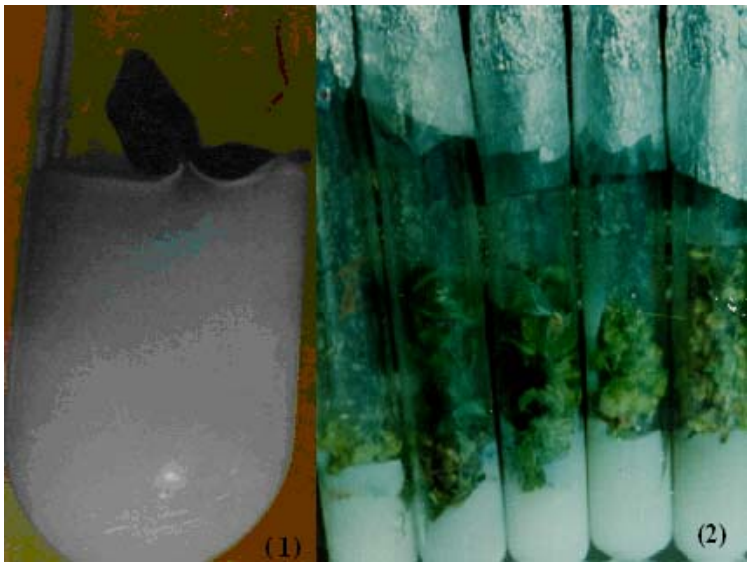

Figure 1: Proliferation of the plum explants on the medium clotted with starch. (1 - First day of planting 2- Six weeks after planting).

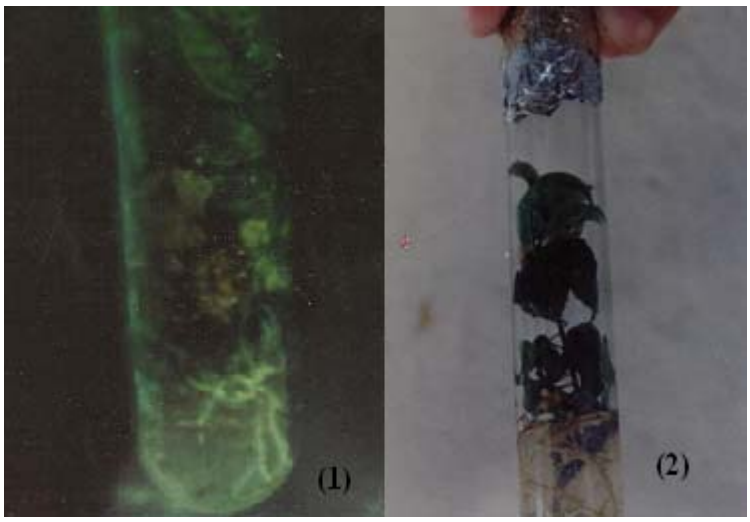

Figure 2: The growth of roots 6 weeks after transplanting. (1 - In the medium clotted with corn starch (1, 2 - In the medium clotted with agar).

In conclusion, only for the production of shoots in vitro, it is recommended the use of corn starch as a solidified substance instead of the use of agar, while for the rooting of the shoots the substrate should be thickened with agar.

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