INFLUENCE OF METHANOL EXTRACT IN SHOOT PROLIFERATION OF PLUM (*PRUNUS DOMESTICA* .) CV "SHENGJINE" BY IN VTRO PROPAGATION

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Abstract

Trials were undertaken to develop a method for rapid propagation of this commercial early cultivar of plum and to overcome supply problems for extending it. A micropropagation method is described using single-node and shoot apical explants from plants grown in the field. Shoot proliferation was obtained on modified MS medium supplemented with 0.5 mg GA_3, 0.1 mg IAA and different concentrations of BAP (0.5; 1; 1.5 and 2 mg/l). Explants were rooted on a medium similar to that for shoot development, but without cytokinins and with IBA at 0.5;1and 1.5 mg/l. Shoot proliferation and shoot length was improved by the addition of crude extracts from shoot apicals of the same plants,collected in March, to the nutrient media at 30 mg/l. Rooted explants were transferred to pots containing equal volumes of peat and perlite. About 75% survived.

Key words: plum, Shengjine, crude extract, indol butiric aciod, benzilaminepurine, explant

1. Introduction

Micropropagation is being used extensively for the rapid clonal propagation of many fruits, nuts and ornamental trees, since it enables rapid propagation and hastens the availability of new cultivars [12]. Plum is an important fruit tree of Albania. "Shengjine" is an albanian native plum variety that matures in the month of April. It is the earliest fresh fruit in Albanian market. Plumes are propagated by budding or grafting on to seedling or clonal rootstocks. The Prunus genus is one of the major difficulties with respect to in vitro organogenesis from mature tissues. Micropagation from shoot cuttings of plum cv, Kantimirovskaja has been reported by Leontiev- Orlov et al [7]. Also, a protocol for in vitro propagation of *Prunus domestica* was developed by H.Y Yan et al [11]. Nodal segments were used as the explants in their study. For the initiation of culture, B_5 medium was better than Murashige and Skoog (MS) medium. Influence of different raw ingredients as juices of various fruits and plant extracts' ingredients has been proved by many researchers on the proliferation and rooting of explants. The effectiveness of coconut water, BAP, or kinetin, as possible zeatin substitutes in olive micropropagation protocols, was investigated [8]. Higher multiplication rates were observed with zeatin at 9.12 mM, or with BAP at 8.87 mM together with 50 mg /l coconut water. The combination of olive knot extract at 25 or 50 mg, l–1 with cytokinins suppressed shoot proliferation of olive cv. Koroneki [10]. Crude extract from olive ovules has been used in proliferation and rooting of olive cv "Kalamon" by Rama and Pontikis [9]. The present work was undertaken to develop a method for rapid propagation of prunus domestica cv "Shengjine" an important precoce cultivar difficulty to propagate by cutting.

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 25x150mm culture tubes with 10 ml of agar medium. After four weeks cultures were transferred to 250 ml erlenmeyer flasks containing 125 ml of the same medium. The cultures were grown at 25±2 ^oC under 16h photo- period and intensity 4000 lux from cool white fluorescent tubular lamps. The medium was

from MS with macroelements and microelements reduced at half. The medium supplemented with cystine 0.5 mg/l, pantotheneic acid calcium 10 mg/l,thiamine HCl 0.5mg/l, glycine 2mg/l, myoinositol 100mg/l, GA_3 0.5mg/l, IAA 0.1mg/l and sacharose 30mg/l. The Ph was adjusted to 5.6 before adding 6.0 g/l Oxoid agar no3. Benzyladenine (BAP) at concetrations of 0.5, 1, 1.5 and 2 mg/l were tested in the initiation and multiplication stages. Also, crude extracts from apical shoots of plum cv "Shengjine" at concentration 20, 30, 40 mg/l in combination with 1mg/l BAP were tested to determine the optimum crude extract concentrations in the multiplication stage. For rooting, shoots at least 1.5 cm long were transferred to a rooting medium similar to the multiplication medium but without cytokinins and with indol butyric acid (IBA) at concentration 0.5, 1 and 1.5 mg/l.Crude extract at concentration 40, 50, 60 mg/l in combination with 1mg IBA were tested to determine the optimum crude extract concentration in the rooting. The effect of crude into the rooting medium in combination with auxins was determined and by the mung bean test [6].

Crude extract was prepared with apical shoots harvested in March. The shoots were cut into 2-3 mm pieces and fresh tissue macerated in pre-chilled absolute methanol, at ratio of 50g fresh tissue to 150ml of solvent, and extracted for 24h at 4 ^{o}C to reduce possible enzymatic reaction [3] . The methanol extract with the apical shoots tissue was then shaken mechanically for 30 min at 4 ^{o}C and clarified by filtration through Whatman no.1 filter paper. The methanol extract was evaporated at 40 ^{o}C to a small residue. This powder was stored at -15 ^{o}C.

Experiment with plum explants were arranged in a randomized complete block design with 30 explants per treatment and four replications. The dates were analyzed for statistical significance by analysis of variance with mean separation by Duncan's multiple range test.

3. Results and discussion

Seventy to eighty per cent of shoot apicals explants survived sterilization with $HgCl_2$. The mean shoot number and mean shoot length of different medium are summarized in Table 1. The culture medium may not be critical, since many species can be propagated well on MS or a slightly modified Ms medium. Differences in the adaptability to in vitro culture have been observed between prunus species [2] and it seems that requirements for mineral elements, growth regulators and aminoacids may differ between plum cultivars.

With the plum cv. "Shengjine" a diffcult species to propagate in vitro a medium with macro and micro elements reduced at half was more effective.

The explants prepared from tender shoots, born in 1-year branches were more effective than those prepared from tender shoots born in new spring growths .The mean number and mean length of the shoots per explants was higher (2.45, 1.68 and 2.05,1.37 respectively) (see Table 2).

That means that physiological state of the donor plants or donor part of the plant has a great influence on the behaviour of the meristematic explant [9, 4].

In determination the aptimum benzyladenine requirements for multiplication, greatest proliferation was achieved at 1mg/l benzyladenine (Table 2), but best results were obtained when BAP were combined at their optimum concentration with 30mg/l crude extract (Table 3).

The optimum crude extract concentration for multiplication (30mg/l), was determined in a test with benzyladenine at 1mg/l and in combination with crude extract at concentration of 10, 20, and 30 and 40mg/l (Table 2)

The mean number of shoots per explant in 6 weeks was highest at 1mg/l BAP (2.5). Benzyladenine in combination with crude extract at concentration (30 mg/l) increased significantly the mean number of shoots per explant with the means of 3.5 in 6 weeks

Table 1: Effect of different medium in shoot proliferation of plum explant "in vitro" (after 6 weeks)

Different medium	The mean shoot number per explant	The mean s hoot length
Ms. comlet	3.3+	1.6 +
Ms. with microelemnts reduced at half	3.3	1.88
Ms. with macro and microelements reduced at half	4.17*	2.45 *

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

Table 2: The mean shoot number and mean length of shoot of different explants (after 6 weeks)

Explant origin	The mean shoot number per explant	The mean shoot length
From tender shoots, born on 1-year branches	3.95a*+	2.55a*+
From tender shoots, born on new spring growths	3.37b	2.1b

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

Table 3: Interaction of BAP and plum crude extract on shoot number per explant and shoot length, (after 6 weeks)

Treatments	The mean shoot number per explant	The mean shoot length
BAP 1ppm	2.5a	2.95a
BAP 1ppm+20ppm extract	2.5a	3.3b
BAP 1ppm+30ppm extract	3.2b*+	3.9c*+
BAP 1ppm +40ppm extract	2.8a	3.15a

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

Table 4: Interaction of IBA and plum crude extract on root number per shoot and root length (after 6 weeks)

Treatments	The mean root number per shoot	The mean root length
IBA 1ppm	2.45a	2.57a
IBA 1ppm+40ppm extract	2.34a	2.27a
IBA 1ppm+50ppm extract	2.2a	2.4 a
IBA 1ppm +60ppm extract	1.9a	2.45a

(Tab3). Crude extract at 40 mg/l in combination with benzyladenine decreased the mean shoot number per explant significantly (2.9)(P= 0,05). The mean shoot length was increased significantly (P=0.05), by combination of the optimum crude extract concentration to 30 mg/l with 1mg/l BAP

The annual shoot formation rates were about 739 for benzyladenine 1ppm, and 1230 for benzyladenine in combination with crude extract at the optimum concentration (30mg/l).

The mean number of roots per shoot and mean length of root was higher at 1mg/l IBA.

However, the mean root number and length of the roots was not markedly different between IBA alone and IBA 1 ppm in combination with crude extract at concentration of 40, 50, 60 ppm (Table 4).

This indicates that indol compounds were destroyed or the concentration of the crude extract used in medium was lower, because the biotest with *Phaseolus aureus* confirmed that in this crude extract there were root formation compounds.

Our results indicate that growth substances are contained in the crude extract. The substances may be indol or phenolic compounds and citokinins. It appears that an explantation other than the inhabitation of auxin destruction should therefore be sought. In the case of shoot growth citokinins interact with indol or /and phenolic compounds present in the crude extract and stimulate growth.

Shoot growth and root induction effects of crude extract confirms the results reported by many authors [8, 10, 9, 6, 5, 1].

Plant survival was about 75% when rooted explants were transferred from aceptic culture to pots containing equal volume of peat and perlite

The evidence of this study confirms the favourable use of plant extract for growth and rooting of this specie. Studies to elucidate the identity of the growth and root promotive substance(s) in the shoot apicals extract of the plum should greatly enhance their value in shoot growth and root induction.

4. References

1. Diagneault.T and Chong.C: **Characterization of the root promoting activity in willow extract**. *The international Plant propagator' scombined proceeding* 1985(35).509-518.
2. Guo Gui Ning, Xiao Li Fan, Wen Jun Huang, Man Zhu Bao, Jin Bo Zhu: **Micropropagation of six Prunus mume cultivars through axillary shoot proliferation and ISSR analysis of cloned plants**. *Acta biologica Crasoviensia Series Botanica* 2007, 49(1): 25
3. Harboner J.: **Phytochemical methods**. *A guide to moderntechniques of plant analysis*, 1st edition, Chapman and Hall. London, (1973)195-198
4. Inmaculada Vila, Ester Sales: **Micropropagation of Oleander (Nerium Orleander.L)** *HortScience* 2010. 45(1):98-102.
5. Jones.O.P: **Effect of phlorizdinand phloroglucinol on apple shots.** *Nture*, UK1976 (262), 392-393.
6. Kawase M: **Root promoting substances in Salix alba**. *Physiologia Plantarum* 1970, 23 (1): 159-170
7. Leontiev-Orlov O., Mossi A.J., Cansian R. L., Rogalski M., Vendruscolo T: **Effect of different growth regulators on in vitro propagation of plum (Prunus domestica L.) cv. Kantimirovskaja**. *Revista Brasileira de Fruticultura* 2000 22(2): 268-271.
8. Peixe A, Raposo A, Lourenco R, Cardoso H, Macedo E: **Coconut water and BAP successfully replaced zeatin in olive (Olea europaea L.) micropropagation**, Scientia Horticulturae 2007, 113(1): 1
9. Rama. P, Pontikis C. A: **In vitro propagation of olive (Olea europea sativa. L) 'Klamon'.** Jurnal of horticultural science 1990, 65 (3): 347-353.
10. Roussos P.A, Pontikis C.A: **In vitro propagation of olive (Oleaeuropaea L.) cv. Koroneiki.** *Plant Growth Regulat* 2002. 37(3): 295.
11. Yan H.Y, Wu Y.X, Liao K, Geng W.J, Li J, Xu Z, Wang T: **In vitro propagation of wild European plum(PrunusxDomestica L.)**, *a rare and endangered species* 2008. Acta Horticulturae 839.
12. Zimmerman R.H: **Micropropagation of fruit plants.** *Acta Horticulturae* 1981.(120):217-222.

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