En Vitro Propagation of Prunus serotina

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ABSTRACT -- Scions from mature Prunus serotina trees were grafted onto seedling root stocks and grown in a greenhouse for approximately five years. Lateral buds were excised just as the buds were beginning to swell. Bud elongation was initiated on Murashige and Skoog inorganic salts, modified with 100 mg/liter i-Inositol, 0.4 mg/liter thiamine-HCL, LO mg/liter N-6-benzylaminopurine (BA) , 0.1 mg/liter gibberillic acid (GA_3), 0.1 mg/liter indole-3-butyric acid (IBA), 2% sucrose, and 7.0 gms/liter phytagar. Buds elongated in 2 to 4 weeks. Adventicious shoot multiplication was induced on the same basal medium as above, modified with 0.75 mg/liter BA, 0.2 mg/-liter GA3, and 0.01 mg/liter IBA. The sucrose concentration was increased to 3%. An average shoot multiplication rate of 5 to 1 was achieved every 3 to 4 weeks. Root formation was induced in vitro on half strength Murashige and Skoog inorganic salts, 100 mg/liter i-Inositol, 0.4 mg/-liter liter thiamine-HCL, 1.0 mg/liter IBA, 2% sucrose, and 7.0 gms/liter phytagar. Rooting was significantly enhanced by incubating the cultures in continuous darkness. Roots develop in 7 to 10 days.

INTRODUCTION

The use of tissue culture for micropropagation of woody species has become increasingly successful within the past few years. The majority of this work has been done on woody deciduous fruit and nut trees (Lane 1982). In vitro propagation of a number of prunus cultivars and species have been accomplished by Quoirin et al., (1977). Tabachnik and Kester (1979) have propagated P. Amygdalus in vitro. Tissue culture has been successfully used to produce plantlets of Prunus myrobalan (Nementh 1979), P. inistita and P. avium (Jones 1979) and P. salicina (Rosati et. al, 1980).

This paper is concerned with tissue culture propagation of mature Prunus serotina Ehrh. trees.

P. serotina is one of the most valuable hardwoods in the Northeastern United State. If successful, micropropagation of black cherry will allow for rapid increase of selected, superior

clones, which could be used in one of two ways. First, they could be used to develop seed orchards of elite trees. Secondly, if tissue cultured plants can be produced at a competetive price, large numbers of select trees could be produced for field planting in clonal mixes.

MATERIALS AND METHODS

Establishment of cultures (Stage 1)

Scions from mature P. serotina trees were collected at the experimental station of the College of Environmental Science and Forestry at Syracuse. These scions were grafted onto seedling rootstocks and grown in a greenhouse for approximately 5 years. The scions continued to flower, indicating that the material was still in a mature state. Twigs were collected just when the lateral buds were beginning to swell. Stem segments were prepared by sectioning the twigs so that each segment contained 1-2 lateral buds. The segments were rinsed in running tap water for 60 minutes, and then soaked for 10 minutes in benomyl 50% W.P. (1.5 mg/ml) (Bonide Chemical Co., Yorkville, NY 13495). The sections were disinfested in a 0.525% solution of sodium hypochlorite for 20 minutes followed by one rinse in sterile distilled water. With the aid of a dissecting microscope, the bud scales were removed and the bud was reduced to 0.1 to 2.0 mm. The excised tissue was transferred to culture tubes containing ten mls. of Murashige and Skoog inorganic salts supplemented with 2% sucrose, 0.4 mg/liter thiamine-HCL, 100 mg/liter i-Inositol, 1.0 mg/liter N-6-Benzylaminopurine (BA), 0.1 mg/liter gibberillic acid (GA_3), 0.1 mg/liter Indole-3-butyric acid (IBA), and 0.7% phytagar (Grand Island Biological Co., Grand Island, N.Y. 14072). The medium was adjusted to, ph 5.6 with 0.1 N KOH or HCL. Cultures were incubated at 26^0C, with a photoperiod of 16 hours light, 8 hours dark, under 3 klux cool white fluorescent lamps.

Shoot Multiplication (Stage II)

Adventicious shoot multiplication was induced by transferring shoots which were 2-3 cm in length to medium consisting of Murashige and Skoog inorganic salts supplemented with 3% sucrose, 0.4 mg/liter thiamine-HCL, 100 mg/liter i-Inositol, 0.0-2.0 mg/liter BA, 0.2 mg/liter GA3, 0.01 mg/liter IBA, and 0.7% phytagar. The ph and incubation conditions were the same as those listed for Stage I.

Pretransplant Stage (Stage III)

Individual shoots 3-4 cm in length were removed from the cluster of shoots that developed in Stage II, and were transferred to rooting medium. This medium consisted of half-

strength Murashige and Skoog inorganic salts, supplemented with 0.4 mg/liter thiamine-HCL, 100 mg/liter Inositol, 2% sucrose, 0.7% phytagar, and the following concentrations of IBA; 0.0, 0.5 and 1.0 mg/liter. The cultures were incubated under a photoperiod of either 16 hours light and 8 hours darkness, or continuous darkness, and a temperture of 26 ^{0}. The effects of two phenolic compounds, Rutin and Quercetin were investigated by adding 10^{-3}M of one or the other to Stage III medium containing LO mg/liter IBA.

RESULTS

Establishment and multiplication

Lateral buds elongated to approximately 1-2 cm within 2-4 weeks. After transferring them to Stage II medium, they produced adventicious shoots within 3-4 weeks. As these shoots were repeatedly subcultured, the rate of adventicious shoot development increased, until it reached a five fold increase every 3-4 weeks. The optimal shoot development occurred on medium containing 0.75 mg/liter BA (see Table 1). BA concentrations below this level gave reduced adventicious shoot development. BA concentrations greater than or equal to 1.0 mg/liter gave reduced shoot elongation. This led to the development of short, tight, clusters of buds that failed to elongate. Only if these clusters were tediously separated into individual buds, did they begin to elongate.

Table 1. The effects of benzyladenine on shoot production of *P. serotina* in vitro. (Gibberillic acid, and IBA supplied at 0.2 and 0.01 mg/liter respectively.)

Benzyladenine mg/liter	No. of shoots/division
0.00	1.2
0.25	1.7
0.50	4.2
0.75	5.3
1.00	3.4
1.50	1.8
2.00	1.4

Pretransplant stage

Shoots rooted within 10-14 days on Stage III medium consisting of LO mg/liter IBA. The percentage of rooted shoots was the greatest (90%) when the shoots were incubated in continuous darkness. Light given by a 16 hour light, 8 hours

dark photoperiod greatly reduced rooting (30%) (see Table 2). The addition of Rutin or Quercetin enhanced rooting in the light. Shoots placed on Stage III medium containing Rutin or Quercetin gave 70% and 65% rooting respectively, when the shoots were incubated under 16 hour light, 8 hours dark, and 85% and 90% rooting respectively when the shoots were incubated under continuous darkness.

Table 2. The effect of IBA, Rutin Quercetin and photoperiod on rooting of P. serotina in vitro after 14 days.

Treatment			% Rooting	
IBA mg/liter	Rutin molar	Quercetin molar	16 hr. light 8 hr. dark	Continuous darkness
0.0	0.0	0.0	0.0	20.0
0.5	0.0	0.0	25.0	86.0
1.0	0.0	0.0	30.0	90.0
0.5	10^{-3}	0.0	20.0	60.0
1.0	10^{-3}	0.0	70.0	85.0
0.5	0.0	10^{-3}	35.0	75.0
1.0	0.0	10^{-3}	65.0	90.0

DISCUSSION

The results of this work demonstrates that tissue culture can be used to rapidly multiply mature trees of P. serotina. The initial work was done with scions from mature trees, which were grafted onto seedling root stocks. However, I presently have cultures which were initiated from "elite" trees of P. serotina which are approximately 40-50 years old.

In vitro rooting was the most difficult part of the procedure. Nemeth (1979) rooted P. myrobalam on a medium containing 5 um IBA approximately 1 mg/liter). Ivanicka and Pietrova (1980) found that shoots of P. avium rooted best when 0.2 mg/liter naphthalene acetic acid NAM plus 0.1 mg/liter GA3 was used. Hammerschlag (1982) used 5.0 mg/liter 3-Indoleacetic acid (IAA) to root shoots of P. cerasifera. The results of my experiments showed that shoots of P. serotina showed maximum rooting on 1.0 mg/liter IBA. The results also show the importance of photoperiod on the rooting of P. serotina in vitro. For example, when shoots were placed on Stage III medium without hormones, no roots developed under a photoperiod of 16 hr. light and 8 hour dark. However, 20% of the shoots rooted on this medium when the cultures were incubated under continuous darkness. Likewise, when 1.0 mg/liter IBA was added to the

medium and cultures were incubated under 16 hours of light and 8 hours of darkness 30% of the shoots rooted. However, 90% of the shoots rooted when cultures were incubated under continuous darkness. Shoots that were rooted under continuous darkness showed no leaf expansion as compared with shoots rooted in the light. Therefore, the effects of two different phenolic compounds, Rutin and Quercetin were investigated. Chancel, Macheux and Jonard, (1980) demonstrated the effect of Rutin and Quercetin on enhancing the rooting of peach in vitro. James (1979) showed that the phenolic compound phloroglucinol improved the number of roots produced in vitro of a hybrid of Rubus ursinus x R. idaeus.

The results of the phenolic study show that both Rutin and Quercetin can be used to partially overcome the effects of continuous darkness. Quercetin is believed to act as an inhibitor of IAA oxidase. (Salisbury and Ross 1978.) It is, therefore, possible that when shoots are rooted in the dark, endogenous IAA, which moves basipetally within the plant, accumulates in the medium at the base of the shoot. Although some of the endogenous IAA is broken down by IAA oxidase, none is degraded by light, therefore, enough accumulates to stimulate rooting. The accumulating IAA, along with the exogenously supplied IBA, may be responsible for the enhancement of rooting in the darkness. In light, the accumulating IAA is broken down by both IAA oxidase, and by light (Galson 1950), thereby decreasing rooting. When either phenolic is added to the medium the amount of IAA degradation by IAA oxidase is decreased. This allows IAA to reach a concentration at which rooting is stimulated. The theory is supported by a study which showed that the addition of exogenously supplied IAA to Stage III medium, increased rooting in the light, especially at concentrations of 2.5 mg/liter (See Table 3). Shoots placed on any of the rooting media did not show appreciable growth during the rooting phase. It is known that high auxin concentrations inhibit shoot growth (Lane 1980). Many plantlets showed severe leaf drop within 2 weeks of being placed in Stage III. This is a common problem encountered when rooting woody species in vitro. Preliminary work indicated that the addition of kinetin at 0.8 mg/liter to Stage III medium stimulates continous growth of the shoots in the light. It also appears to reduce the amount of leaf drop. This provides a more vigorous plantlet for transplanting to the greenhouse.

Table 3. Effect of exogenous IAA on rooting of P. serotina vitro (IBA supplied at 1.0 mg/liter)

IAA mg/liter	% rooting
0.0	20
2.5	60
5.0	50
10.0	50

a-as the IBA concentration increased, the roots originated from callus tissue not thestem, and shoot elongation decreased.

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