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# MICROCLONAL MULTIPLICATION OF WILD CHERRY (*PRUNUS AVIUM* L.) FROM SHOOT TIPS AND ROOT SUCKER BUDS

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The effects of different combinations and concentrations of the growth regulators: 6-benzylaminopurine (BA), 6-furfurylaminopurine (KIN), $N^6$–(2-isopentenyl) adenine (2iP), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and α-naphthaleneacetic acid (NAA) on axillary shoot multiplication rates for wild cherry (*Prunus avium* L.) shoot explants were determined. Apical shoot tips and axillary buds from juvenile trees (5-year old) and from root suckers of mature trees (55-year old) were used as initial explants for establishment of multiple shoot cultures. Factorial tests for growth regulator interactions with the two genotypes showed significant differences in shoot multiplication rate with numerous auxin- and cytokinin-amended basal media. For genotype P5, the most effective growth regulator combination was 5.0 mg/L KIN and 1.0 mg/L IAA. Genotype 5/11 responded best to 1.0 mg/L BA and 5.0 mg/L IBA. Both clones multiplied easily in culture, and shoot multiplication of the mature genotype was not inhibited.

## Introduction

In central Europe, there is increasing interest in reforestation with wild cherry for both economic and ecological attributes of this valuable hardwood species (M e i e r - D i n k e l 1987b). Traditional methods of wild cherry vegetative propagation by suckers or softwood cuttings have not been sufficient for achieving mass propagation for intensive planting of selected mature genotypes (B o n g a 1981) for various ecosystems. The use of micropropaga-

tion techniques may enable propagators to increase quickly the number of propagules of superior genotypes (A b b o t t 1978). Wild cherry micropropagation has been attempted previously using explants from seedlings and mature trees (C o r n u a n d C h a i x 1981, C o r n u et al. 1981, R i f f a u d and C o r n u 1981) although extensive growth regulator studies were not attempted.

Since our previously described investigations showed the unsuitability of mature wild cherry specimen to establish multiple shoot culture, we tried to micropropagate mature trees using root sucker explants (P e v a l e k - K o z l i n a and J e l a s k a 1987).

Determination of optimal growth regulator ratios is important in the development of optimal culture media for mass propagation. The two groups of plant growth regulators, cytokinins and auxins, have a major control over organogenesis, and the selection and optimal ratio of each are necessary for maximizing multiplication rates. Therefore, the purpose of this research was to test the interaction of various cytokinins and auxins on shoot multiplication rates, and to develop an optimal culture medium for micropropagation of explants from mature wild cherry tree.

## Materials and Methods

### Plant material

Wild cherry shoot cultures were established by previously described methods (P e v a l e k - K o z l i n a and J e l a s k a 1987, P e v a l e k - K o z l i n a 1991) for use in these investigations. Briefly, cultures were initiated in May and June from actively growing shoots from a 5–year old tree (genotype 5/11), and from root suckers of a 55–year old tree (genotype P5) on modified Woody Plant Medium (mod. WPM), (M c C o w n and L l o y d 1981) with 20 g/L sucrose, 0.5 mg/L BA, 0.5 mg/IBA, 0.1 mg/L $GA_3$, and solidified with 0.9% Bacto agar. Media were adjusted to pH 5.6 prior to autoclaving. Cultures were grown at 24 ± 2 °C under 40 W fluorescent light (80 $\mu Em^{-2}s^{-1}$) with a 16 h photoperiod.

### Treatments

The following cytokinins and auxins were added to mod. WPM for factorial growth regulator experiments: 6–benzylaminopurine (BA), 6–furfurylaminopurine (KIN), $N^6$–(2–isopentenyl) adenine (2iP), indole–3–butyric acid (IBA), indole–3–acetic acid (IAA) and α–naphthaleneacetic acid (NAA) in concentration ranges of 1–10 mg/L. According to A n d e r s o n (1980), the growth regulator treatments were tested in three separate experiments: (1) determination of the most appropriate cytokinin with IAA, (2) determination of the most appropriate auxin (with the best cytokinin from Exp. 1), and (3) determination of the best cytokinin-auxin ratio. For each experiment, single axillary shoots (1.5 cm) grown for four weeks in multiple shoot culture were severed and placed vertically into test tubes (30 × 160 mm) filled with 15 ml of mod. WPM supplemented with 20 g/L sucrose, 0.1 mg/L $GA_3$, and the

tested growth regulator combination. After inoculation, test tubes were capped with cotton plugs and aluminium foil. All media were tested with at least 10 shoot explants through two subcultures. Axillary shoots (>0.5 cm) were counted after 4–week incubation.

Effects of cytokinin treatments were tested with 0 (control), 1.0, 5.0 and 10.0 mg/L each of KIN, BA or 2iP with IAA (1.0 mg/L). Effects of auxin treatments were tested with 1.0, 2.5 and 5.0 mg/L IAA, IBA or NAA with the best cytokinin type and concentration determined (5.0 mg/L KIN) in the initial factorial test. Finally, the interaction between IAA (1.0, 2.5 and 5.0 mg/L) or IBA (1.0 and 5.0 mg/L) with BA (1.0 and 5.0 mg/L) or KIN (1.0, 2.5 and 10.0 mg/L) was analysed.

### Statistics

Statistical analysis of treatment effects on shoot multiplication rates between two genotypes was done by analysis of variance of the data using the Studentized Range Method. This method is appropriate for an initial experiment because there is no nominated control or reference medium. However, a reference medium for later experiments is likely to arise from this preliminary experiment. Analyzed data from all experiments were combined in Fig. 1 and Table 1.

## Results

### Cytokinin effects

Results of data analysis from tests of cytokinin effects on the shoot multiplication are presented in Fig. 1A. The multiplication rate of both genotypes was higher on all media supplemented with cytokinin in comparison to the control medium (1.0 mg/L IAA). Genotype 5/11 was more sensitive to the exogenously added cytokinin than genotype P5. 2iP was the least effective cytokinin for both genotype shoot proliferation, but on the medium with 2iP shoots elongated frequently and spontaneously. The highest shoot multiplication for genotype 5/11 was obtained with 1.0 mg/L BA (4.2 shoot/explant). For genotype P5, 5.0 mg/L KIN was the most effective (5.2 shoot/explant).

### Auxin effects

IAA and IBA were more effective auxins than NAA for shoot production with both genotypes on KIN-supplemented media (Fig. 1B). On media supplemented with NAA, proliferation of red-greenish coloured callus was observed, which inhibited axillary shoot production. Genotype 5/11 was tolerant even to the high concentrations of synthetic auxins, while genotype P5 was sensitive to them and it responded best on 1.0 mg/L IAA (5.2 shoot/explant). The highest shoot multiplication for genotype 5/11 was observed on medium with 2.5 mg/L IBA (3.1 shoot/explant).

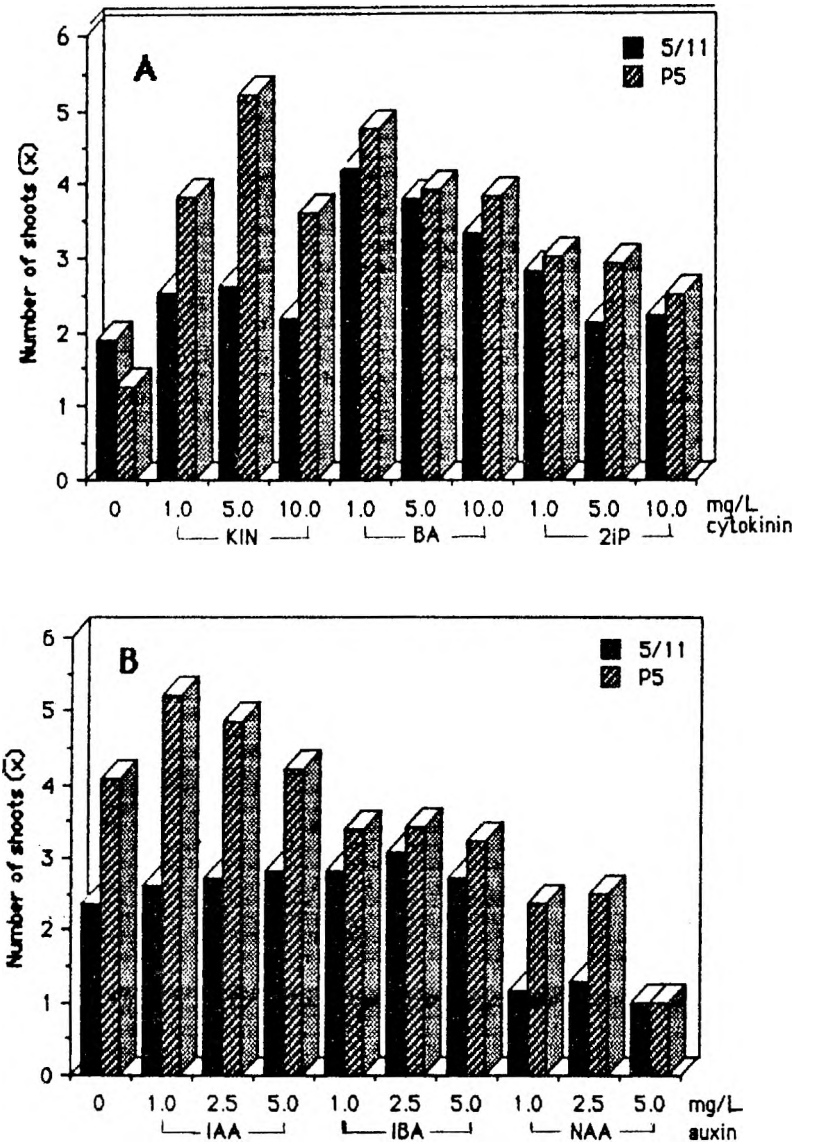


Fig. 1. Effect of different cytokinin and auxin combinations on shoot multiplication in wild cherry culture. Basal medium: mod. WPM, 2% sucrose, 0.9% agar and 0.1 $mgL^{-1}$ $GA_3$ (estimated after four weeks in culture).

A. Basal medium supplemented with 1.0 $mgL^{-1}$ IAA
B. Basal medium supplemented with 5.0 $mgL^{-1}$ KIN

Table 1. Effect of cytokinin and auxin on mean shoot multiplication of two wild cherry genotypes, 5/11 and P5. Basal medium: mod. WPM, 2% sucrose, 0.9% agar and 0.1 mg/L $GA_3$ (average shoot number from two subcultures estimated after 4 weeks in culture; only shoots over 0.5 cm in height counted).

| Growth requlators (mg/L) | | | | Genotype 5/11 | | P5 | |
|---|---|---|---|---|---|---|---|
| Cytokinin | | Auxin | | $\bar{x}$ | σ | $\bar{x}$ | σ |
| BA | 1.0 | IBA | 1.0 | 4.54 | 0.78 | 4.52 | 0.87 |
| | | | 5.0 | 4.81 | 0.81 | 4.81 | 0.80 |
| | | IAA | 1.0 | 4.19 | 0.72 | 4.73 | 0.83 |
| | | | 2.5 | 3.81 | 0.80 | 3.88 | 0.77 |
| | | | 5.0 | 3.62 | 0.64 | 3.69 | 0.74 |
| | 5.0 | IBA | 1.0 | 2.59 | 0.67 | 2.62 | 0.75 |
| | | | 5.0 | 2.81 | 0.57 | 2.81 | 0.79 |
| | | IAA | 1.0 | 3.78 | 0.77 | 3.90 | 0.58 |
| | | | 2.5 | 2.39 | 0.66 | 3.50* | 0.71 |
| | | | 5.0 | 1.89 | 0.76 | 2.89* | 0.76 |
| KIN | 1.0 | IBA | 1.0 | 3.62 | 0.90 | 3.61 | 0.72 |
| | | | 5.0 | 2.32 | 0.72 | 2.31 | 0.63 |
| | | IAA | 1.0 | 2.52 | 0.73 | 3.82* | 0.73 |
| | | | 2.5 | 2.68 | 0.89 | 3.46* | 0.64 |
| | | | 5.0 | 2.81 | 0.79 | 2.89 | 0.83 |
| | 5.0 | IBA | 1.0 | 2.81 | 0.83 | 3.39* | 0.66 |
| | | | 5.0 | 2.70 | 0.70 | 3.23* | 0.73 |
| | | IAA | 1.0 | 2.61 | 0.70 | 5.20* | 0.78 |
| | | | 2.5 | 2.70 | 0.82 | 4.86* | 0.54 |
| | | | 5.0 | 2.81 | 0.83 | 4.20* | 0.78 |
| | 10.0 | IBA | 1.0 | 2.41 | 0.67 | 2.36 | 0.50 |
| | | | 5.0 | 2.08 | 0.76 | 2.12 | 0.59 |
| | | IAA | 1.0 | 2.18 | 0.73 | 3.59* | 0.59 |
| | | | 2.5 | 2.11 | 0.75 | 3.10* | 0.63 |
| | | | 5.0 | 2.41 | 0.51 | 2.93 | 0.88 |

$\bar{x}$ = average number of shoots per culture
σ = standard deviation
* significant difference between shoot multiplication of genotypes ($P < 0.01$)

## Cytokinin-auxin interactions

For genotype 5/11, 1.0 mg/L BA with IBA supplemented media were the most effective for shoot proliferation. However, media supplemented with 5.0 mg/L KIN and IAA were the most suitable for genotype P5. The highest

concentrations of BA and KIN were inhibitory to both genotypes. On most media, genotype P5 produced significantly more axillary shoots than 5/11 (Table 1).

## Discussion and Conclusion

Vegetative propagation of mature trees is generally preferred to juvenile trees even though the former are often more difficult to propagate (Bonga 1981). Franclet (1980) emphasizes that *in vitro* culture of mature trees requires the use of explants from parts of the tree that remain more juvenile. Bonga (1982) states that the base of the trunk apparently remains relatively physiologically more juvenile than the rest of the tree. This is supported by the results on the *in vivo* propagation of oak and beech. An acceptable percentage of rooted cuttings was obtained from young mother stocks or from stump-shoots of mature trees after felling (Cornu et al. 1977). Similar source material of mature chestnut was suitable for *in vitro* micropropagation (Biondi et al. 1981, Vieitez et al. 1983). An initial culture was established successfully with the shoots sprouted in the glasshouse, but not with explants collected in the field. Ballester et al. (1989) propose partial etiolation of branches as a suitable pretreatment for *in vitro* propagation of selected mature chestnut trees. Recently, several authors (Favre and Juncker 1987, Meier–Dinkel 1987 a, b, San-José et al. 1988) reported on successful use of stump sprouts as an initial material for *in vitro* culture of oaks.

In our experiments root suckers from a mature genotype were more responsive to a growth regulator stimulus for shoot proliferation than the 5–year old genotype established from shoot tips. With IBA, 1.0 mg/L BA was equally effective for both genotypes, but 5.0 mg/L KIN with IAA was equally effective for the genotype 5/11. With micropropagation of *Prunus* and other hardwood species, other investigators (Cornu et al. 1981, Cornu and Chaix 1981, Riffaud and Cornu 1981, Vieitez and Vieitez 1982) have also found BA to be an effective cytokinin for stimulating axillary shoot multiplication.

We demonstrated the multiplication potential of wild cherry shoots originated from not completely mature shoot tips and rejuvenated material (root suckers) of mature trees on media with appropriate adjustment of growth regulators and their concentrations. Our results showed that optimal growth regulators combination was significantly different in two genotypes tested. By using buds from sucker shoots, we overcame problems usually associated with micropropagation of mature trees.

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SAŽETAK

MIKROKLONSKO RAZMNOŽAVANJE DIVLJE TREŠNJE (*PRUNUS AVIUM* L.) IZ VEGETACIJSKOG VRŠKA STABLA I IZDANKA KORJENJAKA

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Istražen je utjecaj različitih kombinacija i koncentracija regulatora rasta: 6–benzilaminopurina (BA), 6–furfurilaminopurina (KIN), $n^6$–(2–izopentenil) adenina (2iP), indol–3–maslačne kiseline (IBA), indol–3–octene kiseline (IAA) i α–naftalenoctene kiseline (NAA) na stopu umnožavanja izdanka divlje trešnje (*Prunus avium* L.). Kao početni eksplantati za postavljanje kulture upotrebljeni su vršni i bočni pupovi mlade biljke stare pet godina (genotip 5/11) i izdanci razvijeni iz korjenjaka stabla starog 55 godina (genotip P5). Uočene su statistički značajne razlike u stopi umnožavanja izdanaka na podlogama s različitim kombinacijama regulatora rasta. Za umnožavanje izdanaka genotipa P5 najbolja je bila podloga s 5,0 mg/L KIN i 1,0 mg/L IAA, dok su se izdanci genotipa 5/11 najbolje umnažali na podlozi s 1,0 mg/L BA i 5,0 mg/L IBA. Oba klona mogla su se uspješno umnožavati u kulturi, a umnožavanje izdanaka odraslog genotipa nije bilo inhibirano.

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