

MICROPROPAGATION OF BLACKBERRIES (*Rubus* sp.) CULTIVARS

BOBROWSKI, Vera L.¹; MELLO-FARIAS, Paulo C.² & PETERS, José A.¹

¹UFPEL/IB/Laboratório de Cultura de Tecidos de Plantas - Campus Universitário - Caixa Postal 354 - Tel. (0532) 757316 - CEP 90010-900 - Pelotas/RS, Brasil.

²UFPEL/FAEM/Deptº de Ciencia e Tecnologia Agroindustrial - Campus Universitário - Caixa Postal 354 - Tel. (0532)757258 - CEP 96010-900 - Pelotas/RS, Brasil.

(Recebido para publicação em 20/12/95)

ABSTRACT

Experiments were carried out aiming to develop techniques for *in vitro* propagation of blackberries (*Rubus* spp.) plants. Multiplication were done from axillary buds which were placed in MS (MURASHIGE & SKOOG, 1962) medium, supplemented with BA (benzylaminopurine), NAA (naphthaleneacetic acid) and GA₃ (gibberellic acid) in different concentrations and combinations. Subsequently, studies were done to determine the influence in rooting of the MS medium with all the salts reduced to 1/3, and with different concentrations of IBA (indolbutyric acid). Results indicated that the best multiplication of shoots was obtained with BA (1.0 and 2.0 mg/l). The addition of NAA and GA₃ in media in these experiments didn't improve the multiplication rate. Rooting was successfully achieved with 100% rooted plants in all cultivars, with no differences significant differences among rooting media.

Key words: *Rubus*, blackberry, fruit, micropropagation

RESUMO

MICROPROPAGAÇÃO DE AMORA PRÊTA (*Rubus* spp.). Com o objetivo de desenvolver técnicas de propagação *in vitro* de plantas de amora-preta (*Rubus* spp.), foi realizada a multiplicação de brotos com gemas axilares, os quais foram excisados e inoculados, em meio MS (MURASHIGE & SKOOG, 1962), suplementado com diferentes combinações e concentrações de BAP (benzilaminopurina), ANA (ácido naftalenoacético) e AG₃ (ácido giberélico). No enraizamento, utilizou-se meio MS reduzido a 1/3 da sua concentração de sais minerais, adicionado com AIB (ácido indolbutírico) em diferentes concentrações. As avaliações permitiram concluir que a multiplicação de brotos foi mais estimulada por BAP (1,0 e 2,0 mg/l). A presença de ANA e AG₃ nas concentrações utilizadas não melhorou as taxas de multiplicação. A taxa de enraizamento foi de 100% para todas as cultivares testadas, não havendo diferença significativa entre os meios utilizados.

Palavras-chave: *Rubus*, amora-preta, fruta, micropropagação

INTRODUCTION

Blackberry belongs to the *Rosaceae* family, *Rubus* (Tourn) L. genus and *Eubatus* subgenus. Blackberry is a shrubby tree with erect, semierect or creep grown habit, and most cultivars have thorny stems. Thornless blackberries are commercially propagated by tip layers or stem cuttings (BROOME & ZIMMERMAN, 1978; CALDWELL, 1984). According to Moore and Clark (1989), mentioned by FERNANDEZ & CLARK (1991), shoot emergence from root cuttings of the erect thornless blackberry "Navaho" has been poor, setting of rooted plants or closely spaced root cuttings was suggested to achieve adequate stands.

Tip layering propagation requires a sizeable planting for the layering bed, few tips are available per plant, and weed control among the layers is a problem. Propagation by hardwood stem cuttings is simple but rooting is not always satisfactory. Softwood cuttings root readily, but require considerably more care for successful plant production (BROOME & ZIMMERMAN, 1978).

The use of tissue-cultured plants would eliminate the need for root pieces and allow uniform spacing of plants in the field (FERNANDEZ & CLARK, 1991). Nowadays, the tissue culture have been used in scions propagation, supplying thousands virus-free and genetically uniform plants, and in a reduced time.

However according to SKIRVIN *et al.* (1981), successful tissue cultures have been reported for many members of the genus *Rubus* involving callus cultures, shoot tips growth, parthenocarpic fruit and roots development. Despite these studies, no good system for the rapid proliferation of the trailing blackberry has been developed. Tissue culture may be the only practical method of a quickly redistributing virus-free material if strict certification programs for *Rubus* are put into effect. Tissue culture also offers additional opportunities for the rapid dissemination of new cultivar releases. Furthermore, plants can be produced during the entire

year rather than during limited periods (CALDWELL, 1984).

These experiments were carried out aiming to determine the best culture medium for blackberry micropropagation, cultivars "Ebano", "Tupi", "Guarany" and 3 and 79 selections.

MATERIALS AND METHODS

Blackberry shoots of "Ebano", "Tupi", "Guarany" cultivars and 3 and 79 selections were utilized, coming from shoot tip culture and kept *in vitro*. Initial explants were with 1.0 cm length and contained axillary buds were placed in MS (MURASHIGE & SKOOG, 1962) medium supplemented with sucrose (3%), myo-inositol (100 mg/l) and agar (6 g/l) with different concentrations of growth regulators. Medium A: MS + BA (2.0 mg/l); Medium B: MS + BA (2.0 mg/l) + NAA (0.1 mg/l) + AG₃ (0.5 mg/l); Medium C: MS + BA (1.0 mg/l); Medium D: MS + BA (1.0 mg/l) + NAA (0.1 mg/l) + GA₃ (0.5 mg/l); Medium E: MS without growth regulators. The pH of the media was adjusted to 5.8 after the media were autoclaved at 121 °C and 1.5 atm for 20 min.

The cultures were kept in a growth chamber for 45 days, at 27 ± 1 °C, with 16 hours photoperiod and 3000 lux of light intensity. Six explants per vessel flask of medium (containing 40 ml) were utilized. The result evaluations were done by count shoots number since initial explant in incubation for 45 days. Factorial experiment was utilized with 2 factors (medium and genotype) in a completely casual planning, with 4 repetitions (flask) in each treatment. The variable average number of shoots per flask was transformed according to (\sqrt{x}). The analysis of variance putted into effect to evaluated differences between culture media and genotypes, in relation shoots proliferation. Later, Duncan's multiple range test was utilized to determine differences among treatments.

In the rooting experiment was used the same

cultivars and selections, and shoots with 2.0 cm in length as initial explants. The mineral basic medium used was MS, reduced at 1/3 mineral salts, and indolbutyric acid (IBA) in 0.3; 0.5 and 0.8 mg/l (medium 1, 2 and 3 respectively). The pH adjustment, sterilization media and incubation conditions were the same as the proliferation experiment.

Six explants per flask of medium were placed in each vessel, and rooting evaluation was done by counting and measuring shoots and roots after 45 days in incubation. In this evaluation was utilized a completely casual experiment with the same factors and a planning kind described in multiplication, but with 3 repetitions of each treatment. To evaluate differences between culture media and genotypes in relation to rooting average length and shoots average size was using analysis of variance. Later, Duncan's multiple range test was used to compare treatment effects on rooting.

RESULTS AND DISCUSSION

The highest shoot proliferation for all cultivars was in the A and C media with 2.0 and 1.0 mg/l BA, respectively, differing significantly to the rest media. Table 1 shows that NAA (0.1 mg/l) and GA₃ (0.5 mg/l) additions did not increase the multiplication rate of the shoots, and the best results independent from the used concentration, were in media with only BA. Similar results in pear, were obtained by LEITE *et al.* (1993), where media with BA, NAA and GA₃ produced lower multiplication of shoots than media containing BA and NAA. PASQUAL *et al.* (1991), working with cv. "Ebano" using equidistant BA concentrations (0.1 and 1.0 mg/l), got similar results as in our experiment with different BA concentrations (1.0 and 2.0 mg/l). Higher multiplication rates (20 and 30 shoots/explant) were reported by BABIC' & NESKOVIC' (1984), using 1.0 and 3.0 mg/l of BA in "Smoothstem" and "Thornless" cultivars, and when BA was absent or in low quantity (0.1 and 0.2 mg/l) there aren't significantly multiplication.

Table 1. Effects of different culture media on shoot multiplication rates of different blackberry cultivars.

Medium	Cultivar				
	Ebano	Tupi	Guarany	Sel. 3	Sel. 79
A	7.21 a	5.96 a	12.15 a	8.59 a	5.14 a
B	6.07 a	3.85 b	2.76 bc	2.74 c	2.63 b
C	7.60 a	7.02 a	10.65 a	6.92 a	6.60 a
D	3.81 b	1.65 c	2.14 c	1.68 c	4.62 a
E	1.16 c	1.00 c	4.03 b	4.59 b	1.09 c

Mean separation in columns by Duncan's multiple range test, $P \leq 0,05$.

Constancy in high multiplication rates were observed in "Guarany" and "Ebano" cultivars with the best multiplication results in 12.3 shoots/explant for cv. "Guarany" in A medium and 7.2 shoots/explant in C medium for "Ebano" cultivar (Figure 1). According to

SCHUCH *et al.* (1993), BA necessity in apple cultivation depends on the amount of cytokinin endogenous in each cultivar, and this interaction influenced shoot multiplication, this may explain similar results using BA at 1.0 and 2.0 mg/l in distinct blackberry cultivars.

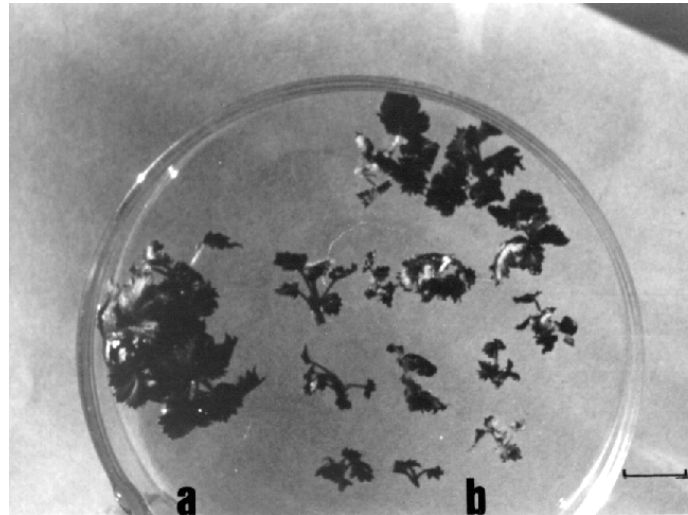


Fig. 1. Shoots multiplication of Guarany cultivar, after 45 days in MS medium with BA (2.0 mg/l). A - Group of shoots from one microexplant. B - Individual shoots. Scale = 1 cm.

All tested cultivars had successfully 100% rooting of shoots. There was not a statistical difference among IBA levels (0.3; 0.5 and 0.8 mg/l). Rooting percentages in our study were higher than those obtained by BROOME & ZIMMERMAN (1978), with blackberry in vitro propagation using BA with IBA and GA₃. However,

with "Black Satin", "Dirksen Thorneless", "Smoothstem" and US 64-39-2 cultivars, these authors got only 64% rooted shoots using MS + IBA (1.0 mg/l). However, FERNANDEZ *et al.* (1991), working in a more complex medium (MS + 8.9 μM BA + 0.5 μM IBA + 0.29 μM GA₃) with cv. "Navaho" obtained only 90% of rooting.



Fig. 2. Rooted plants in MS medium, diluted in 1/3 of total concentration. A - Selection 3. B - Ebano. C - Tupi

In relation of roots average length, Selection 3 shows the best results, with a variation between 8.76 to

9.93 cm, while "Tupi" cultivar shows a variation of 5.0 to 8.2 cm (Table 2), according to the used media. Even not

occurring statistical by significant differences in majority of tested cultivars in relation roots average length/IBA concentration, we verify that IBA in lower concentrations (0.2 mg/l) induce higher roots average length, exception to "Tupi" cultivar. This correlation was observed also by MAGALHÃES Jr. & PETERS (1991), working with plum, in which they found that auxin is responsible for roots

induction and had inhibit the same roots. Another important observation is the qualitative aspect of roots, that has great significance in scions surviving. In this way, Selection 3 resulted in better average root length, but very thin roots, while cv. "Ebano" in spite of that, had fewer roots, but the quantity of root hairs was very higher than other tested cultivars (Figure 2).

Table 2. Average root length for different blackberry cultivars in three tested media.

Medium	Cultivar				
	Ebano	Tupi	Guarany	Sel. 3	Sel. 9
1	2.43 a	5.03 b	3.50 a	9.93 a	1.50 a
2	2.10 a	5.23 b	2.76 a	9.16 a	1.76 a
3	2.16 a	8.23 a	2.93 a	8.76 a	1.33 a

Mean separation in columns by Duncan's multiple range test, $P \leq 0,05$.

In relation of average shoot size, "Ebano" cultivar had longer shoots than others cultivars with an average length of 2.9 cm (Figure 3), and the other cultivars had low than 2.5 cm. This variation is probably caused by genotype differences, where endogenous levels of GA₃ may differ among the cultivars, favouring a differential elongation.

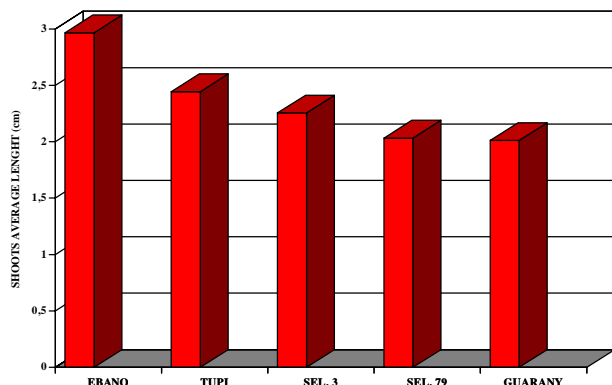


Fig. 3. Influence of culture media on different shoot average length of blackberry cultivars.

CONCLUSIONS

The results in this experiment allow to conclude:

The multiplication rate changes with genotype utilized;

The shoot multiplication of Ebano, Tupi, Guarany, Sel. 3 and Sel 79 is higher when the medium was supplemented with BA (benzylaminopurine);

The addition of auxin (IBA) in the medium favour the rooting phase, although the responses varies with the genotypes and concentrations.

REFERENCES

- BABIC', V.& NESKOVIC', M. Propagation of three blackberry cultivars from small apical buds *in vitro*. **Journal of Hortic. Science**, 59(2): 183-185, 1984.
- BROOME, O.C. & ZIMMERMAN, R.H. *In vitro* propagation of blackberry. **HortScience**, 13(2): 151-153, 1978.
- CALDWELL, J.D. Blackberry Propagation. **HortScience**, 19(2): 193-195, 1984.
- FERNANDEZ, G.E. & CLARK, J. *In vitro* propagation of the erect thornless "Navaho" blackberry. **HortScience**, 26(9): 1219, 1991.
- LEITE, D.L.; PETERS, J.A. & NAKASU, B.H. Micropropagação de pereira (*Pyrus* spp.) cultivar Carrick. **Rev. Bras. Fisiologia**, 14(1): 149-154, 1992.
- MAGALHÃES Jr., A.M. & PETERS, J.A. Cultura *in vitro* de ameixeira: Efeito do ácido indolbutírico, tipo de

lâmpada e intensidade luminosa no enraizamento. **Rev. Bras. Fisiol. Vegetal**, 3 (1): 57-61, 1991.

MURASHIGE, T. & SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, 15: 473-497, 1962.

PASQUAL, M.; PEIXOTO, P.H.P.; SANTOS, J.C. & PINTO, J.E.B.P. Propagação *in vitro* da amora-preta (*Rubus* sp.) cv. Ebano: uso de reguladores de crescimento. **Ciênc. e Prát. Lavras**, 15(3): 282-286, 1991.

SCHUCH, M. & PETERS, J.A. Multiplicação *in vitro* de brotações de macieira cultivares Marubakaido (*Malus prunifolia*, Willd, Borkh.) e Megumi (*Malus domestica*, Borkh.). **Pesq. Agrop. Bras.**, 28(4): 433-437, 1993.

SKIRVIN, R.M.; CHU, M.C. & GOMEZ, E. *In vitro* propagation of thornless trailing blackberries. **HortScience**, 16(3): 310-312, 1981.