MICROPROPAGATION AND MORPHOGENESIS OF ARRACACHA (*ARRACACIA XANTHORRHIZA* BANCROFT)

SLÍVA Š.[1], VIEHMANNOVÁ I.[1], VÍTÁMVÁS J.[2]

[1]*Institute of Tropics and Subtropics, Czech University of Life Sciences, Prague Czech Republic*
[2]*Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Czech Republic*

Abstract

Arracacha (Arracacia xanthorrhiza) belonging to Apiaceae family is one of the most important side crops in Southern America. It is planted for its storage roots containing valuable starch. The goal of these experiments was to establish optimal protocol for micropropagation of arracacha. For multiplication phase MS medium, half-strength MS medium and MS medium supplemented with different concentrations of BAP and NAA was used. Indirect morphogenesis in callus cultures, induced from petioles, was tested on MS medium containing 2,4-D, KIN and on MS medium without PGR. For rooting of regenerated shoots MS medium without PGR and media containing NAA, 2.4-D, IAA and AC were used. For multiplication of culture, MS medium supplemented with combinations of NAA and BAP was found to be the most effective (4.2 ± 0.73 new shoots per explant within four weeks). On the contrary, no shoots were formed on half-strength MS medium. Higher frequency of callogenesis and shoot regeneration was achieved when explants were cultivated on the medium containing 2.4-D and subsequently transferred to MS medium without PGR. Low frequency of spontaneous rooting was observed in shoots on MS medium with/without AC.

Key words: arracacha, *Arracacia xanthorrhiza*, micropropagation, callogenesis, organogenesis

Abbreviations:
2.4-D = 2.4-dichlorophenoxyacetic acid
AC = activated charcoal
BAP = 6-benzylaminopurine
IAA = 3-indoleacetic acid
KIN = kinetin
NAA = 1-naphthaleneacetic acid
PGR = plant growth regulators
RH = relative humidity
MS = Murashige & Skoog (1962) medium

INTRODUCTION

Arracacha is supposed to be one of the oldest plants, cultivated in the Andean region and is one of the most important side crops of Southern America. It is a perennial plant, cultivated for its storage roots. The most important is high content of valuable and quality starch in the storage root. Among the nine minor Andean root and tuber species arracacha has the widest range of culinary uses and it appears to be free from undesirable substances which could limit its acceptability. Arracacha adds an interesting texture and flavour to a variety of dishes (pastry, soups, purées, stews, alcohol beverage) (Hermann et al., 1997). In agriculture practice arracacha is propagated vegetatively by propagules, i.e. cormels that have buds on their surface. Multiplication rate of arracacha is expressed as number of cormels obtained from a mature plant through the conventional field method. It varies between 9 and 48 (Flores, 2003). Using *in vitro* cultures could help to improve the reproduction and propagation cycle of the plant. In arracacha, callus cultures were studied (Pessoa et al., 1994), however only roots were recovered.

The aim of our study was to establish a proper protocol for micropropagation of arracacha, which could enable rapid multiplication of clones with desirable characteristics.

MATERIALS AND METHODS

Plant material

A landrace of *Arracacia xanthorrhiza* (2n = 44), obtained from Locotal region, Bolivia was used in this study. Plants were cultivated in pots in the subtropical greenhouse of Botanic garden – Institute of Tropics and Subtropics, Czech University of Life Sciences Prague.

Establishment of in vitro culture

Roots and leaves were removed from the leaf rosette and the basal part containing meristem was dipped into 96% (v/v) ethanol for 15 s, 1% (v/v) sodium hypochlorite with 0.1% (v/v) Tween 20 for 20 min and then rinsed three times with sterile distilled water. After sterilisation, basal meristem was isolated under stereo microscope with the use of preparation needle and was transferred into Erlenmeyer flasks (50 ml) containing 20 ml MS media (Murashige & Skoog, 1962) supplemented with 3% sucrose and 0.8% agar. Cultures were maintained at 25/23°C under a 16/8 h light/dark regime with 36 µmol per m^2/s cool white fluorescent light (NARVA LT 36 W/010). Regenerated shoots were transferred to fresh medium every 4 weeks and cultivated in the same conditions.

Multiplication

For multiplication phase shoots were transferred on MS media supplemented with 3% sucrose, 0.8% agar and BAP (1 mg/l) or with combinations of NAA and BAP (0.1 mg/l + 1 mg/l, 0.1 mg/l + 2 mg/l). MS medium and half-strength MS medium (supplemented with 3% sucrose and 0.8% agar) without PGR were used as controls.

Callogenesis

Petiole segments 10–12 mm in length were used for callus formation. MS media with 2.4-D (0.1 mg/l or 1 mg/l) and with 2.4-D and KIN (0.1 mg/l + 0.2 mg/l) were tested. Explants were cultivated in test tubes (150 × 25 mm) at 25/23°C under a 16/8 h light/dark regime or in the dark at 20 ± 2°C.

Morphogenesis

To regenerate shoots, calli were subsequently transferred onto MS medium without PGR and on MS medium supplemented with 0.2 mg/l KIN. Regenerated shoots were separated from calli and subcultivated on rooting media.

Rooting

Rooting of shoots was initialized on MS medium containing 3% sucrose, 0.8% agar and different concentrations of NAA (1, 2 mg/l) or 2.4-D (0.1, 1 mg/l), combinations of NAA and AC (1 mg/l + 1 g/l), NAA and IAA (0.5 mg/l + 2 mg/l), NAA, IAA and AC (0.5 mg/l + 2 g/l + 2 g/l), MS medium supplemented with AC (10 g/l) or without PGR.

Transfer to soil

Plantlets with properly developed roots were rinsed with tap water to remove the adhering medium and transferred separately to pots, filled with a soil-perlite 1 : 1 (v/v) mixture. The plants were grown in the glasshouse under high (85%) RH for 7 days. For the next 14 days, RH was reduced and light intensity was gradually increased.

Design of statistical evaluation of experiments

Each treatment was applied to two replications of ten explants. Significance was determined by analysis of variance (ANOVA) and the least significant ($p < 0.05$) differences among mean values were estimated using Fisher's LSD test [StatSoft STATISTICA 8.0]

RESULTS AND DISCUSSION

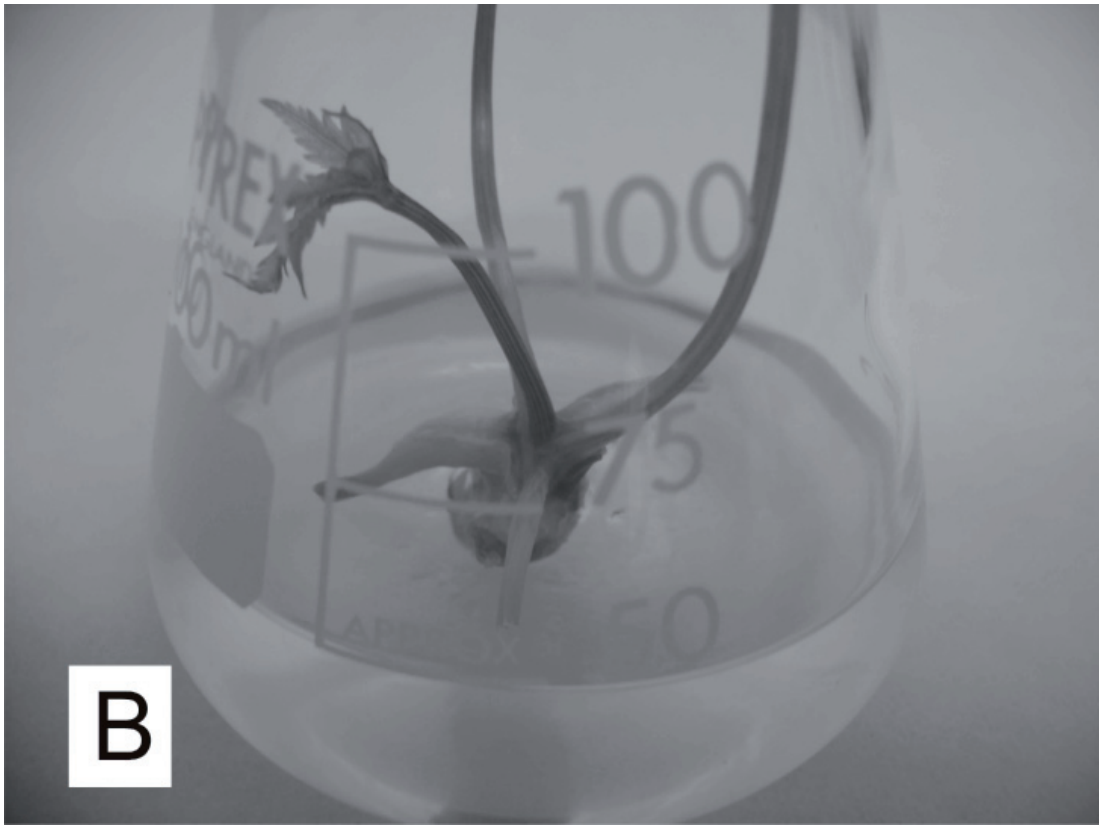
Multiplication

For multiplication phase MS medium supplemented with NAA (0.1 mg/l) and BAP (1 mg/l) was found to be the most effective combination. Within 4-week cycle 100% of plants produced minimal 4 new shoots per plant. Mean number was 4.2 ± 0.73 new shoots per plant, which is statistically significantly higher than in other variants. Explants showed more vigorous growth compared to control plants on MS medium (Figure 1). The medium supplemented with 0.1 mg/l NAA and 2 mg/l BAP showed very good regeneration ability (3 ± 0.63 shoots per plant) as well. Nevertheless, plant growth and the regeneration of leaves were rather slower and explants intensively produced callus. MS medium supplemented with BAP (1 mg/l) and without auxins inhibited growth of callus tissue and stimulated good shoot proliferation (2.2 ± 0.37 shoots per plant). On half-strength MS and MS medium without PGR the formation of new shoots was significantly lower compared to treatments with PGR (0.2 ± 0.1 shoots per plant in MS, or no new shoots in half-strength MS, respectively).

Positive effect of combinations with NAA and BAP on multiplication phase of micropropagation in *Apiaceae* family was also reported in *Thapsia garganica* (Makunga et al., 2003), where 1.5 mg/l BAP and 0.5 mg/l NAA significantly improved the production of multiple shoots.

Influence of BAP was described by Grigariadou and Maloupa (2008) in *Crithmum maritimum* L. (*Apiaceae*),

Figure 1: Comparison of arracacha plantlets:
A) on MS medium supplemented with 0.1 mg/l NAA and 1 mg/l BAP; B) on MS medium free of PGR



Tab. 1: Regeneration via organogenesis from arracacha calli

Treatment		Observation after 4 weeks on MS medium free of PGR
Medium	Culture conditions	
0.1 mg/l 2.4-D	16/8 h (light/dark)	in 15% of explants formation of shoots
	dark	in 30% of explants formation of roots
1 mg/l 2.4-D	16/8 h (light/dark)	in 5% of explants formation of shoots
	dark	in 100% of explants formation of roots
0.1 mg/l 2.4-D + 0.2 mg/l KIN	16/8 h (light/dark)	in 25% of explants formation of roots
	dark	in 40% of explants formation of roots

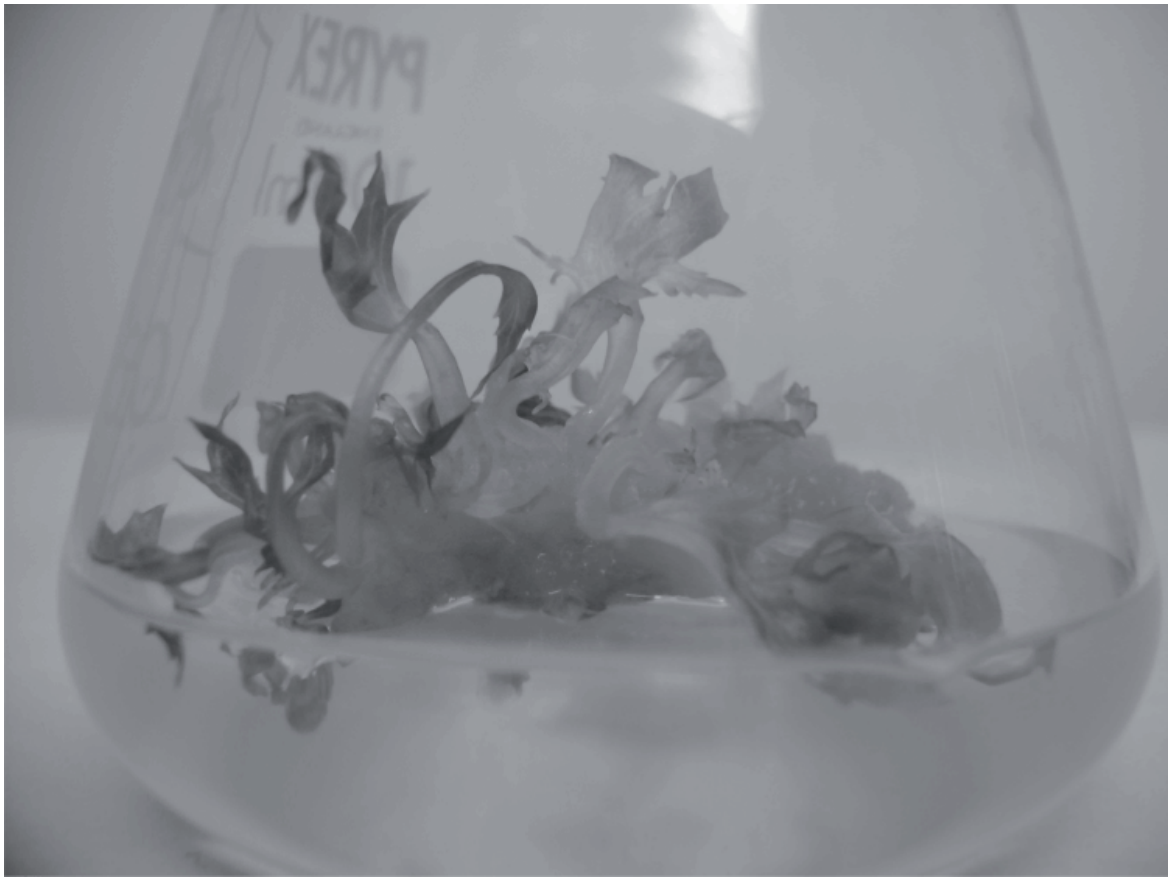
where 0.5 mg/l BAP increased shoot proliferation. These results correspond with Madeira (2005) who carried out experiments to evaluate the effect of BAP concentration on *in vitro* arracacha development. The increase in the BAP concentration reduced the callus formation; however, inhibited shoot development.

Callogenesis and organogenesis

Experiments using petiole segments showed very good ability to produce callus cultures in all treatments. Callus production started 2 weeks after placing of petioles on these media. The best regeneration of calli on explants was observed on medium supplemented with 1 mg/l 2.4-D, where callus was formed at 70% of explants after 18 days of culture.

Calli produced in the dark had predominantly compact surface with smaller grains and white colour. Calli produced under light had more grainy surface and was yellow to greenish. Higher frequency of callus regeneration was observed on basal parts of the explants (segments of petioles).

Figure 2: Shoots regeneration from callus induced on MS medium supplemented with 0.1 mg/l 2.4-D and subsequently transferred on MS medium

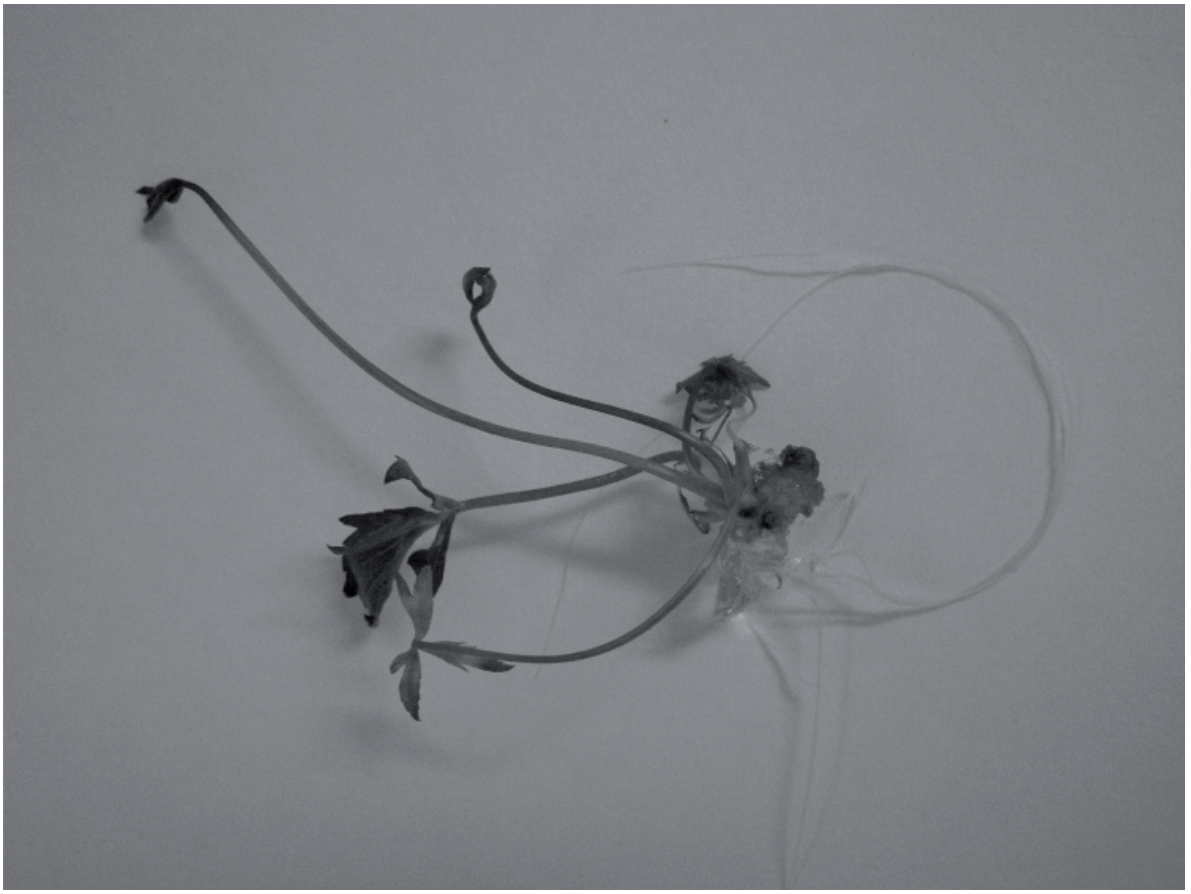


All calli cultivated on regeneration MS medium supplemented with 0.2 mg/l KIN showed very low survival rate. On the contrary, when transferred on MS medium without PGR, they showed good ability to recover organs (Table 1). Shoot regeneration was achieved from calli cultivated on media supplemented with 2.4-D (0.1 mg/l, 1 mg/l) under light conditions (Figure 2). Fast regeneration of roots was observed in these treatments in the dark. Calli of arracacha obtained on MS medium and supplemented with 0.1 mg/l 2.4-D and 0.2 mg/l KIN showed good ability to regenerate roots both, under dark and light conditions. Makunga *et al.* (2005) referred that all cultures of *Thapsia garganica* L. (*Apiaceae*) failed in shoot regeneration using supplements of 2.4-D. According to Azza and Noga (2002) callus was induced from hypocotyl and primary leaf explants of cumin (*Cuminum cyminum* L.) seedlings on a medium with 0.9 mg/l 2.4-D alone or plus 0.4 or 0.8 mg/l KIN. An embryogenic callus developed in cumin within 2 weeks after transferring the callus to medium lacking PGR. Even using same PGR supplements arracacha failed in producing any embryogenic callus formation. More detailed results on arracacha regeneration from calli are available in the Table 1.

Rooting

Media containing 0.1 mg/l 2.4-D and 1 mg/l 2.4-D negatively influenced the vigour of plants within 3 weeks. After two more weeks significant callus production occurred on the plant basis. Leaf growth was stopped, shoots turned brownish and later on died. These results do not correspond with study of Pessoa et al. (1994), where roots in arracacha were developed on MS medium containing 2.4-D and BAP. Experiment using 1 mg/l NAA supplement and 1 mg/l NAA with AC showed negative effects on plants within 4 weeks. Our experiments showed just high callus production on the basis of explants after two week period. Calli were white and friable. Experiment using 2 mg/l NAA showed high callus growth after the first week. In two-week period 50% of explants regenerated roots, however on the base of callus, not on the mother explants. In four-week period 50% of plantlets died, just white callus remained. On the contrary, Abdulaziz (2001) obtained the highest rooting frequency on a medium containing 1 mg/l NAA. Medium supplemented with 0.5 mg/l NAA and 2 mg/l IAA showed better results in lowering the callus production in

Figure 3: Rooted plant (on MS medium) prepared for transfer to greenhouse



arracacha. However no rooting explants were obtained. Roots were produced after three weeks just on the callus. *In vitro* rooting of hypocotyl segments of *Daucus carota* using AC and auxins 2.4-D (1.0 mg/l), NAA or IAA (0.5 mg/l) was investigated (Pan & van Staden, 2002). Rooting occurred after 7 days. Our experiments using 0.5 mg/l NAA + 2 mg/l IAA and 2 g/l AC showed low callus production. Leaves remained green, but no root production occurred within three weeks. On MS medium without PGR supplements and on MS medium with 10 g/l AC plants showed normal growth of leaves and no callus production on the basis. Rooting occurred at those variants spontaneously and rarely (1.32 ± 0.43 roots/plant) (Figure 3).

Ex vitro transfer

Plants obtained from multiplication experiments and rooted on MS medium free of PGR and on MS medium supplemented with 10 g/l AC were transferred to *ex vitro*. Survival rate after 2 weeks was 62.5%. All plants showed ability to form new leaves (1.98 ± 0.05 new leaves/plant within 2 weeks).

Transplanting of non-rooted plants obtained on medium containing 1 mg/l NAA was also tested. After 2 weeks, 50% of plants survived. It indicates that root formation started in the soil. The lowest surviving rate (25%) was achieved in plants obtained by organogenesis on MS medium supplemented with 1 mg/l 2.4-D.

ACKNOWLEDGEMENT

This research was financially supported by Department of Crop Sciences and Agroforestry in Tropics and Subtropics, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague.

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Received for publication on February 3, 2009
Accepted for publication on June 6, 2010

Corresponding author:

Ing. Iva Viehmannová, Ph.D.
Czech University of Life Sciences Prague
Institute of Tropics and Subtropics,
Kamýcká 129, 165 21 Prague 6
Czech Republic
e-mail: viehmann@its.czu.cz