IECPS 2021
The 2nd International Electronic Conference on Plant Science
01–15 DECEMBER 2021 | ONLINE
10th Anniversary 2012 – 2021
Chaired by DR. ADRIANO SOFO
plants
MDPI

The phenotypic reactivity of *Passiflora incarnata* L. on various content of mineral salts and regulators during micropropagation and acclimatization

Marcin Ożarowski[1,*], Elżbieta Bilińska[2], Mariola Dreger[1], Milena Szalata[1], Tomasz M. Karpiński[3], Artur Adamczak[2], Douglas Siqueira de Almeida Chaves[4]

[1] Department of Biotechnology, Institute of Natural Fibres & Medicinal Plants National Research Institute, Wojska Polskiego 71b, 60-630 Poznań, Poland;
[2]Dept. of Botany, Breeding and Agricultural Technology for Plants, Institute of Natural Fibres & Medicinal Plants National Research Institute, Kolejowa 2; 62-064 Plewiska, Poland;
[3]Chair and Department of Medical Microbiology, Poznań University of Medical Sciences, Wieniawskiego 3, 61-712 Poznań, Poland;
[4]Dept. of Pharmaceutical Sciences, Institute of Biological and Health Sciences, Federal Rural University of Rio de Janeiro, BR-465, Km 7, Seropédica, Rio de Janeiro, CEP: 23897-000, Brazil;
* Corresponding author: marcin.ozarowski@iwnirz.pl

Abstract:

Passiflora incarnata is ornamental and medicinal plant that contains a valuable active chemical derivatives of apigenin and luteolin. Conventional cultivation of this plant in Poland is a very problematic, caused by the low percentage of seed germination, viability of seedlings, and plant diseases which can seriously reduce the productivity of *P. incarnata*. An alternative and promising way to solve these problems may be used the technique of micropropagation, which may have applied for the plant multiplication under controlled conditions and have offered the production of healthy, pathogen-free and true-to-type plants.

The aim of this study was to determine 1) the influence of IAA (0.1-1.0 mg L^{-1}), and IBA (0.1-1.0 mg L^{-1}) on Brazilian seed germination, and 2) the influence of various concentrations of mineral salts in Murashige and Shoog (MS), Gamborg (B-5), Shenk-Hildebrandt (SH) and Phytamax media on growth, development and condition of plant *in vitro*, 3) induction of adventitious shoots using nodal fragments under influence of BAP (0.1-1.0 mg L^{-1}), TDZ (0.1-1.0 mg L^{-1}), KIN (0.1-1.0 mg L^{-1}) with IAA (0.1 mg L^{-1}).

Results showed that 1) MS medium with IAA (1.0 mg L^{-1}) has been most effective in induction of seed germination (60%); 2) Gamborg (B-5) medium has been more favorable for plant growth and development, and 3) SH with BAP (1.0 mg L^{-1}) and TDZ (0.1 L^{-1}) with IAA induced more adventitious buds and new regenerated plantlets. After rooting, 100% obtained plants have been acclimatized to *ex vitro* conditions and have been observed in greenhouse.

Keywords: *Passiflora incarnata*; *in vitro* cultures; seeds; micropropagation

Material and methods

1. SEEDS

1.1. Seeds were obtained from the Federal Rural University of Rio de Janeiro. This primary plant culture was obtained from disinfected seeds by surface-sterilization with soaked in a sterilizing solution (20% NaOCl) for 20 min. under agitation and then seeds were washed three times with sterile deionized water in horizontal laminar airflow cabinet.

1.2. These seeds were placed in basal medium MS with auxins: IAA and IBA (0.1; 0.5; 1.0 mg L^{-1}).

2. PLANT IN VITRO CULTURE

2.1. After 4 weeks, *s*hoot tips and nodal explants were excised from *in vitro* germinated plants and were cultured within 60 days on four kind of media such as: Murashige and Shoog (MS), Gamborg (B-5), Shenk-Hildebrandt (SH) and Phytamax media. All media have been supplemented with BAP (0.1-1.0 mg L^{-1}), TDZ (0.1-1.0 mg L^{-1}), KIN (0.1-1.0 mg L^{-1}) with IAA (0.1 mg L^{-1}). They were incubated under a 16-h photoperiod in plastic containers placed in phytotron. Control explants were cultured on a MS medium devoid of plant growth regulators.

2.2. Elongated shoots obtained from the nodal explants have been transferred to MS medium with (or without) one auxin in various concentrations for rooting.

3. ACCLIMATIZATION

3.1. Next, regenerated plants hase been placed plastic pots with sterile soil in order to acacclimatization of plants.

3.2. After this stage, plants have been transferred to greenhouse.

IECPS 2021

Results and Discussion

Results showed that

1) MS medium with IAA (1.0 mg L^{-1}) has been most effective in induction of seed germination (60%);

2) Gamborg (B-5) medium has been more favorable for growth and development of plantlets.

3) MS and SH with BAP (1.0 mg L^{-1}) or TDZ (0.1 L^{-1}) with IAA induced more adventitious buds and new regenerated plantlets.

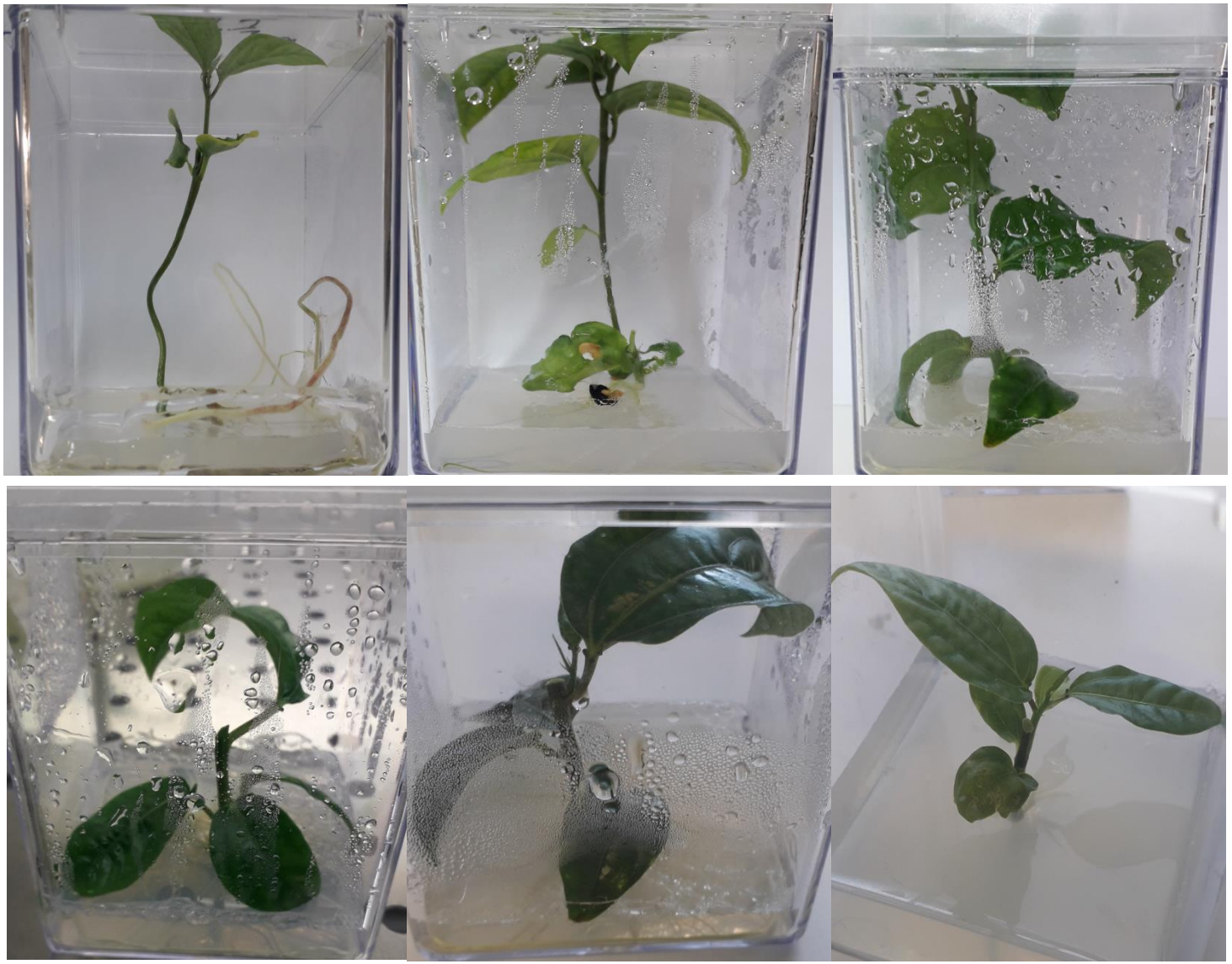
4) All (100%) eelongated shoots have been rooting on MS with IAA (0.5 mg L^{-1}).

5) After rooting, 100% obtained plants have been acclimatized to *ex vitro* conditions and have been observed in greenhouse.

MS medium with IAA (1.0 mg L^{-1}) has been most effective in induction of seed germination (60%)

LP	½ MS MEDIUM WITH AUXINS	N	RESPONSE [%] (N)
1	IAA 1,0 mg/l	50	60 (n = 30)
2	IAA 0,5 mg/l	50	2 (n = 1)
3	IAA 0,1 mg/l	50	4 (n = 2)
6	IBA 1,0 mg/l	50	no
5	IBA 0,5 mg/l	50	no
4	IBA 0,1 mg/l	50	no
7	MS 0 - CONTROL	50	o
4	½ MS - CONTROL	50	no

Induction of seed germination on MS medium with IAA (1.0 mg L^{-1})

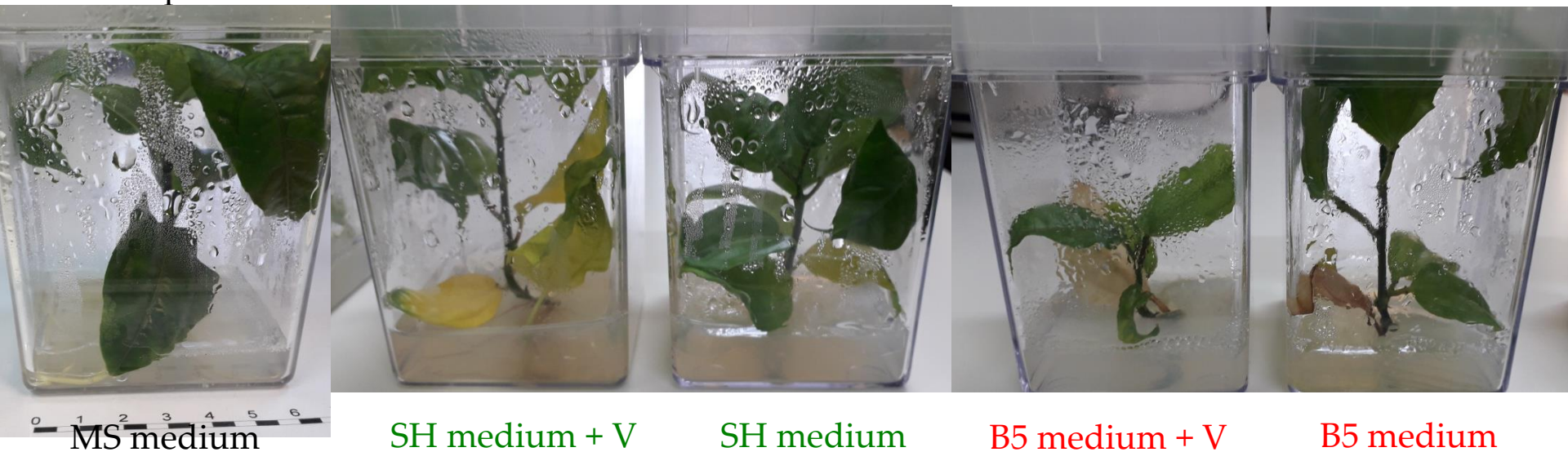


IECPS 2021

2) Gamborg (B-5) medium has been more favorable for growth and development of plantlets.

EFFECT OF VITAMINS AND MINERAL SALTS IN BASAL MEDIA ON THE MOPHROLOGICAL DEVELOPMENT OF THE SHOOT CULTURE

The aim of the experiment was to indicate an alternative medium for the development of shoot culture and to show whether the increased content of thiamine and the presence of ammonium sulfate (B-5 medium) or ammonium phosphate (SH medium) - other salts than in the Murashige and Skoog medium (ammonium nitrate) will have influence on induction and development of shoots and roots.



MS medium | SH medium + V | SH medium | B5 medium + V | B5 medium

In addition, it was tested whether the lower content of calcium, zinc, boron, manganese (in the B-5 and SH media), magnesium ions (in the B-5 medium), iron ions (in the SH medium), and higher content of potassium ions (in in B-5 and SH media), sodium ions (B-5 medium), as well as cobalt, copper, magnesium, molybdenum ions (in SH medium) will affect the development of shoots and roots.

3) MS and SH with BAP (1.0 mg L^{-1}) or TDZ (0.1 L^{-1}) with IAA induced more adventitious buds and new regenerated plantlets.

ASSESSMENT OF THE INFLUENCE OF CYTOKININS ON THE DEVELOPMENT OF BUDS FROM THE NODAL FRAGMENTS OF THE SHOOTS

	MS MEDIUM WITH PLANT GROWTH REGULATORS	N	RESPONSE [%] (N)	NUMBER OF SHOOTS WITH LEAVES	LENGTH OF THE SHOOTS
1	BA 1,0 mg/l + IAA 0,1 mg L^{-1}	15	100	2,25 ± 0,5	2,52 ± 2,34
2	BA 0,1 mg/l + IAA 0,1 mg L^{-1}	15	100	1,0 ± 0	1,16 ± 0,78
3	KIN 1,0 mg/l + IAA 0,1 mg L^{-1}	15	100	1,0 ± 0 #	5,95 ± 2,14
4	KIN 0,1 + IAA 0,1 mg L^{-1}	15	100	1,0 ± 0	1,8 ± 0,88

IECPS 2021

Morphological assessment of adventitious shoots and new leaves

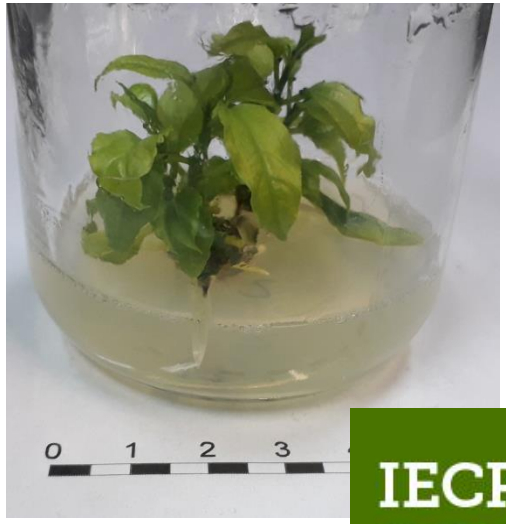
MS + BA 1,0 mg L^{-1} + IAA 0,1 mg L^{-1}

OBSERVATION

Average number of leaves on adventitious shoots
- indicator for photosynthesis
= 5,1 ± 0,88

OBSERVATION

Leaves properly developed= caudal, no distortion= no root growth (at low auxin concentration)



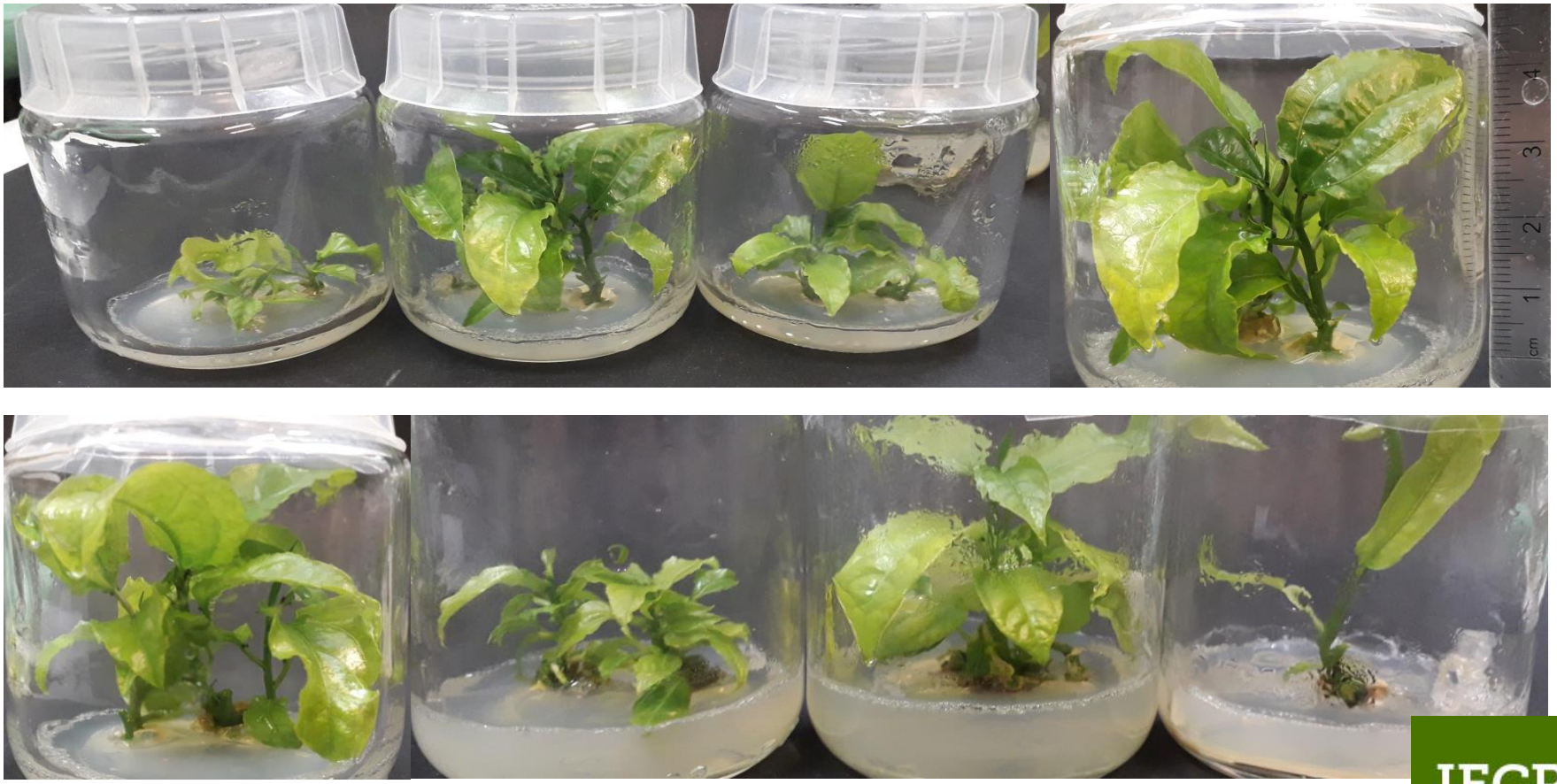
IECPS 2021

Morphological assessment of adventitious shoots and new leaves

MS + BA 0,1 mg/l + IAA 0,1 mg/l

OBSERVATION
Average number of leaves on adventitious shoots - indicator for photosynthesis
= 5,4 ± 0,96

OBSERVATION
The leaves are correct
= caudal, no distortion, large
= no root growth (at low auxin concentration)



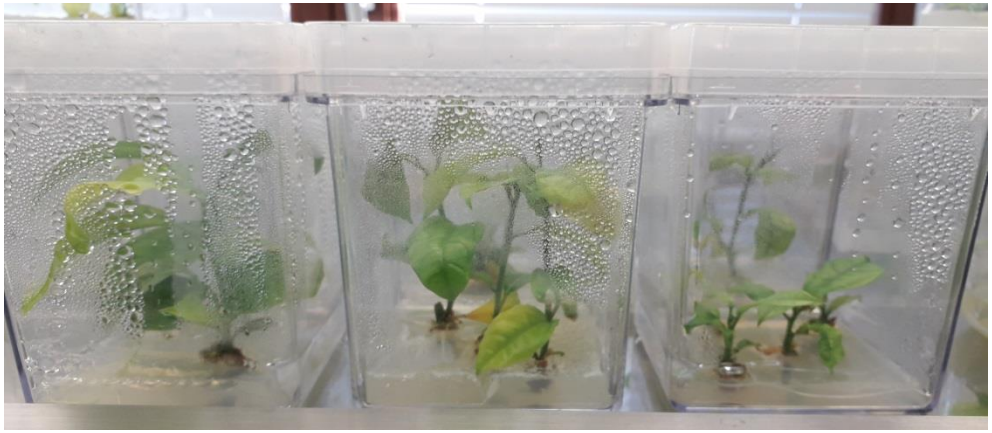
IECPS 2021

Morphological assessment of adventitious shoots and new leaves

MS + KIN 1,0 mg L^{-1} + IAA 0,1 mg L^{-1}

OBSERVATION

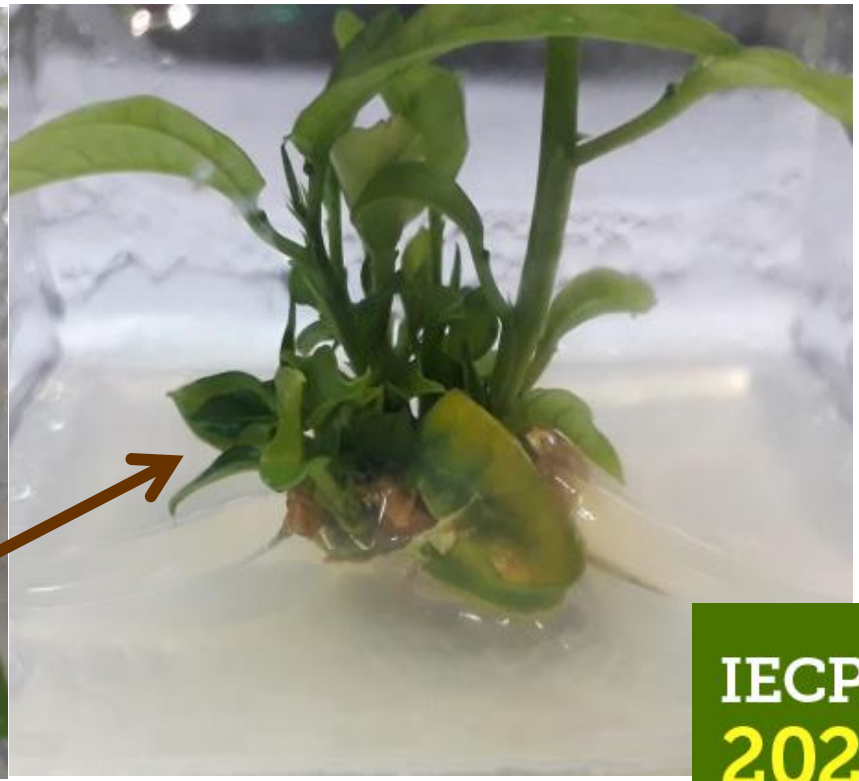
Average number of leaves on adventitious shoots
- indicator for photosynthesis
= 4,1 ± 0,88



OBSERVATION

The leaves are correct
= caudal,
no distortion

Smaller sizes
= no root growth
(at low auxin concentration)



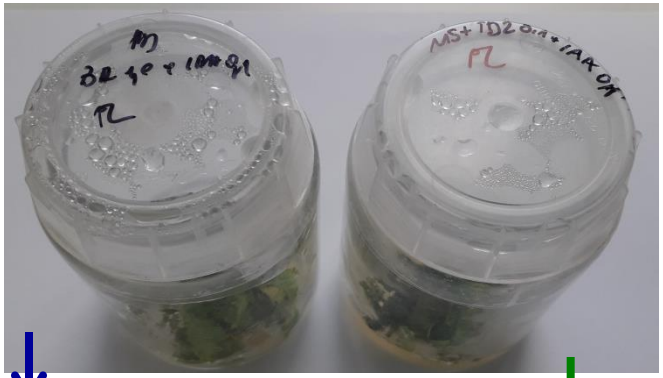
IECPS 2021

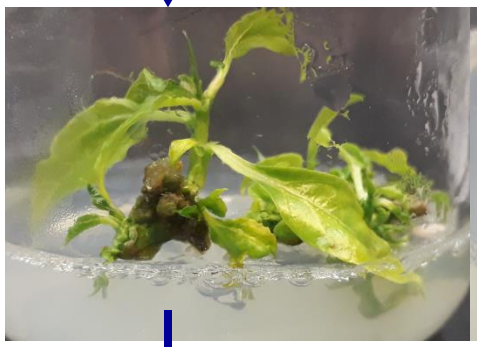
INFLUENCE OF CYTOKININS ON THE DEVELOPMENT OF ADVENTITIOUS BUDS IN THE WAY OF MORPHOGENESIS ON LEAF BLADES

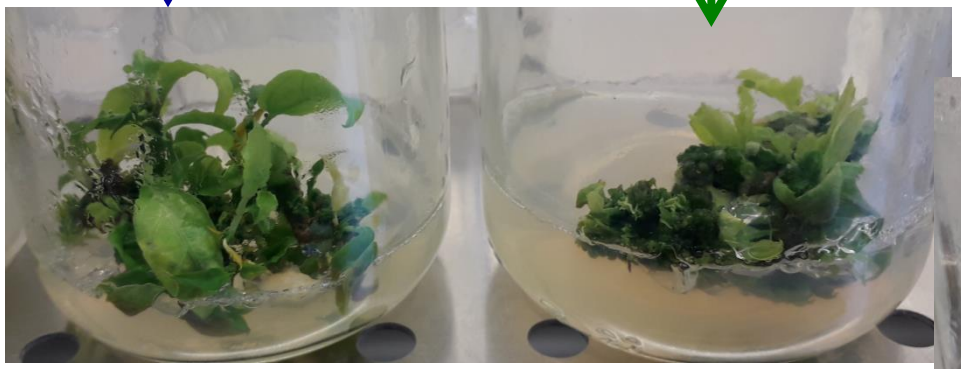
	MS MEDIUM WITH PLANT GROWTH REGULATORS	N	RESPONSE [%] (N)	NUMBER OF SHOOTS WITH LEAVES	LENGTH OF THE SHOOTS
1	BA 1,0 mg/l + IAA 0,1 mg L^{-1}	15	100	> 5	> 0,5 cm
2	BA 0,1 mg/l + IAA 0,1 mg L^{-1}	15	100	< 5	< 0,5 cm
3	TDZ 1,0 mg/l + IAA 0,1 mg L^{-1}	15	100	< 5	< 0,5 cm
6	TDZ 0,1 + IAA 0,1 mg L^{-1}	15	100	> 5	> 0,5 cm

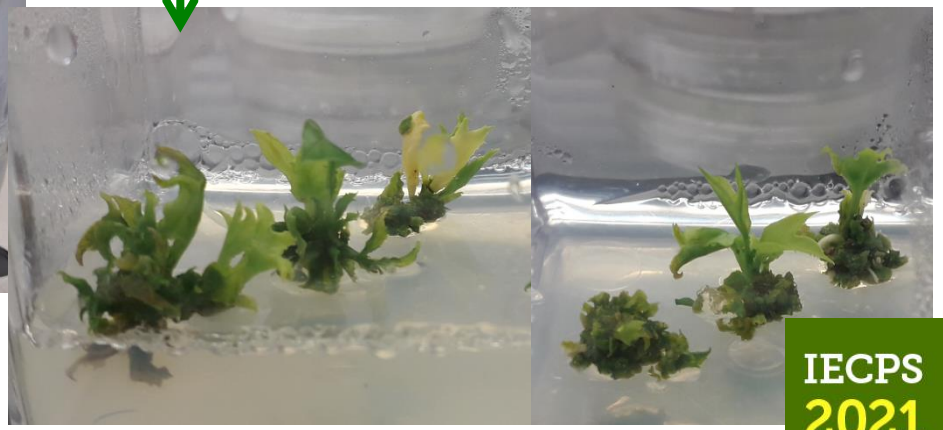
IECPS 2021

MS + BA 1,0 mg L^{-1} + IAA 0,1 mg L^{-1}









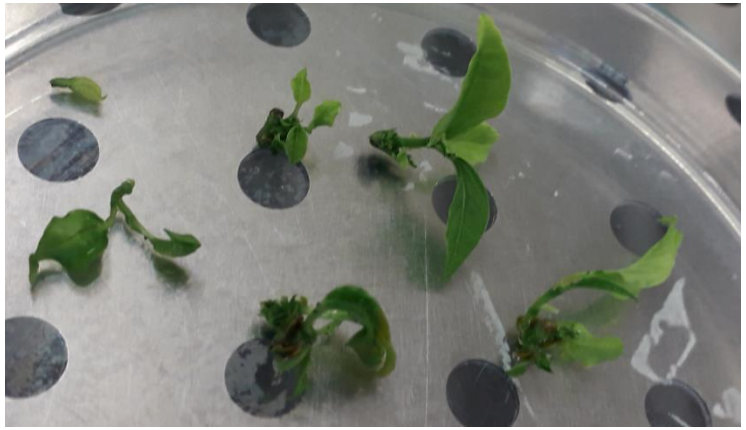
MS + TDZ 0,1 mg L^{-1} + IAA 0,1 mg L^{-1}

IECPS 2021

Morphological assessment:

- No callus
- Number of adventitious shoots = 15 (> 0.5 cm long)
- Properly developed leaf blades

MS + BA 1.0 mg L^{-1} + IAA 0.1 mg L^{-1}



IECPS 2021

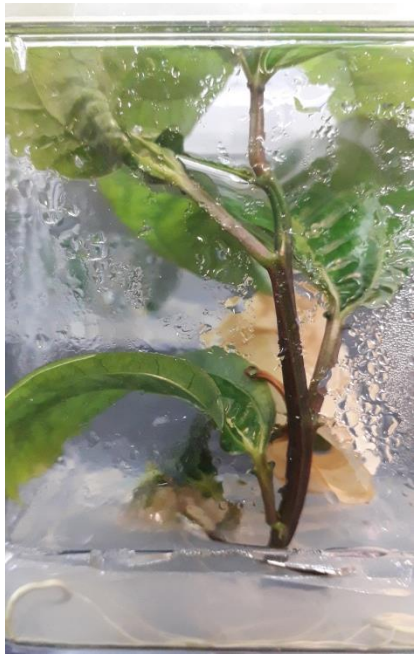
4) All (100%) elongated shoots have been rooting on MS with IAA (0.5 mg L^{-1}).

IECPS 2021

4) All (100%) elongated shoots have been rooting on MS with IAA (0.5 mg L^{-1}).








IECPS 2021

5) After rooting, 100% obtained plants have been acclimatized to *ex vitro* conditions and have been observed in greenhouse.

CONCLUSIONS

1. Various techniques used in plant biotechnology, especially plant in vitro cultures, are an effective way to obtain healthy plants of *Passiflora incarnata* by vegetative reproduction.

2. Studies have shown that both the nodal parts and the leaf blades are a promising source of adventitious shoots that effectively rooting and acclimatizing to *ex vitro* conditions.

3. In the process of micro-propagation of *P. incarnata*, not only Murashige and Shoog (MS), but also Gamborg (B-5), Shenk-Hildebrandt (SH) media can be used, despite the differences in the amount of mineral salts.

4. Morphological examinations showed that *in vitro* regenerated plants fast grew with normally developed leaves, without signs of disease. The plants were able to effective photosynthesis.

We kindly invite you to cooperation

Plants obtained by using *in vitro* methods

https://www.iwnirz.pl/

IECPS 2021