

Application of the Jerusalem artichoke (*Helianthus tuberosus* L.), as a plant origin medium additive, during the micropropagation of *Ada keiliana*

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Summary: A procedure for *in vitro* propagation of *Ada keiliana* seedlings are suited for acclimatization, was worked out. M medium was supplemented, with Jerusalem artichoke, as plant origin complex additive. The apply of JAD (1,5g/flask) gave the best response, considering the shoot (29 mm), and the root development (24,9 mm) too. The plantlets with satisfying growth (25–30 mm, 4–5 roots) were transferred in small pine bark : Novobalt peat : coconut fibres : perlit (2:3:1:1) mix, among greenhouse circumstances.

Key words: *Ada keiliana*, acclimatization, complex additives, Jerusalem artichoke, protocorm, seed sowing

Abbreviations: CM (coconut milk), PE (potatoe extract), TC (turnip cubes), PEE (pine-apple), CE (corn extract), RBH (ripe banana homogenizate), GBH (green banana homogenizate), JAC (Jerusalem artichoke's cubes), JAD (dried Jerusalem artichoke), PLBs (protocorm-like-bodies)

Introduction

The Orchid Family (*Orchidaceae*) has an important role from the point of view of horticulture and nature conservation. On the whole word hybrids are improved and botanical species are propagated on purpose to genetical preservation and commercial realization.

It's possible to increase the success of breeding by supplement the basal medium with natural origin complex additives. A lot of plants are suitable for producing extracts, homogenizates or additives. Coconut milk (coconut water) is known for a long time as a material, which is good for plant tissues because of its cytokinin contain and the reciprocal effects of its components (Maróti, 1976). The positive effects of unripe fruit's extracts (*Ginkgo biloba*, *Zea mays*, *Aesculus hippocastanum*) and different saps of trees (*Betula pendula*, *Juglans regia* etc.) already have proved (Tillyné, 2005). Malmgren (2004) portioned out 1 cm³ turnip cubes (TC) in every flask and 20 ml pine-apple extract (PEE) added to one litre medium during the *in vitro* cultivation of *Ophrys* species. In case of the propagation of *Cypripedium flavum* the Harvais medium was completed with PE (Yan et al., 2006). Hadley (1970) examined the effect of phytohormones, symbiotic fungus, CM, PE and Yeast extract comparing to each other in sterile culture of some hardy orchids. In a comprehensive article reported Islam (2003) and his collaborators about their experiments with PE, CE, papaya extracts.

In the Sigma-Aldrich's catalogue (2007) PE is recommended for culture of yeast-fungus and mould-fungus. In the general description its high carbohydrate, amino acids, proteins, mineral salts contain is pointed out (Anonymous 2008). Arditti (1977) in a table reviewed the successful applications of complex additives at different orchid genera.

The sterile culture of *Ada keiliana* seedlings hadn't shown suitable root-development on the maintained medium after 4-5 transfer, so there wasn't possibility to transplant them into greenhouse. Therefore plant origin organic compound was added to the growing medium.

The tuber of Jerusalem artichoke is rich in mineral materials (1,7g/100g), especially potassium content is high (478mg/100g). Its carbohydrate content is 15-20g/100g, primary inulin which is polymer comprising fructose units, that is good soluble in water. Among the B vitamin group are found thiamine, riboflavin, niacin in it, and essential amino acids are presented too, as lysine, arginine, histidine, tryptophan, aspartic acid (Angeli, et al., 2000). By its compounds it seemed suitable for experiment, for all that it was easy available and was controlled quality (own culture).

Materials and methods

The origin of the seeds for sowing was the seed change's service of „The Orchid Seedbank Project” (PO Box 7042, Shandler, AZ 85246). The seeds which were derived from the

hybridization of the selected clones of *Ada keiliana* 'Black Creek' AM/AOS x *Ada keiliana* 'Herman' were sowed at 04. 12. 2002. on Fast medium (Fast, 1982). For seed sterilization the filtrate of 10g/90ml calcium-hypochlorite was used, supplemented with 2–3 drops of Tween 80 before filtration. The seed sowings were cultured in glass jars containing 35 ml medium, which were closed with double semi-permeable plastic foil. All cultures were kept in the culture room at 24 °C, direct natural light but not sun. After germination, on 06. 01. 2003. the culture was placed on 10/14 photoperiod, under 1000 lux exposition. The developing protocorm were transplanted on KM medium (Eszéki & Györvári, 2000). During the seedling's cultivation, the shoot-growth, PLB formation and clusters of shoots were observed. Simultaneously with weak root-formation the browning of the basic part of the plantlets was noticed. The protocorm formation often came together with the necrosis of the growing shoot. After the 4.–5. passing, satisfying root-development, suitable for their acclimatization, wasn't observed.

Experiment was adjusted on 22. 02. 2007., with 3–4 leaves seedlings, without root. The basic medium was (Table 1), M medium, which is a modified ½ MS medium (Murashige & Skoog, 1962), the basic of the changes was the Orchimax medium of the Duchefa catalogue, where tryptone was applied as medium compound (Anonymus, 2003–2005).

Table 1. Applied basal media during the experiments

	KM	M
	mg/L	mg/L
Ca(NO ₃) ₂ x 4H ₂ O	500	—
CaCl ₂	—	150
(NH ₄) ₂ SO ₄	250	—
NH ₄ NO ₃	500	850
KH ₂ PO ₄	250	100
KNO ₃	—	950
MgSO ₄ x 7H ₂ O	250	100
Myo-Inositol	100	100

The JAC medium was complemented with 10 g of 0,5 cm³ fresh Jerusalem artichoke cubes in every flasks.

Dried Jerusalem artichoke was added in the case of JAD medium. The dried one was prepared from fresh tubers; the cleaned ones were cut into thin sheets, first the material was dried on 85 °C for 4 hour, after that on 65 °C for 4 hour. After drying 20,15 g dried weight became from 100 gram fresh weight. The dried material was ground fine, after that 1,5 g was added to every flask.

The other basic medium was KM (R. Eszéki, Györvári, 2000). 35 ml medium was poured in every 200 ml glass flasks, which were closed with double semi-permeable plastic foil. During the experiments 5 repeating were applied. The cultures were kept in the culture room at 24 °C,

10/14 photoperiod, under 1000 lux exposition.

The evaluation of the experiments was at 25. 07. 2007., the tables and the histograms were completed by Microsoft Excel. Because of the pozitiv results, on the Jerusalem

artichoke complemented medium the plantlets became suitable for acclimatization, with good rate of the shoot and root (5 pieces) development and adequate plantsize (>2,5cm). The medium was regularly cleared away from the roots with tapwater, then the plantlets were planted in transparent plastic box, in small pieces pine-bark : Novobalt peat : coconut fibres : perlite, (2:3:1:1) mix. Against *Botrytis* contaminated fungicid (Merpan 50 WP) was applied, after that the cultures were covered with transparent cap. The plantlets were grown in greenhouse, on 22–26 °C, during 90% relative humidity.

Results and discussion

During the experiments plantlets of *Ada keiliana* 'Black Creek' AM/AOS x *Ada keiliana* 'Herman' derived from seeds, with 4–5 leaves were cultured on 4 different media (Table 2).

Table 2. Root and shoot growth of *Ada keiliana* plantlets at evaluation

Basic medium	Plant origin additive		Shoot growth (mm)	Root growth (mm)	Number of root (db)	Wet weight (g)
	Dried jerusalem artichoke (g/flask)	jerusalem artichoke's cubes (g/flask)				
KM	—	—	17	7,85	1,8	1843,75
M	—	—	14,8	6,125	2	2113,6
JAC	—	10	23,2	20,8	6,6	3041
JAD	1,5	—	29	24,9	8	5790

The seedling development was varied. Between KM and M media, the KM seemed a bit more suitable in respect the shoot and the root growth, but the root number was better on the M medium but any case, we didn't get serious different.

On the M medium with Jerusalem artichoke additives were observed better results in respect the shoot-and the root growth (Figure 1), and regarding the root number too (Figure 2). The best response was on JAD (Figure 3).

There wasn't any study about the apply of Jerusalem artichoke product, therefore we had to base our results to the comparison of tests with other tuber plant's additives.

The result on ½ MS medium supplemented with PE (200 g/L), for seedlings of *Dendrobium strongylanthum* wasn't better than the control. The best response was given by the BH (100g/L) (Kong et al., 2007). Islam and his team (2003) added different concentration of PE to NP (New Phalaenopsis) medium, at the callus culture of *Doritaenopsis*. 100ml/L concentration produced the best effect. This was the optimal concentration in the case of the PLB regeneration, at the same time the 200ml/L concentration inhibited callus growth, but it was better compared to the controll.

During the regeneration of *Doritaenopsis*' PLBs the PE (100ml/L) stimulated the shoot growth, and CE in the same concentration the root growth (Rahman et al., 2004).

In various development stage of the flower stalks culture's *Ascofinetia*, *Neostylis* and *Vascostylis* were kept on VW (Vacin&Went) medium supplemented with different additives: CM (150ml/L); GBH (100g/L), RBH (100g/L), PE

(200ml/L). The PE in the regeneration and maintain media was the most efficient (Intuwong & Sagawa 1973).

Arditti (1977) in the context the use of organic additives emphasized, that determination of the punctual effectual quantity as complex materials isn't possible. Very important is the stage and maturity of the stock, and the response of the orchid species are different too. The time of harvest of tubers or storage organs, which are used to prepare additives is important in the aspects of their nutrient's optimal level too. After harvest, the best time of processing the Jerusalem artichoke is September and October (Angeli et al., 2000).

The results of our experiments are raised more problem to solve.

The dosage of Jerusalem artichoke into flasks, whether in rough, or in dry stage is laborious, so dosage in extract form to prepare on the base of literature would be better (Islam et al., 2003).

Aim to study the efficiency of Jerusalem artichoke's additives by the propagation of other orchid species.

Project specifying, which components of Jerusalem artichoke's compound have an effect, on the growing of the orchid plantlets.

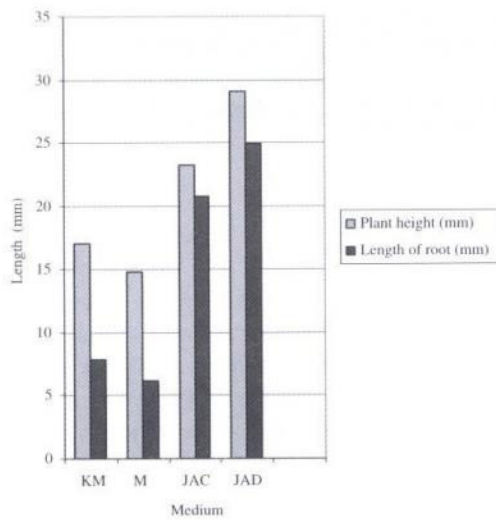


Figure 1. Effect of Jerusalem arthichoke additives on the *Ada keiliana*'s root and shoot growth

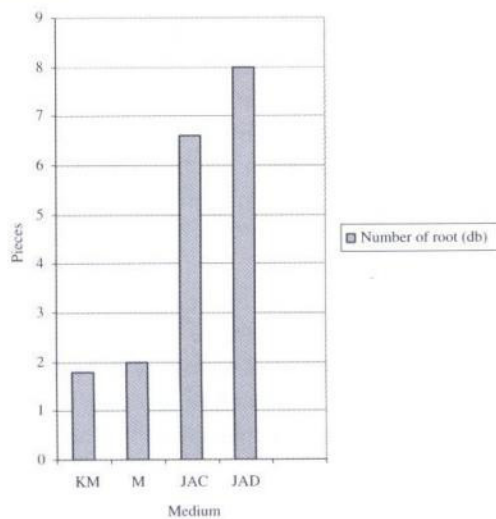


Figure 2. Effect of Jerusalem arthichoke additives on the number of roots of *Ada keiliana*

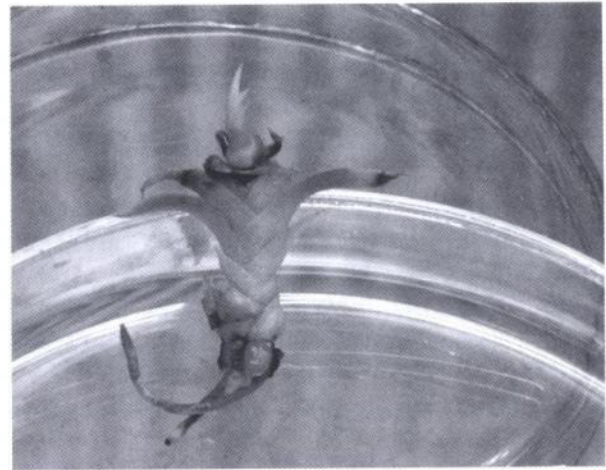


Figure 3. *Ada keiliana* seedling on M medium

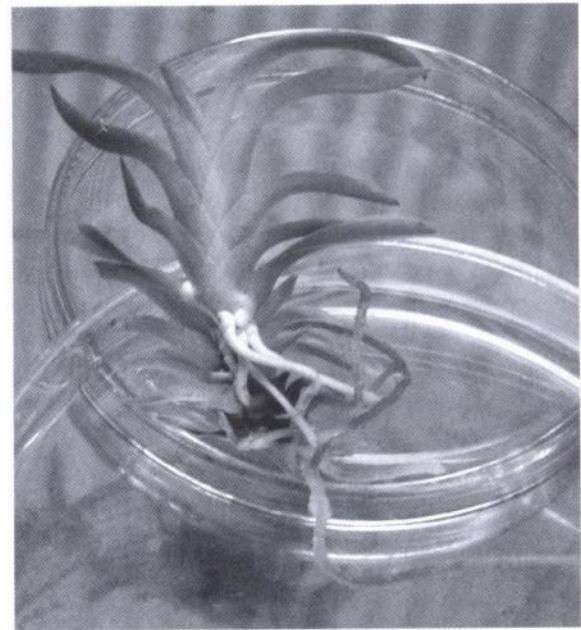


Figure 4. *Ada keiliana* seedling on JAD medium



Figure 5. Well-rooted plantlet, derived from JAD medium one year after acclimatization

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