Influence of the physico-chemical factors, plant growth regulators, elicitors and type of explants on callus cultures of medicinal climbers of *Passiflora* L.

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S u m m a r y

This paper reviews the available research results published in 2006–2011. The attention has been focused on biological processes in the *in vitro* callus cultures which were obtained from various species of medicinal climbers of *Passiflora*. Procedures of callus induction on different parts of plants including physico-chemical conditions, among others light, photoperiod, temperature, basal medium, concentration and composition of plant growth regulators: auxin – 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphtaleneacetic acid (NAA), picloram – PIC (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid), cytokinin – benzyladenine (BA), kinetin (KIN), thidiazuron (TDZ), and gibberellin GA_3 were analyzed. The occurrence of complex interactions between these factors and the efficiency of callus induction as well as its proliferation and development were described. The experiments in callus culture (induction of somatic embryogenesis, indirect organogenesis, cell suspension cultures) including studies concerning the effect of the elicitor (methyl jasmonate, MeJA) and the precursor (L-tryptophan) as well as phytochemical investigations were summarized. It has been found on the basis of reviewed bibliography for a systematic research in this area.The important role of callus cultures in search of an alternative source of plant material (biomaterial) for traditional crop of plants from *Passiflora* spp. without the involvement of climatic factors. The following species were studied in the callus cultures: *P. alata, P.*

caerulea, P. cincinata, P. edulis, P. edulis var. *flavicarpa, P. foetida, P. gibertii, P. incarnata, P. quadrangularis, P. pohlii, P. setacea, P. suberosa* has been shown. Systematic review showed that only *P. edulis* and *P. edulis* var. *flavicarpa* were most frequently investigated. Few studies were performed for *P. incarnata* which is the most important source of raw material in Europe. Summarizing it can be concluded that the biotechnological methods including the plant *in vitro* techniques may play important role in development of pharmacognosy and phytotherapy.

Key words: *Passiflora spp., physico-chemical factors, plant in vitro techniques, callus culture, shoot culture, suspension culture, embryogenesis, morphogenesis, plant growth regulators, elicitors, precursors, secondary metabolites, C- glycosylflavones, alkaloids, plant extracts*

BIOLOGICAL AND PHARMACEUTICAL VALUE OF *PASSIFLORA* L.

The *Passifloraceae* family (the Passion flower) consists of 16 genera and 650 species which grow in range from large woody lianas longer than 35 m to delicate climbers [1]. *Passiflora* L. is the largest genus in the *Passifloraceae*, ranging from 400 [2] to 520 species [3]. Recent data shown that there are 576 *Passiflora* species of vines, lianas and small trees; moreover, there are over 700 known *Passiflora* hybrids [4]. According to Vanderplank [5] 585 species were described. Further, new species are still discovered [6, 7]. The plants of this genus are distributed in the tropical regions of the South America (e.g. in Argentina, Brazil, Colombia, Ecuador, Peru, Venezuela); they are much rarer in Asia, Australia, and tropical Africa [8, 9].

Recently, a real „boom" of passionflowers has been noted (the golden age of *Passiflora*) and their popularity becomes amazing not only for gardeners, but also for ethnobotanists and phytotherapists [10]. Apart from beautiful ornamental flowers, species of *Passifloraceae* family produce giant and edible fruits. In Poland these plants are cultivated also as greenhouse species [11]. These plants are very attractive for pharmaceutical industry due to the presence of valuable and multiactive C-glycosyl flavones (e.g. vitexin, isovitexin, orientin, isoorientin) [12-14], saponins [15], alkaloids [16-20], and cyanogenic glycosides [21-24].

According to ethnobotanical data, these plants influence the central nervous system and cardiovascular system, acting as an anxiolytic, sedative, analgesic, antiepileptic agents and as well as remedy for alcohol, hallucinogens, opiate withdrawal and for hypertension [25-32]. At least, it was shown that numerous pharmacological effects of *Passiflora incarnata* are mediated *via* modulation of the GABA-ergic system including affinity to GABA receptors, and effects on GABA uptake [32]. Recent research showed that isovitexin exerts neuroprotective and antiapoptotic effects and decreases caspase-8 activity in 6-OHDA-induced PC12 cells (Parkinson's disease model) [29-33]. This compound showed also antiproliferative activities in human cancer cell lines (amelanotic melanoma) [34].

Many studies have reported *Passiflora incarnata* L. (PI) as a source of pharmacopoeial raw material showing mainly anxiolytic and sedative effects [35, 36]. Currently, there is an increasing demand is in all age groups on such raw materials

and herbal medicinal products. This is due to stressful lifestyle and increasing consumption of medicinal products, diet supplements and additive-potential drugs.

IMPORTANCE OF PLANT TISSUE CULTURES

Plant tissue culture is a technique of growing plant cells, tissues or organs isolated from the mother plant on artificial, sterile nutrient media. It includes techniques and methods used to research in many botanical aspects [37]. According to George's 'Tissue culture' is commonly used as a collective term describing all kinds of *in vitro* plant cultures, although strictly it should refer only to cultures of unorganised aggregates of cells. In practice the following kinds of cultures are most generally recognized: callus (or tissue) cultures and suspension (or cell) cultures. Moreover, there are plant tissue cultures which aim to obtain microplants *via* propagation of true-to-type plants [37].

The production of therapeutically valuable secondary metabolites from callus or cell suspensions has been intensively studied since the 1960s [38]. Plant tissue culture methods as a part of biotechnology offers an alternative way to herbal and pharmaceutical industries in the production of economically important medicinal plants and valuable secondary metabolites, e.g. pharmaceuticals, flavors, fragrances, and natural pigments [39, 40]. Use of well-established technique of plant cell culture may be promising for the enhancement of plant biomass and bio-compounds production not only in laboratory scale but primarily in industrial bioreactors as large-scale processes [41, 42]. Moreover, plant cell culture offers the manufacturer irrespectiveness of fluctuations in the raw plant material supply that might have been created by changes in the climate, and agriculture activities of the source country [41]. Furthermore, it is of great importance in the case of *Passiflora* species that naturally occur only in tropical and subtropical region [8, 27]. In a moderate climate (e.g. in Poland), these species are cultivated as ornamental plants in greenhouses and botanical gardens [11, 43], but more often these plants can be found in home gardens. It seems that in a case of plant cell culture from climbers of *Passifloraceae* it is interesting to obtain the large scale of biomass rich in C-glycosyl flavones because these compounds may have therapeutic properties valuable for central nervous system diseases. It can be assumed that a well-stabilized cell and tissue cultures as well as regenerated plants can provide an alternative source of medicinal raw material for well-known *P. incarnata*.

INDUCTION AND GROWTH OF CALLUS CULTURE

The first step in initiating tissue cultures is effective elimination of microorganisms from explants and seeds [44]. This problem is very important for passionflower *in vitro* culture because vines of *Passilor*a sp. are often infected by bacteria

Xanthomonas axonopodis pv. *passiflorae*, one of the most important phytopathogens of this vine [45]. In order to obtain sterile starting materials, different protocols of surface sterilization of seeds or fragments of intact plants of *Passiflora* spp. were used.

The sterilization procedures of seeds and explants are complex and multistage. This may result from difficulties of effective sterilized starting plant material. A wide-range and different plant organs can be used as a source of explants for the initiation of callus culture of *Passiflora* spp. Sterile seedling derived from seeds could be an alternative source of leaf, root and shoot explants. Currently, several protocols have been developed for sterilization of seeds to induce plant *in vitro* cultures from aseptic seedling explants [46, 47, 54-56].

The mature seeds were treated with 2–15% sodium hypochlorite solution (for 10–70 min.) [53, 55, 58], or 2% calcium hypochlorite solution (20 min.) [47], 0,1% $HgCl_2$ (for 5 min) [52] with or without antibiotic (cefotaxime) [53], or fungicide (benomyl) [48] and then seeds were rinsed few times in deionized and autoclaved water. Several authors used additionally the GA_3 [54, 55, 63] as well as mechanical and chemical scarification for better induction of seed germination also [48, 54, 55]. In order to sterilize parts of intact plants, they used the same solution as in the case of *Passiflora* spp. seeds, but the processes were carried out in shorter time.

It's well known that the growth of callus cultures includes various biological processes (e.g. dedifferentiation, frequent lose of the ability to photosynthesize, habituation, genetic instability) that mostly distinguish them from other plant cell and tissue cultures. Currently, there are several studies available concerning on the callus induction from different explants of passiflora plant, but not many studies have been performed to determine the chemical composition of callus cultures. Available studies in callus cultures are summarized in table 1.

DISCUSSION

This review showed that in most cases of *Passiflora* spp. explants were isolated from seedlings derived from dry seeds germinated in sterile conditions (*in vitro* germination). Different methods have been used including immersion in 70% ethanol, surface sterilization in commercial sodium hypochlorite. Additionally, several authors used antibiotics or fungicides. In a few cases the seeds were treated with GA_3. Moreover, mechanical and chemical scarification for water immbibition and better induction of seed germination were applied.

The variety and divergence of presented results makes drawing clear conclusions about most effective protocol for callus culture induction of *Passiflora* spp. impossible. The callus cultures were induced from different types of plant explants mainly on leaf disc [53, 56, 57, 60-62, 64-69], root segments [60, 65, 66, 69], shoot fragment (internodes) [57, 60, 65, 66], hypocotyl fragments [58, 66, 69],

Table 1.

Overview of *in vitro* culture studies for *Passiflora* spp.

Species	Subject of studies	Media with phytohormones (mg/l)	Explants	Light conditions and temperature	None (results/ comments) (mg/l)	Time cours of culture initiation	Biblio-Ggaphy
p. alata	callus induction, TLC, HPLC and NMR analysis of callus extract to detect harmane alkaloids	MS + 2,4-D (2.0) and KIN (3.0) and BA (3.0) with or without the L-tryptophan (50, 100, 200) as precursor	leaf segment	16/8 h light/dark photoperiod with 40–50 μmol $m^{-2} s^{-1}$ irradiance, 28±1°C	- MS + precursor (L-tryptophan) → no effect on the biosynthesis of β-carboline alkaloids in callus culture - absence of harmane, harmin, harmol, harmalol, and harmaline in callus extract	30 days	56
P. alata	establishment of callus cultures and cell suspensions	MS + PIC (1.0, 2.0, 3.0, 4.0, 5.0, 7.0, 10.0) MS + NAA (1.0, 2.0, 3.0, 4.0, 5.0)	leaf and shoot fragments of seedling *in vitro*	presence or absence of light, 25°C	- all variants → effective (100%) callus induction on shoot and leaf explants with the exception of leaf fragments cultured on MS+NAA, which did not show morphogenesis - MS + PIC (7.0) → most effective accumulation of biomass of friable callus induced on leaf fragments	30 days	57
P. cincinnata	indirect/direct organogenesis, anatomical study of bud formation	MS + 6-BA (0.0, 0.5, 1.0, 1.5, 2.0) + 5% coconut water	leaf, root segments and the seedlings itself obtained from *in vitro* seed germination	16/8 h light/dark photoperiod, 25°C	- indirect organogenesis *via* meristemoids in callus – leaf and root fragments - direct organogenesis – root and regenerants - the highest induction of organogenesis – BA (0.5 and 1.5) on leaf fragments - organogenesis on root fragments –all cases required BA	28 days	49
P. cincinnata	induction of indirect somatic embryogenesis; callus observation	MS + 2,4-D (1.0, 2.0, 3.0, 4.0, 5.0, 6.0) + BA (1.0) + Phytagel	mature zygotic embryos	darkness, 27±2°C	- MS + different concentration of 2,4-D + BA (1.0) → callus induction mainly in the cotyledonary region - MS + 2,4-D (4.0) → largest number of somatic embryos - the first embryogenic callus → yellowish, friable with white proembryogenic masses	30 days	49

Species	Subject of studies	Media with phytohormones (mg/l)	Explants	Light conditions and temperature	None (results/ comments) (mg/l)	Time cours of culture initiation	Biblio-Ggaphy
P. cincinnata	embryogenic callus induction, evaluate the genetic stability of plants regenerated *via* somatic embryogenesis	MS + 2,4-D (4.0) + BA (1.0)	mature zygotic embryos	16/8 h light/dark photoperiod with 36 μmol $m^{-2}s^{-1}$ irradiance 26±2°C	- effective induction of embryogenic callus - regeneration plants *via* somatic embryogenesis → generation of mostly genetically true-to-type plants	30 days	51
1. P. edulis *2. P. cincinnata*	influence of selected antibiotics and gelling agents on the plant regeneration from hypocotyls	MS + BA (1.0) for *P. edulis* MS + BA (0.5) + 10% coconut water for *P. cincinata* + kanamycin B (200) + ticarcillin (300) + higromycin (20, 24) + Phytagel (2,5 g/l) or agar (8 g/l)	hypocotyl fragments of seedlings *in vitro*	n.i.	- MS + all antibiotics → absence of influence the frequency of buds on the explants - Phytagel and agar → influence the efficiency of hygromycin selection	n.i.	58
P. edulis var. *flavicarpa*	callus induction; studies of cell growth for the cell suspension culture; formation of callus from suspension culture	MS + NAA (0.5 and 2.0) for callus culture MS + NAA (0 - 2.0) + 30 g/l sucrose for cell suspension culture	n.i.	n.i.	- friable, globular and yellowish-white and morphogenic callus - MS + NAA (2.0) → the best medium for growth of cell suspension culture - white, slimy type of callus from cell suspension culture on hormone-free medium + 60 g/l sucrose	20 days	59

Species	Subject of studies	Media with phytohormones (mg/l)	Explants	Light conditions and temperature	None (results/ comments) (mg/l)	Time cours of culture initiation	Biblio-Ggaphy
P. edulis *P. gibertii*	analyze *in vitro* plant regeneration and characteristic the callus structure	MS + BA MS + GA_3 MS + 2,4-D MS + KIN MS + PIC Quantitative composition and ratio between the phytohormons were not disclosed	leaf , nodal and root segments	darkness	- MS + BA (2.0) → callus formation on leaf and nodal explants (except root segments) - MS + GA_3 → callus formed buds after transfer - PIC and KIN in medium MS → larger callus formation on leaf explants than 2,4-D + KIN - MS + BA (0.5) + the light → formation the organogenic callus - MS + 2,4-D → embriogenic callus	30 days	60
P. edulis	embryogenic callus induction and somatic embryogenesis	MS+2,4-D (4.0 – 25.0) + BA (1.0)	cotyledonary lamina	darkness, 27±2°C	- MS + 2,4-D (16.0) + BA (1.0) → highest frequencies of embryogenic callus in the dark at 27 ± 2°C	30 days	52
P. foetida	callus induction, antioxidant activity study	MS + 2,4-D (2.0) and KIN (0.5)	leaf segment	n.i.	- green and friable callus - MS + low sucrose and salt concentration → 65% response of callus induction in leaf explants - EtOH extract of callus → significant *in vivo* antioxidant activity in CCl_4 induced hepatotoxicity in rats model Comments: phytochemical analysis of callus extract were not yet performed	16 days	61
P. incarnata (PI) *P. edulis* (PE) *P. quadrangularis* (PQ)	callus induction; elicitation of callus culture; phytochemical analysis of MeOH callus extracts (HPLC); antioxidant activity	MS + 2,4-D (0.25-3.0) + KIN (0-2.0) + BA (0-2.0) + 3% sucrose + 2.5% Phytagel elicitors: UV-B, 100 μM MeJA	leaf segment (the midrib and lamina)	16/8 h light/dark photoperiod with 50 μmol m^{-2} s^{-1} irradiance 22±1 °C	- MS + KIN (2.0) + 2,4-D (3.0) → 80–90% callus induction in midrib and 32 to 45% → in lamina - faster growth of callus PQ - increase in the biosynthesis of orientin by 100 μM MeJA (after 7 days) and isoorientin by UV-B 25.3 kJm^{-2} (after 7 days) in callus PQ - antioxidant activity (after UV-B dose)	14-28 days	62

Species	Subject of studies	Media with phytohormones (mg/l)	Explants	Light conditions and temperature	None (results/ comments) (mg/l)	Time cours of culture initiation	Biblio-Ggaphy
P. pohlii	callus induction, optimization of this process conditions	MS + PIC (1.0, 3.0, 5.0, 7.0, 10.0)	intermodal fragments of seedlings *in vitro*	16/8 h light/dark photoperiod 20˚C – 16h 30˚C – 8h	- MS + PIC (5) → the best result in induction of friable callus from internodal segments and in greatest accumulation of biomass in comparison with other callus cultures	60 days	63
P. quadrangularis	callus induction; optimization of plant cell culture conditions	(1) MS + BA (2.0) + GA_3 (1,0) (2) MS+BA (0.5) (3) MS+NAA (2.0 + KIN 0,1) (4) MS+2,4-D (0.25) + KIN (0,25) (5) B5+2,4-D (2.0)	leaf segments	16/8 h light/dark photoperiod, 25±1˚C	- morphological changes induced in the initial callus cultures: (1) compact, hard, organogennic with silver-dark-green collor (2) from light green to light yellow color and then to light brown, loose and soft (3) dark yellow, loose and soft (4) from light yellow to light green (5) light grown, loose and soft - MS + BA (2.0) + GA_3 (1.0) and MS+BA (0.5) → the best callus induction	more than 30 days	53
P. quadrangularis (PQ) *P. caerulea* (PC) *P. racemosa* (PR)	callus induction; optimization of plant cell culture conditions and phytochemical investigations	MS+2,4-D (2.0) MS+NAA (2.0) + KIN (0.1) MS+ NAA (0.1) + KIN (0.25) MS+BAP (2.0) + GA_3 (1.0)	leaf fragments	PQ - 16/8 h light/dark photoperiod, PC, PR - darkness 25±1˚C	- the highest increase of callus biomass growth → on MS + 2,4-D (2.0) for PC and PQ, and on MS + NAA (0.1) + KIN (0.25) for PR - 2-D TLC → 6-8 spots for flavonoids and 3 spots for fenolic acids in callus methanol extracts	more than 30 days (PQ, PR), within 30 days (PC)	64
P. quadrangularis (PQ) *P. caerulea* (PC)	callus induction; optimization of plant cell culture conditions and phytochemical investigations	MS+2,4-D (2.0) MS+NAA (2) + KIN (0.1) MS+NAA (0.5) + KIN (0.1) MS+BA (2.0) + GA_3 (1.0) MS+BA (0.5)	leaf and root and internodes fragments	PQ - 16/8 h light/dark photoperiod, PC - darkness 25±1˚C	- MS + NAA (0.5 and 2.0) + KIN (0.1) – rhizogenesis on organogenic callus -MS + BA (0.5) and MS+BA (2.0) + GA_3 (1.0) → caulogenesis on organogenic callus - 2-D TLC → spots for flavonoids and phenolic acids in callus methanol extracts	more than 30 days (PQ), within 30 days (PC)	65

Species	Subject of studies	Media with phytohormones (mg/l)	Explants	Light conditions and temperature	None (results/ comments) (mg/l)	Time cours of culture initiation	Biblio-Ggaphy
P. quadrangularis (PQ) *P. caerulea* (PC)	callus induction optimization of callus culture and phytochemical investigations	MS+2,4-D (2.0) MS+BA (2.0) + GA_3 (1.0)	epicotyl, hypocotyl leaf, internodes and root fragments	PQ - 16/8 h light/dark photoperiod, PC - darkness 25±1°C	- epicotyls and hypocotyl fragments of PC → the best callus induction - MS+ BA (2.0) + GA_3 (1.0) → the highest value of growth index (GI) for PQ - MS+2,4-D (2.0) → the highest value of growth index (GI) for PC - photoperiod → effective condition for GI value (compared with darkness)	more than 30 days (PQ), within 30 days (PC)	66
P. quadrangularis	elicitation in callus culture, HPTLC and HPLC-DAD analysis	MS+BA (2.0) + GA_3 (1.0) + 100 and 200 μM MeJA for 7 days	leaf fragments	16/8 h light/dark photoperiod, 25±1°C	- increase of isovitexin and hiperoside level by 200 μM MeJA (after 7 days) - 100 and 200 μM MeJA → diminish the growth of callus. - callus methanol extract → vitexin, apigenin, luteolin, rutin and chlorogenic and rosmarinic acid. - absence → alkaloid (harman)	more than 30 days	67
P. quadrangularis	elicitation in callus culture, HPTLC and HPLC-DAD analysis	MS+2,4-D (2.0) + 100 and 200 μM MeJA for 7 days	leaf fragments	16/8 h light/dark photoperiod, 25±1°C	- increase of isovitexin and vitexin, rutin, chlorogenic acid level by 100 μM MeJA (after 7 days) - 100 and 200 μM MeJA → diminish the growth of callus - absence → alkaloid (harman)	more than 30 days	68

Species	Subject of studies	Media with phytohormones (mg/l)	Explants	Light conditions and temperature	None (results/ comments) (mg/l)	Time cours of culture initiation	Biblio-Ggaphy
P. setacea	callus induction, optimization of organogenesis conditions	MS + BA MS + TDZ MS + BA + TDZ MS Quantitative composition and ratio between the phytohormons were not disclosed	leaf, root and hypocotyl fragments of seedlings *in vitro*	16/8 h light/dark photoperiod	- all variants → effective callus induction on leaf and hypocotyl explants under photoperiod 16h - indirect organogenesis - MS + BA or BA + TDZ→ more shoots regeneration from the callus	30 days	69

Abbreviations: BA – benzyladenine; B5 – Gamborg's medium [71]; CCl_4 - carbon tetrachloride; 2,4-D – 2,4-dichlorophenoxyacetic acid; 2-D-TLC – two dimensional TLC, GA_3 – gibberellin GA_3; TDZ – thidiazuron; HPLC-DAD – high performance liquid chromatography with diode-array detection; HPTLC – high performance thin layer chromatography; KIN – kinetin; LS - culture medium according to Linsmaier et Skoog [70]; MeJA - methyl jasmonate; MS - culture medium according to Murashige et Skoog [69]; NAA – 1-naphtaleneacetic acid; n.i. – no information; PC - *P. caerulea*; PE - *P. edulis;* PI - *P. incarnata;* PIC – picloram; PQ - *P. quadrangularis;* PR - *P. racemosa*; TLC – thin layer chromatography

cotyledonary lamina [52] and also embryo tissues [50, 58]. The plant explants can react differently to the composition of plant growth regulators (auxin, cytokinin, gibberellin) and their concentrations, as well as to the physio-chemical factors. This proves that there are differences between species. It was observed relationship between species of *Passiflora,* kind of explants and phytohormones inducing callus. The callus initiation may occur both in photoperiod condition (16/8 h light/dark) [51, 53, 56, 57, 62-67, 69] and in total darkness [49, 52, 60]. The percentage of explants responding to callus formation ranged from 32 to 100% among tested treatments. It was reported that time of callus induction was also different and ranged from 16 [61] to 60 days [63]. Moreover, there were no significant adverse effects of antibiotics (kanamycin B, ticarcillin, higromycin) on the cultures induction [58].

In recent years, the callus cultures of *P. incarnata* have not been well studied. Only Antognoni et al. [62] observed callus induction on leaf explants after 2-4 weeks, but better callogenesis was evaluated on midrib (64%, 73%, 82% after 2, 3, 4 weeks respectively) if compared with callus induction on lamina. The best results were achieved on MS medium supplemented with KIN (2.0 mg/l) and 2,4-D (3.0 mg/l). Moreover, this composition of plant growth regulators induced callus in leaf fragments of *P. quadrangularis* and *P. edulis* also.

As in the case of *P. incarnata*, there is little number of studies on callus cultures carried out for the *P. caerulea.* Studies [66] showed that the homogenic callus was initiated efficiently on epicotyls and hypocotyls of seedlings *P. caerulea* on MS medium with NAA (0.5 mg/l) + KIN (0.1 mg/l), but the highest value of growth index was obtained on the medium supplemented with 2,4-D (2.0 mg/l). Similar influence of this auxin (2 mg/l) on callus initiation from leaf fragment of *P. foetida* [61], and *P. alata* [56] was also shown.

On the other hand few authors showed positive effect of 2,4-D on induction of embryogenic callus for *P. gibertii* [60], *P. cincinnata* [49, 51], and *P. edulis* [52]. Until recent times, there have been few studies on somatic embryogenesis of *Passiflora*. Da Silva et al. [49] reported that different concentrations of 2,4-D plus BA (1 mg/l) induced formation of pro-embryogenic callus on cotyledonary region of mature zygotic embryos of *P. cincinnata.* Moreover, mature zygotic embryos of *P. cincinnata* cultured on 2,4-D (4 mg/l) plus BA (1 mg/l) produced yellowish embryogenic calli with whitish pro-embryogenic masses. The highest response of embryogenic callus formation was observed when explants (cotyledonary lamina) of *P. edulis* were incubated in MS medium containing highest concentration of 2,4-D (16.0 mg/l) with BA (1.0 mg/l) [52]. Cytological and histological analyses of pro-embryogenic callus allowed to state the presence of two types of developing cells: thin-walled, small, isodiametric cells with large nuclei and dense cytoplasm, typical for intense metabolic activity; and elongated and vacuolated cells, with small nuclei and less dense cytoplasm [52].

Numerous studies revealed the influence of other plant growth regulators on homogenic or organogenic callus formation. According to recent studies [53]

callus was efficiently induced on leaf fragments of *P. quadrangularis* under the influence BA (2.0 mg/l) with GA_3 (1.0 mg/l) and only BA (0.5 mg/l) added to MS medium. In the first case callus was organogennic, compact, hard, of silver-dark-green color, in another case callus was of from light green to light yellow and then to light brown. It was also loose and soft. Furthermorethe highest increase of callus biomass on BA (2.0 mg/l) with GA_3 (1.0 mg/l) and 2,4-D (2.0 mg/l) was observed [64, 65]. Callus induction was also observed in the presence of BA in MS medium for *P. setacea* [69]. On the other hand, another studies showed that not every species of passionflower responded to cytokinin BA in callus formation. De Figueiredo [60] observed that the use of BA (2 mg/l) *P. gibertii* reacted better to the callus formation than *P. edulis* which do not form callus.

Anatomical studies of organogenic callus of *P. cincinata* [48] showed that under the influence of different concentrations of benzyladenine, the buds formation on leaf discs and root fragments from meristemoids may originat. It was observed that the growth of bud may result from cell proliferation in the chlorophyll parenchyma of leaf. But in the case of root fragments, the buds were initiated directly in the pericycle and the vascular cambium. Lombardi et al. [48] showed also the correlation between BA level and indirect organogenesis. In the absence of BA in callus-induction medium, no bud formation on leaf fragments was demonstrated and the higher concentration of this hormone (2.0 mg/l) was also not effective. Moreover, the highest induction of organogenesis was observed on medium MS supplemented with BA (0.5 and 1.5 mg/l). For *P. quadrangularis* both concentrations 0.5 mg/l and 2.0 mg/l BA (with GA_3 1.0 mg/l) [65] and for *P. gibertii* (BA 1 mg/l) resulted in ogranogenic callus formation [60]. Moreover, MS medium supplemented not only with BA but also with GA_3 induced growth of buds on callus of *P. gibertii* [60]. Apart from the BA and GA_3 other plant growth regulators also can induce the organogenic callus. For *P. caerulea* this callus was obtained under the influence of NAA (0.5 mg/l) and KIN (0.1 mg/l) in MS medium [66]. It was also reported that TDZ induced indirect organogenesis on callus of *P. setacea* and better shoots formation on root explants was observed on MS medium supplemented with TDZ and BA [69]. On the other hand few studies showed the influence of picloram (PIC) on callus induction [63, 57]. Merhy et al. [63] observed that the best of callus initiation from intermodal fragments of *P. pohlii* was assessed for 5 mg/l PIC. For *P. alata* the highest accumulation of biomass of friable callus was observed in the present of 7 mg/l PIC. Moreover, Lugato et al. [57] estimated that all tested concentrations of PIC had ability to callus induction.

Other interesting feature of the work on callus culture was the investigation of precursor (L-tryptophan) and elicitors (methyl jasmonate, UV) influencing on biosynthesis of secondary metabolites (alkaloids, C-glycosyl flavones) in biomass. Only some authors carried out studies on the phytochemical composition of callus cultures and the influence of plant growth regulators and physico-chemical conditions on the secondary metabolites concentrations in them. Studies showed that callus cultures of *P. quadrangularis* and *P. cearulea* are available to biosynthesize the

secondary metabolites [64]. Although, Antognoni et al. [62] indicated only small amounts of isoorientin in callus cultures of *P. quadrangularis*, while the concentration of other flavonoids was below the detection limit. In one study with the use of L-tryptophan as a precursor in medium MS, the influence on the biosynthesis of β-carboline alkaloids in callus culture of *P. alata* when callus were incubated in a medium containing 2,4-D (2 mg/l) or KIN (3.0 mg/l), or BA (3.0 mg/l) was not shown [56]. Moreover, recent investigation with HPLC phytochemical analysis did not show any alkaloids in calluses of *P. quadrangularis* both after and without elicitation [62, 67, 68]. It was shown that elicitation with use of methyl jasmonate is an effective method to increase of C-glycosyl favones (isovitexin, vitexin orientine) concentrations in callus of *P. quadrangularis* [62, 67, 68]. The HPLC analysis [68] of methanol extract of callus of *P. quadrangularis* growing on MS medium supplemented with 2,4-D 2 mg/l demonstrated that 100 μM of MeJA for the highest production of vitexin, isovitexin, rutin, chlorogenic acid in callus culture compared with the control is more effective. Moreover, it was evaluated that 200 μM of MeJA was most effective in increasing the secondary metabolites (isovitexin, hyperoside) level in callus incubated in a medium containing BA (2 mg/l) and GA_3 (1 mg/l). Further HPLC analysis showed the presence of vitexin, apigenin, luteolin, rutin, chlorogenic, and rosmarinic acid in this methanol extract. Antognoni et al. [62] showed that UV-B irradiation of callus of *P. quadrangularis* growing on medium MS supplemented with 2,4-D (3.0 mg/l) and KIN (2.0 mg/l) was able to increase the production of orientin, isoorientin, vitexin and isovitexin. It was evaluated that the production of isoorientin by *P. quadrangularis* callus cultures reached concentrations similar to that found in fresh leaves. Moreover, elicitation with use the methyl jasmonate (100 μM) enhanced strongly orientin concentration but had no influence on the isovitexin level.

Based on the results of the few studies, it was shown that callus of *P. quadrangularis* [62] and *P. foetida* [61] exhibited antioxidant activity.

CONCLUSION

In conclusion, it seems that more systematic studies are needed in order to obtain the valuable biomaterial for pharmaceutical purposes and to explain the influence of the bio-physical-chemical conditions on induction, biomass growth and secondary metabolites synthesis in callus cultures obtained from different explants of *Passiflora* sp. The results of indirect embryogenesis of species of *Passiflora* suggest a promising protocols for induction of somatic embryogenesis (e.g. for *P. edulis*). Moreover, according to Pinto et al. [51] *P. cincinnata* plants regenerated *via* somatic embryogenesis maintained true-to-type ploidy. This result indicates the possibility of callus culture application in order to obtain plants of *Passiflora* sp. regenerated *in vitro*.

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WPŁYW CZYNNIKÓW FIZYKOCHEMICZNYCH, REGULATORÓW WZROSTU I ROZWOJU, ELICYTORÓW I RODZAJU EKSPLANTATÓW NA KULTURY KALUSA LECZNICZYCH PNĄCZY Z RODZAJU *PASSIFLORA* L.

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Streszczenie

W pracy dokonano przeglądu dostępnych wyników badań opublikowanych w latach 2006-2011. Zwrócono uwagę na procesy biologiczne zachodzące w kulturach kalusowych *in vitro* otrzymanych z różnych gatunków leczniczych pnączy z rodzaju *Passiflora* L. Przeanalizowano procedury indukowania kalusa na różnych fragmentach roślin z uwzględnieniem warunków fizykochemicznych, takich jak światło, fotoperiod, temperatura, podstawowe podłoża oraz rodzaj, stężenie i kompozycja roślinnych regulatorów wzrostu i rozwoju, tj.

auksyny (kwas 2,4-dichlorofenoksyoctowy (2,4-D), kwas naftylo-1-octowy (NAA), pikloram - PIC (kwas 4-amino-3,5,6-trichloropikolinowy), cytokininy, tj. benzyloadenina (BA), kinetyna (KIN), tidiazuron (TDZ) oraz giberelina GA_3, wskazując na złożone zależności pomiędzy tymi czynnikami a efektywnością indukowania i prowadzenia kultur kalusowych. Podsumowano eksperymenty prowadzone w kulturach kalusowych (indukcja embriogenezy somatycznej, organogeneza pośrednia, zawiesinowe kultury komórkowe) z uwzględnieniem badań z zastosowaniem elicytora (jasmonian metylu, MeJA) lub prekursora (L-tryptofan) oraz analiz fitochemicznych. Na podstawie analizowanej bibliografii wykazano potrzebę przeprowadzenia systematycznych badań w tym zakresie oraz ważny udział kultur kalusowych w poszukiwaniu alternatywnego źródła surowca roślinnego (biomateriał) w stosunku do tradycyjnych upraw gatunków z rodzaju *Passiflora*, bez udziału czynników klimatycznych. W kulturach kalusowych badano następujące gatunki: *P. alata, P. caerulea P. cincinata, P. edulis, P. edulis var. flavicarpa, P. foetida, P. gibertii, P. incarnata, P. quadrangularis, P. pohlii, P. setacea, P. suberosa*. Systematyczny przegląd bibliografii wykazał, że tylko *P. edulis* oraz *P. edulis* var. *flavicarpa* były najczęściej badane w tym zakresie. Niewiele badań przeprowadzono dla *P. incarnata*, gatunku który jest najważniejszym źródłem surowca zielarskiego w Europie. W pracy podkreślono rolę metod biotechnologicznych dotyczących roślinnych kultur *in vitro* w rozwoju farmakognozji i fitoterapii.

Słowa kluczowe: *Passiflora spp., czynniki fizyko-chemiczne, techniki roślinnych kultur in vitro, kultura kalusowa, kultura pędowa, kultura zawiesinowa, embriogeneza, morfogeneza, regulatory wzrostu i rozwoju, elicytory, prekursory, metabolity wtórne, C-glikozydy flawonowe, alkaloidy, ekstrakty roślinne*