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Influence of plant growth regulators on development and polysaccharide production of cell cultures of *Pelargonium sidoides*

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Pelargonium sidoides **is a traditional medicinal plant from South Africa. An aqueous-ethanolic formulation of the roots and tubers is approved for the treatment of acute bronchitis. Therefore propagation of the plant material by cell cultures and the extraction of potential pharmaceutical active compounds are of great interest. Calli were established on different media from roots and shoots of seedlings and softness and colour of the tissue were compared. Optimum growth of callus cultures was achieved in MS-medium containing 1 mg/L 2,4-D and 0.2 mg/L kinetin or 2.2 mg/L TDZ supplemented with 50 mg/L ascorbic acid and 50 mg/L citric acid. Accumulation of phenolic deposits, responsible for inhibition of growth was avoided by addition of ascorbic and citric acid and a short period of sub-culture. Furthermore, the influence of different phytohormones [2,4-D, kinetin, 6-benzylaminopurine (BAP), *α*-naphthaleneacetic acid (NAA), Thidiazuron (TDZ)] on the polysaccharide composition of the liquid media of suspension cultures was investigated. For the first time, arabinogalactan-proteins (AGPs) as bioactive components were isolated from cell cultures of *P. sidoides.***

Key words: *Pelargonium sidoides*, cell culture, arabinogalactan-protein, 2,4-dichlorophenoxyacetic acid, kinetin, *β*-glucosyl Yariv reagent.

INTRODUCTION

Plant cell culture is an important system to produce secondary metabolites and to investigate biosynthetic pathways besides propagation of plants. Furthermore, the isolation of primary and secondary metabolism products from plant cell cultures has a certain industrial relevance in medicine, pharmacy and food technology. Successful examples for production of pharmaceuticals in plant suspension cultures are secondary metabolites like

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Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic acid; **A,** ascorbic acid; **AG,** arabinogalactan; **AGP,**arabinogalactan-protein; **BAP,** 6-benzylaminopurine; **C,** citric acid; **IBA,** indole-3-butyric acid; **K,** kinetin; **MS,** Murashige and Skoog medium (1962); **NAA,** *α* naphthaleneacetic acid; **PA6,** polyamide 6; **TDZ,** Thidiazuron; **Yariv,** *β*-glucosyl Yariv reagent.

ginseng saponins, diosgenin and shikonin (Weathers et al., 2010), the biotransformation of methyldigitoxin to methyldigoxin in cell cultures of *Digitalis lanata*, as well as the production of the anti-cancer agents podophyllotoxin and paclitaxel (Malik et al., 2011; Nosov, 2012). Initially, paclitaxel was isolated from the bark of *Taxus brevifolia*, leading to die back of the slow growing trees. Since 2002, the active agent is mainly isolated from suspension cultures of *Taxus*. On one hand, this secures the long-ranging requirement of paclitaxel with high quality; while on the other hand, it ensures conservation of *T. brevifolia*. Considering the importance of plants like *Pelargonium sidoides* it is essential to look for conservation strategies and guarantee availability of high quality pharmaceutical products (Moyo and van Staden, 2014).

Pelargonium sidoides DC. (Geraniaceae) belongs to the pool of medicinal plants of South Africa, and an aqueous-ethanolic extract from the roots of this plant is approved in Germany and other countries for the treatment of acute bronchitis. This extract is characterized by different pharmacological effects (Moyo and van Staden, 2014). Diverse antibacterial and antiviral activities of the *Pelargonium* root extract have been proven to date and gallic acid and other phenolic substances seem to be responsible for these effects (Helfer et al., 2014; Kolodziej, 2011). It has been postulated that the also known immunomodulatory activities are probably a result of synergistic effects of polyphenols and coumarins (Brendler and van Wyk, 2008; Kolodziej, 2011). On the other hand, it is known, that *Pelargonium* root extracts contain high amounts of carbohydrates (Schoetz et al., 2008), which have not been characterized accurately up to now. Recently, special glycoproteins, the so-called arabinogalactan-proteins (AGPs) have been detected in *P. sidoides* (Duchow, 2012). These hydroxyprolin-rich cell wall glycoproteins are characterized by heterogeneity of carbohydrate chains, complexity of the protein backbone as well as the different possibilities in linking both parts (Nothnagel, 1997; Seifert and Roberts, 2007). Besides involvement in growth and developmental processes in plants (Goellner et al., 2013), AGPs play a commercial role especially as Gum Arabic, which is used in food and pharmaceutical industry due to its emulsifying, adhesive and water-binding attributes (Showalter, 2001). AGPs from other species, especially from *Echinacea purpurea*, are considered as stimulator of the human immune system and show immunomodulating activities in vitro, e.g. binding to human leucocytes (Thude et al., 2006). This implements the consideration that AGPs from *Pelargonium* could also be involved in the immunomodulatory effects of the root extract. For suspension cultures of different plants, accumulation of high amounts of AGPs in the suspension media has been shown (Classen, 2007).

The aim of the present study was the establishment of callus and suspension cultures of the medicinal plant *P. sidoides*. Different media including different phytohormones have been tested to produce cell cultures of highest quality. Furthermore, the polysaccharide fractions of the different suspension media were compared with regard to their monosaccharide composition and used for isolation of AGPs. The possible influence of different phytohormones on the polysaccharide and AGP composition is a special focus of this work. Suspension cultures of *P. sidoides* offer a reproducible source of AGPs as putative immunomodulatory active compounds with high yields and independent of varying plant origin.

MATERIALS AND METHODS

Plant materials

P. sidoides DC. (Geraniaceae) has been grown in the Garden of the Pharmaceutical Institute of the University of Kiel, Germany. Plant material has been identified by Birgit Classen, the Department of Pharmaceutical Biology, University of Kiel, where voucher specimens are deposited in the herbarium (identification number Ps 2012).

Establishment of cell cultures

Calli from shoots of germinating seeds of *P. sidoides* DC. were induced as described by Classen (2007) for *Echinacea*. Seeds were disinfected by using absolute ethanol (8 min) and 10% Domestos®-solution (30 min, Unilever, Hamburg, Germany). After rinsing with distilled water (3 × 5 min) seeds were kept in sterile petri dishes having autoclaved wet filter paper for germination. Hypocotyls, 5 mm pieces, were transferred to solid MS-medium (Murashige and Skoog, 1962) containing 0.4 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and stored at light intensity of 94 µmol m^{-2} s^{-1} (Osram L 58W/77 Fluora) and 25°C for callus induction.

For further callus proliferation, the following phytohormones: A) 0.4 mg/L 2,4-D, B) 1 mg/L 2,4-D + 0.2 mg/L kinetin (K), C) 5 mg/l 6-benzylaminopurine (BAP) + 1 mg/L 1-*α*-naphthaleneacetic acid (NAA) and D) 2.2 mg/L Thidiazuron (TDZ) were used. Calli were subcultured every 4-5 weeks.

In experiments to avoid accumulation of polyphenolic compounds during callus proliferation, medium was supplemented with ascorbic- and citric acid (A+C, 50 mg/L each) or polyamide 6 (PA6). In addition, fast subculturing (every second week) was also attempted to reduce the impact of phenolic compounds on callus growth.

Calli (∅ 2 cm) were transferred into 100 ml liquid MS-medium supplemented with: A) 0.4 mg/l 2,4-D; B) 1 mg/L 2,4-D + 0.2 mg/L K; C) 5 mg/L BAP + 1 mg/L NAA and D) 2.2 mg/L TDZ and ascorbic- and citric acid (50 mg/L each) or PA6 (Roth, Germany) and stored in the dark at 25°C on a shaker (120 rpm, Edmund Bühler GmbH, Hechingen, Germany). Suspension cultures were subcultured every 3 weeks. A small amount of the cultures (16.5 ml) has been utilized as inoculum for the next culture; while the rest has been used for isolation of polysaccharides and AGPs and represents a batch. Micrographs of suspension cultures have been taken with a Zeiss light microscope (number 4760059901, Zeiss, Germany).

Gel diffusion assay for detection of AGPs

A solution containing 1% agarose, 0.9% NaCl a nd 1 mM $CaCl_2$ in

10 mM Tris-HCl buffer (pH 7.3) was autoclaved and poured into sterile Petri dishes. Five holes (Ø 1.2 mm) were punched into the gel with four holes arranged around one central cavity at 1 cm distance. 20 µl of β-glucosyl Yariv reagent solution (1 mg/ml) was poured in the central cavity; the other four holes were filled with solutions of high molecular weight polysaccharide fraction (20 µl) at different concentrations (1, 10, 25 and 50 mg/ml). The plates were stored for 24 h at room temperature.

Isolation of polysaccharides and AGPs from suspension cultures

Suspension cultures (1-2 L) were centrifuged (3875 *g,* 15 min), cells deleted and the cell-free medium used for isolation of polysaccharides. After heating (95°C, 10 min) of small portions (100 ml) of the liquid medium, denatured proteins were removed by centrifugation (5000 rpm, 10 min) and the supernatant was precipitated with ethanol (80% V/V). After centrifugation (20000 *g*, 15 min), the precipitated material was freeze-dried to obtain the high-molecular weight polysaccharide fraction.

The polysaccharide fraction (100-300 mg) was dissolved in distilled water (putative AGP; 1 mg/ml) and precipitated by addition of an equal volume of an aqueous solution of 1 mg/ml β-glucosyl Yariv reagent (Yariv et al., 1962) containing NaCl (0.15 M) to get AGPs. After formation of the AGP-Yariv complex at 4°C overnight, it was centrifuged (20000 *g*, 10 min, 4°C), dissolved in distilled water and degraded by addition of $Na_2S_2O_4$ before heating to 50°C (Kreuger and van Holst, 1995). Final dialysis (molecular weight cut off: 12-14 kDa) against distilled water and freeze-drying resulted in pure AGPs.

Analysis of neutral monosaccharides

Neutral sugar analysis of the high molecular weight polysaccharide fraction and the Yariv reagent-precipitated AGPs was performed according to the method of Blakeney et al. (1983). After acid hydrolysis (1 M trifluoracetic acid, 1 h, 121°C) followed by reduction and acetylation of monosaccharides, the resulting alditol acetates were investigated by gas liquid chromatography with a flame ionization detector (HP 5890 series, Hewlett-Packard, Nürnberg, Germany) and a fused silica capillary column (Optima-OV-225, 0.25 µm, L = 25 m, i.d. = 0.25 mm, Machery & Nagel, Düren, Germany). Nitrogen was used as carrier gas with a flow rate of 1.2 ml/min and temperatures of 230°C for the oven (isothermal), 250°C for the injector and 240°C for the detector.

RESULTS AND DISCUSSION

Establishment of callus cultures on different media

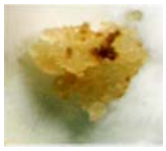
Callus induction was achieved on different MS-media (Murashige and Skoog, 1962) varied by additions of phytohormones and other supplements. Both auxins (2,4-D and NAA) as well as cytokinins (K and BAP) and TDZ with cytokinin like activity in different concentrations led to growth of undifferentiated calli (Table 1).

2,4-D (0.4 mg/L) supplementation led to viable, but slow growing callus tissue with many brown deposits. To improve the growth rate of the calli, 2,4-D concentration was increased to 1 mg/L and the medium was additionally supplemented with the cytokinin K. The combination of both phytohormons (2,4-D and K) resulted in optimum growth rates but calli were still partly brown. The parallel testing of another phytohormone combination with 5 mg/L BAP and 1 mg/L NAA led to comparable growth rates, however resulted in hard and dry callus tissue, interspersed with many brown deposits. These excretions probably consist of phenolic compounds, which led to reduced growth rates. Therefore, to decrease phenolic deposits, media were supplemented with ascorbic and citric acid (50 mg/l each) and the cultures were subcultured in frequent intervals (every two weeks). After three month, light coloured calli having one month sub culturing period were obtained. Although calli obtained from BAP and NAA supplemented MS media recorded faster growth per month with little brown deposits (yielding 3 cm dia-size from 0.5 cm), callus tissue was dry and crumbly. Calli cultured in 2,4-D and K or TDZ supplemented media containing ascorbic and citric acid resulted in soft tissue with high growth rates and represented the optimal basis for permanent culture. In addition to the application of ascorbic and citric acid for inhibition of phenolic excretions polyamide 6 was also tested and supplemented to the solid basic medium with 2,4-D and K in three different concentrations: 0.2, 0.5 and 1 mg/100 ml media. All three concentrations eliminated the occurrence of polyphenolic deposits and resulted in highly soft and lightish callus tissue with good growth rates.

Until now, only little is known about the micropropagation of *P. sidoides*: especially about the establishment of callus and suspension cultures. Investigations concerning shoot organogenesis and hairy root cultures have been done by Moyo et al. (2012, 2013, 2014) and Colling et al. (2010). Successful induction of callus tissue from leaves, stems and seeds of *P. sidoides* under the influence of auxins like 2,4-D and IBA was first achieved by Lewu et al. (2007). The use of media with kinetin in combination with NAA for callus induction from petioles only led to calli with crumbly consistence (Moyo et al., 2012). These first results underline the need for further research with the aim to optimize callus growth and quality. Data for other *Pelargonium* species show that a balanced combination of auxins and cytokinins results in optimal cell growth. The ratio of these phytohormones is most important for the induction of undifferentiated tissues (Brown and Charlwood, 1986): high amounts of auxins result in root development whereas an excess of cytokinins leads to shoot differentiation (El-Nil et al., 1976; Rao, 1994; Beck and Hartig, 2009; Moyo et al., 2012).

Comparable to this, our experiments also show that a balanced combination of auxins (1 mg/L 2,4-D) and cytokinins (0.2 mg/L K) has a favourable effect on the growth of *P. sidoides* callus cultures. The use of 0.4 mg/L 2,4-D as single phytohormone, which was applied for the establishment of soft and lightish callus tissues from *Echinacea purpurea* (Classen, 2007), only led to slow

Table 1. Growth and quality of callus cultures obtained from shoots of *Pelargonium sidoides* on MS-media with different supplements.

Culture	MS-medium +		Characteristics of callus tissue			
	Phytohormones	Other constituents	Size (ø) after 4 weeks (cm)	Colour	Consistence	Growth rate
	0.4 mg/L 2,4-D		1	Brown deposits	Soft	+
	1 mg/L 2,4-D 0.2 mg/L K		2	Brown deposits	Soft	++
	5 mg/L BAP 1 mg/L NAA		2	Brown deposits	Dry Hard	++
	0.4 mg/L 2,4-D	50 mg/L A+C	1	No brown deposits	Soft	+
	1 mg/L 2,4-D 0.2 mg/L K	50 mg/L A+C	2	No brown deposits	Soft	++
	5 mg/L BAP 1 mg/L NAA	50 mg/L A+C	3	Little brown deposits	Dry Hard	+++
	2.2 mg/L TDZ	50 mg/L A+C	2	No brown deposits	Usually soft	++
	1 mg/L 2,4-D 0.2 mg/L K	0.2 g/100 ml PA6	2	No brown deposits	Soft	++
	1 mg/L 2,4-D 0.2 mg/L K	0.5 g/100 ml PA6	2	No brown deposits	Soft	++
	1 mg/L 2,4-D 0.2 mg/L K	1 g/100 ml PA6	2	No brown deposits	Soft	++

Growth rate: +, weak; +, weak; +++, strong.

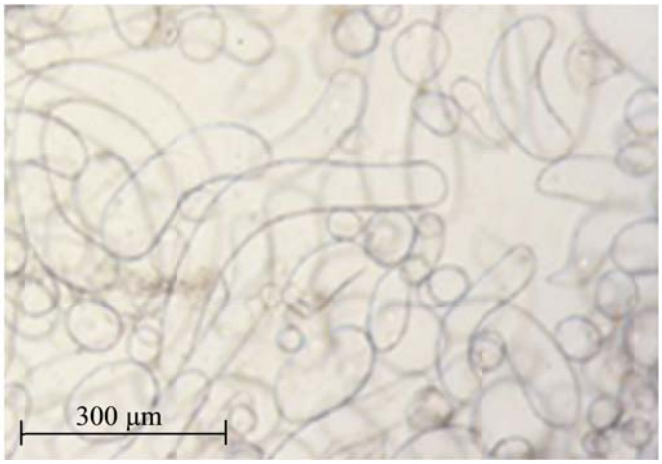


Figure 1. Suspension cultured cells of *P. sidoides* in a medium with 1 mg/L 2,4-D, 0.2 mg/L K and ascorbic and citric acid (50 mg/L each).

growing calli in case of *P. sidoides*. A critical point for all media was the occurrence of brown, fast spreading accumulations which inhibited callus growth, especially in the medium with BAP and NAA. These dark deposits of phenolic origin have often been observed in cell cultures from different plants with high amounts of tannins (Vatanpour-Azghandi et al., 2002; Zagoskina et al., 2003) and have been quantified in tissue cultures from *P. sidoides* by a Folin and Ciocalteu assay (Moyo et al., 2012). For callus cultures from other species it has been shown that supplementation of media with antioxidant compounds like ascorbic and citric acid (50 mg/L) results in suppression of the formation of polyphenolic compounds (Klein, 2004; Sathyanarayana and Varghese, 2007). For *P. sidoides*, addition of ascorbic and citric acid to the media in combination with a shortened subcultivation period (every two weeks) also led to inhibition of phenolic deposits. Ascorbic and citric acid probably act as inhibitors of polyphenoloxidases (Pizzocaro et al., 1993; Jang and Moon, 2011; Suttirak and Manurakchinakorn, 2010). The benefit of short cultivation intervals to reduce the occurrence of brown coloration has also been shown for teak cell cultures (Tiwari et al., 2002). Interestingly, a reduction of the amount of 2,4-D seems to minimise brown coloration in cell cultures of *Curcuma mangga* (Sundram et al., 2012). This could not be proven for *P. sidoides*: 0.4 mg/L 2,4-D as well as 1 mg/L 2,4-D produced comparable amounts of phenolic deposits.

Alternatively, suppression of polyphenols was achieved with polyamide 6 (PA6) which was added to the culture media. Fast growing, soft and bright calli impressively proved the adsorption of phenolic deposits on PA6.

In brief, our investigations have identified MS-media with 1 mg/L 2,4-D and 0.2 mg/L K or alternatively 2.2 mg/L TDZ, both enriched with 50 mg/L A+C each or 2 g/L PA6 as optimal media for permanent callus culture of *P. sidoides*.

Establishment of suspension cultures

Comparable to callus cultures, quality of suspension cultures was investigated with regard to different phytohormones and further supplements. At first, calli from media with 0.4 mg/L 2,4-D were transferred to the liquid medium of the same composition. Although growth was acceptable, the colour of the suspension was rather dark, indicating production of phenolic compounds. Therefore calli from media with 0.4 mg/L 2,4-D, 1 mg/L 2,4-D and 0.2 mg/L K, 5 mg/L BAP and 1 mg/L NAA as well as 2.2 mg/L TDZ, all supplemented with ascorbic and citric acid, were transferred to liquid media and the suspension cultures investigated concerning their growth and colour. Suspension cell lines have been stable over a period of up to 16 subcultivations. Growth of all cultivations was good with comparable medium viscosity after three weeks. Suspensions only differed in their colour: media with BAP/NAA led to gloomy cultures, cells on 2,4-D/K were of bright colour and suspensions with TDZ were even brighter. Microscopic investigation of the cultured cells showed relatively homogeneous suspensions with undifferentiated, more or less round or sausage-like cells (Figure 1).

Isolation and analysis of secreted polysaccharides

The amount of water-extractable polysaccharides in suspension culture media varied between 1 and 4% related to the dry cell mass and was highest for the medium with TDZ (Table 2). This result shows that high amounts of polysaccharides can be isolated directly from suspension culture media with the advantage of a faciliated isolation process in comparison to isolation from native plant material.

Neutral monosaccharide composition of the polysaccharide fractions differed from medium to medium (Table 3). The polysaccharides from the medium with BAP and NAA are characterized by a special composition with glucose and galactose (about 35 and 30%) as the dominating monosaccharides, followed by nearly similar amounts of arabinose and xylose (nearly 15% each) and small amounts of mannose, rhamnose and fucose (2% each). In contrast to that polysaccharides which were isolated from media with 0.4 mg/l 2,4-D or 1 mg/L 2,4-D in combination with 0.2 mg/l K have another principal composition with galactose (about 43%) and arabinose (about 25%) accompanied by smaller amounts of glucose (about 11%), xylose (about 12%), mannose (about 4%) rhamnose (about 2%) and traces of fucose. Galactose (ca. 39%) and arabinose (about 33%) were also the main sugars in media supplemented with TDZ with a different

Table 2. Amounts of isolated polysaccharides and AGPs from different suspension cultures of *P. sidoides* (related to freeze-dried cell mass).

Cultivation with		Amount of polysaccharides (%)	Amount of AGPs (%)	Number of batches
0.4 mg/L 2,4-D		2.66 ± 0.97	0.39 ± 0.19	8
0.4 mg/L 2,4-D	A+C	1.54 ± 0.01	0.41 ± 0.17	2
1 mg/L 2,4-D + 0.2 mg/l K	A+C	1.75 ± 0.78	0.41 ± 0.24	19
5 mg/L BAP + 1 mg/l NAA	A+C	1.20 ± 0.59	0.24 ± 0.11	4
2.2 mg/L TDZ	A+C	4.06 ± 4.00	0.67 ± 0.51	4

Table 3. Neutral monosaccharide composition of polysaccharide fractions from suspension cultures of *Pelargonium sidoides* with different phytohormones.

Neutral monosaccharide (% w/w)	Polysaccharide fractions from suspension cultures with:				
	0.4 mg/L 2,4-D	0.4 mg/L 2,4-D + A/C[a]	1 mg/L 2,4-D + 0.2 mg/L K + A/C[a]	5 mg/l BAP + 1 mg/L NAA + A/C[a]	2.2 mg/L TDZ + A/C[a]
Number of batches	8	2	19	4	4
Gal	43.3 ± 3.8	45.6 ± 4.3	42.8 ± 4.4	29.7 ± 4.2	38.6 ± 10.3
Ara	26.3 ± 3.7	26.4 ± 3.6	24.5 ± 3.4	13.4 ± 1.4	32.5 ± 11.1
Glc	11.6 ± 4.0	7.6 ± 6.8	11.3 ± 5.2	35.5 ± 6.8	12.1 ± 10.9
Xyl	11.2 ± 1.9	12.3 ± 2.8	13.6 ± 3.0	14.7 ± 1.0	8.2 ± 4.4
Man	4.1 ± 0.7	4.3 ± 1.1	3.8 ± 0.8	2.5 ± 0.4	4.7 ± 1.0
Rha	2.4 ± 1.3	3.1 ± 0.8	2.4 ± 0.8	2.2 ± 0.6	2.0 ± 0.8
Fuc	1.1 ± 0.5	1.0 ± 0.4	1.7 ± 1.1	2.1 ± 0.3	1.8 ± 0.7
Ara:Gal ratio	1:1.7	1:1.8	1:1.8	1:2.3	1:1.3

[a]Concentration 50 mg/L.

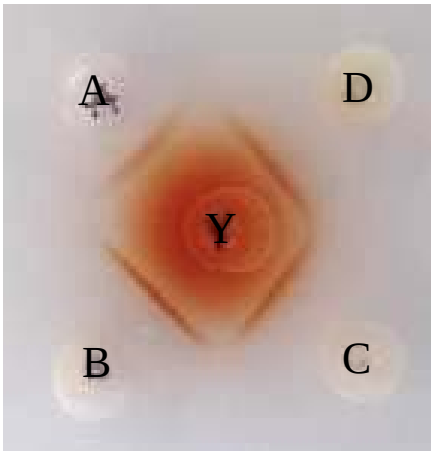


Figure 2. AGP-positive gel diffusion assay with Yariv reagent (Y) and four different concentrations of the polysaccharide fraction from medium supplemented with 0.4 mg/L 2,4-D (A=1 mg/ml, B=10 mg/ml, C=25 mg/ml, D= 50 mg/ml).

ratio of galactose to arabinose. All other monosaccharides occured only in small amounts (Glc 12%, Xyl 8%, Man 5%, Rha and Fuc 2%), indicating that a high percentage of this polysaccharide fraction are possibly AGPs.

Detection of AGPs

Using a gel diffusion assay, the presence of AGPs could be proven to be part of the ethanol-precipitated polysaccharide fraction from cell culture media. AGPs can be detected by formation of a red precipitation line with Yariv reagent (Figure 2), which is a typical feature of many AGPs (Seifert and Roberts, 2007). For suspension cultures from different plants, secretion of high amounts of AGPs into the suspension media has already been shown (Immerzeel et al., 2004; Classen, 2007; Sánchez-Sampedro et al., 2008), but for *Pelargonium* species, the presence of AGPs has not been reported before, neither for the plant nor for suspension cultured cells.

Isolation and characterisation of AGPs

AGPs are predominantly secreted into the extracellular matrix in plants, and this probably is the reason, why they can be found in the liquid media of suspension cultured cells. Precipitation with Yariv reagent yielded purified AGPs which accounted for 0.24-0.67% of dry weight of

Table 4. Neutral monosaccharide composition of AGPs from suspension cultures of *Pelargonium sidoides* with different phytohormones.

Neutral monosaccharide (% w/w)	AGP fractions from suspension cultures with:				
	0.4 mg/L 2,4-D	0.4 mg/L 2,4-D + A/C[a]	1 mg/L 2,4-D + 0.2 mg/L K + A/C[a]	5 mg/L BAP + 1mg/L NAA + A/C[a]	2.2 mg/L TDZ + A/C[a]
Number of batches	8	2	19	4	4
Gal	61.7±0.3	62.2±3.8	62.6±2.3	65.6±2.6	58.6±7.0
Ara	32.5±2.1	30.2±2.2	31.2±1.9	27.2±2.2	31.5±1.6
Glc	0.9±0.4	1.1±1.6	1.5±1.5	1.9±1	1.9±1.8
Xyl	1.1±0.1	1.7±1.6	0.8±0.4	1.4±1	2.9±3.2
Man	2.1±0.4	2.6±1.3	2.4±0.8	2.3±1.8	2.4±2.3
Rha	1.3±0.3	1.6±1.3	1.1±0.3	1.5±0.2	2.0±0.5
Fuc	0.4±0	0.6±0.4	0.5±0.4	0.4±0.2	0.7±0.5
Ara:Gal ratio	1:1.9	1:2.1	1:2.0	1:2.5	1:1.9

[a]Concentration 50 mg/L.

the cell material (Table 2), which is in good agreement with yields of AGPs from liquid media of *Echinacea* suspensions (Classen, 2007). The amount of AGPs was highest for the medium with TDZ and lowest for that with BAP and NAA. Compared to isolation from plant material (Classen et al., 2006), yields of AGPs from suspension cultures are tenfold higher, pointing out that suspension cultures are an excellent source of AGPs.

After isolation of AGPs, the influence of the phytohormones on the neutral sugar composition of AGPs was investigated. In contrast to the composition of high molecular weight polysaccharide fractions which differed with regard to the used phytohormone(s), monosaccharide composition of AGPs was rather stable. AGPs typically consisted of about 60% galactose and 30% arabinose accompanied by low amounts of glucose, xylose, mannose, rhamnose and traces of fucose (Table 4).

Compared to literature, neutral sugar composition of *P. sidoides* AGP reflects the typical qualitative and quantitative distribution of characteristic monosaccharides within an AGP such as high amounts of galactose and arabinose in a ratio of about 2:1 and minor amounts of accompanying monosaccharides like rhamnose and glucose (Showalter, 2001; Ellis et al., 2010). AGPs isolated from media with BAP and NAA might be a little different with a higher Gal:Ara ratio of 2.5:1, but statistical relevance of this should be verified by investigations of further batches.

Conclusion

With this work optimal conditions for growth of callus and suspension cultures of *P. sidoides* could be established. Future work should clarify whether suspension cultures might be a good source for *Pelargonium* coumarins which seem to be responsible for antibacterial, antiviral and immunomodulating effects of the *Pelargonium* root extract together with polyphenols (Brendler and van Wyk, 2008; Kolodziej, 2011; Moyo and van Staden, 2014).

From suspension cultures of *P. sidoides* high amounts of potentially biological active AGPs were isolated. Prospective investigations of the *Pelargonium*-AGP will help to characterize the carbohydrate moiety more precisely; especially the linkage types between the single monosaccharide units as well as the mode of linkage between the sugar and the protein part. Together with the testing of biological activities with focus on immunomodulating effects, structure-activity-relationships are of high interest.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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