Original Research Paper

Flavonoids and Antimicrobial Properties of *Begonia fischeri* var. *palustris in vitro* Plantlets

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Abstract: The objective of this study was to design a protocol of the successful establishment of plants of *Begonia fischeri* var. *palustris* obtained in the *in vitro* seeds culture, to evaluate flavonoid content and antimicrobial properties of *in vitro* plantlets. The significant increase of percentage of seed germination (92.5%) was recorded in the half strength MS medium and 1% agar medium (83.3%) in comparison with 50-60% in greenhouse conditions. Flavonoid composition of the leaves of *in vitro* plantlets and greenhouse stock plants had no substantial differences. Significant differences ($p < 0.05$) were not found between flavonoid contents of the leaves (13.6 and 15.5 mg·g^{-1} of Dry Weight (DW), respectively). Aqueous ethanol extracts of plants showed antimicrobial effects against reference strains of *Bacillus subtilis*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Concentration of flavonoids in acetone and ethanol extracts of exudative compounds of the leaves of *in vitro* plantlets was 0.02 and 2.0 mg·g^{-1} DW, respectively. Our results indicated that this protocol can be used for the production of a large number of plants of *B. fischeri* var. *palustris* with rich flavonoid composition and high quantities of flavonoids, including exudative compounds, with healing and air cleaning properties.

Keywords: Antimicrobial Properties, *Begonia fischeri* var. *palustris*, Flavonoids, Indoor Plant Design, *in vitro* Seed Germination, Metabolic Engineering

Introduction

Begonia fischeri Schrank var. *palustris* (Hartw. ex Benth.) Irmsch. (section *Begonia*) is ornamental shrub-like plant with a vigorous growth. It is implemented in ornamental gardening in greenhouses throughout the world, including Siberia. Its natural habitat is the Atlantic coastal forest in Brazil. It grows mostly in marshy places and forms large thickets under the shadow of forest canopy. Flowering continues throughout the year.

Vegetative multiplication of the species by means of traditional cultivation techniques seems to be highly laborious. *In vitro* raising of many begonias is an alternative efficient system widely used by commercial nurseries and institutes for rapid plants propagation, germplasm conservation, elimination of pathogens and for phytochemical screening of medicine-useful compounds (Murugan *et al.*, 2016). The seed populations *in vitro* can be stored for a long time lacking the problems of bacterial or fungal infections ordinarily associated with long-term maintenance of *Begonia* clonal stock.

In addition to high decorative, *B. fischeri* var. *palustris* contains phenolic compounds, including flavonoids and have prospects for use as medicinal (Karpova *et al.*, 2018). Nowadays, a large number of studies are devoted to antimicrobial activity of plants. Flavonoid-rich plant extracts from many species were shown to possess antibacterial and antifungal activity (Cushnie and Lamb, 2005; Xie *et al.*, 2015).

Despite the increased attention to the biology and pharmacognosy of the genus *Begonia* in the world, data on the chemical composition and biological activity of the species of the genus are still sparse. Antimicrobial properties of the extracts of several species (*B. erythrophylla*, *B. heracleifolia*, *B. semperflorens*, *B. fuchsioides*, *B. picta* and *B. malabarica*) were investigated (Frei *et al.*, 1998; Ramesh *et al.*, 2002; Nisha *et al.*, 2016).

Flavonoids composition of the genus *Begonia* is fragmentary studied. In leaves and flowers, luteolin, luteolin 7-O-glucoside (cynaroside), quercetin, rutin, quercitrin, isoquercitrin, quercetin 3-O-xyloside, kaempferol 3-O-glucoside (astragalin), kaempferol rutinoside, kaempferol 3-methyl ether (isokaempferide) and its 7-O-glucoside, quercetin 3-methyl ether and its 7-O-glucoside, quercetin 3, 7, 3´-trimethyl ether and gossypetin 3,7,8,3´-tetramethyl ether, glycosyl flavones (vitexin, isovitexin and orientin) were found. In anthocyanin composition, glycosides of cyanidin, pelargonidin and peonidin were detected (Chirol and Jay, 1995; Harborne and Williams, 2001; Saffidine *et al.*, 2017). There are very few reports concerning the concentrations of flavonoids in *in vitro* derived plants (Murugan *et al.*, 2016) and the chemical content of *B. fischeri* var. *palustris* (Karpova *et al.*, 2018).

Representatives of the genus *Begonia* are widely presented in the plant design indoor. In this regard, it is important to assess the composition of the surface exudative compounds of the leaves as air cleaners. At this moment, exudative constituents of the leaves, located on the leaf surface, are extensively investigated (Muravnik *et al.*, 2016). Meanwhile, these compounds of representatives of the genus *Begonia* are at the very beginning of study (Ensemeyer and Langhammer, 1982).

The objective of this study was to design a protocol for the plant production using *in vitro* seeds culture and to evaluate flavonoid content and antimicrobial properties of *in vitro* plantlets *B. fischeri* var. *palustris*. This includes initiation and inoculation of aseptic cultures of seeds, examination of the effect of different media on the germination rate of *B. fischeri* var. *palustris*.

Materials and Methods

Seed Sterilization and Germination

The mature fruits of *B. fischeri* var. *palustris* were harvested three months after hand pollination. Seed capsules were surface disinfected with 70% ethanol for 1 min, followed by immersion in 0.1% solution of $HgCl_2$ for 10 min and finally rinsed thoroughly with three times in sterile distilled water.

The nutritious media were enriched by 2.5% sucrose and solidified with 0.6% agar. The pH in all variants of media were adjusted to 5.8 prior to autoclaving at 121°C and 103.4 kPa for 20 min. The cultures were incubated at 22±2°C with light/dark 16/8 hours photoperiod provided by white fluorescent tubes (1500 lx).

Seeds were inoculated onto four variants of imbibition media: 1.0% agar, ½ MS (half strength MS salts) (Murashige and Skoog, 1962), MS and MS supplemented with 0.5 mg·L^{-1} 6-benzylaminopurine (BAP) and 0.3 mg·L^{-1} of α-Naphthaleneacetic Acid (NAA). In each flask, 100±5 seeds were inoculated, using the modified technique, where the disinfected seeds were firstly scattered on a sterile paper and transferred individually to the agar medium with a dissecting needle. In every variant of medium, three replicates were fulfilled.

B. fischeri var. *palustris* seed germination was defined here as hypocotyl emergence (1/3 the length of the seed size). Germination was monitored every day for until no further germination was recorded. Final germination percentage (FG) and Mean Daily Germination (MDG) were calculated using the formulas given below:

$$FG = n \: / \: N \times 100\%, \: MDG = FG \: / \: D$$

Where:
n = The number of germinated seeds
N = The total number of seeds
D = The number of days to final germination

Plant Growth Conditions in Greenhouse

Hardened *in vitro* plantlets of *B. fischeri* var. *palustris* after transfer from sterile substrate grew in greenhouse on soil mixture consisted of the rotted leaf litter mixture with river sand (1:1) in the greenhouse conditions (temperature 20-23°C, humidity 50-60%, illumination 40-60 µmol m^{-2}s^{-1}).

Simultaneously with the beginning of the seed germination *in vitro*, *B. fischeri* var. *palustris* seeds gathered from greenhouse stock plants were sown directly in greenhouse on the same soil mixture under the similar greenhouse conditions (greenhouse plants). Further, experimental design consisted in comparison of the greenhouse plants and *in vitro* plantlets.

Flavonoid Identification and Quantification

The greenhouse-grown plants and *in vitro* plantlets of *B. fischeri* var. *palustris* were grown in the composition of "Collections of living plants indoors and outdoors" USU 440534 of Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russian Federation (CSBG SB RAS) under greenhouse conditions with a temperature of + 15-25 C^0, humidity of 30-60% and illumination of 40-60 µmol m^{-2}s^{-1}.

Leaves from ten of greenhouse plants (L) and ten *in vitro* plantlets (LR) were collected for detection of flavonoids in the beginning of generative state (at 60th day after transfer ex vitro).

For detection of flavonoids, precisely weighed samples of fresh plant material (0.5 g) were exhaustively extracted with an ethanol: water mixture (70:30, v/v) in a water bath at 60-70°C. Aqueous ethanol crude extract was filtered, diluted with the ethanol:water mixture (70:30, v/v) up to the volume of 25 mL in a graduated flask and used for quantification of flavonoids and evaluation of antimicrobial activity. Dry-weight concentration in the samples was calculated by the gravimetric method. The method was reported in detail previously (Karakulov *et al.*, 2018).

For studying the exudate flavonoids of the leaves of *in vitro* plantlets, acetone and ethanol extracts of leaf surface constituents were prepared. For acetone extraction, fresh (not ground) precisely weighed leaves were briefly rinsed with acetone to dissolve the surface constituents (Exudate A). Ethanol extract (Exudate E) was made with analogous wash of fresh leaves with 95% ethanol. The acetone and ethanol solutions were evaporated to dryness and each residue was dissolved in a small volume of 95% ethanol. These ethanol extracts were used for quantification of compounds in leaf exudates and for evaluation of antimicrobial activity. Dry-weight concentration in the samples was calculated by the gravimetric method.

The HPLC system for absolute quantification of flavonoids consisted of an Agilent 1200 with a Diode Array Detector (DAD) and the ChemStation software (Agilent Technologies, Santa Clara, CA, USA) for data processing. The chromatographic separation was conducted at 25°C on a Zorbax SB-C18 Column (4.6×150 mm, 5 μm i.d.) with the Agilent Guard Column Hardware Kit (p.n. 820888-901).

The mobile phase consisted of MeOH (solvent A) and 0.1% orthophosphoric acid dissolved in water (solvent B). Separation of glycosides of flavonoids in crude extracts was performed with gradient. The run via gradient was started with a solvent A–solvent B mixture at 32:68 (v/v) followed by a linear gradient to 33:67 (v/v) for the first 27 min, then to 46:64 (v/v) from minute 28 to minute 38, then to 56:44 from minute 39 to minute 50, then to 100:0 from minute 51 to minute 54. The mobile phase was returned to 32:68 (v/v) from minute 55 to minute 56. The flow rate was set to 1 mL·min^{-1}. The sample injection volume was 10 μL and the absorbance was measured at 210, 255, 270, 290, 325, 340, 360 and 370 nm. The quantification of phenolic compounds was conducted by the external-standard method. Chemical reference standards of quercetin, kaempferol, luteolin, kaempferol rutinoside, vitexin and isovitexin were purchased from Sigma (St. Louis, MO, USA) and hyperoside, isoquercitrin, astragalin were obtained from Fluka (Fluka, Sigma-Aldrich Chemie GmbH, Munich, Germany). Validation of the analytical procedures was performed in accordance with ICH guidelines (2005).

Anthocyanin content was determined by spectrophotometric method on UV-Vis spectrophotometer "Agilent 8453" (Agilent Technologies, Santa Clara, CA, USA). The concentration of anthocyanins (mg·g^{-1} of dry weight) was calculated using the molar absorbtion coefficient of cyanidin-3-rutinoside in 1% HCl in water equal to 28 840. The absorbance of fresh leaves extract (in 1% HCl in water) was measured at wavelengths of 529 and 650 nm (Karpova and Fershalova, 2016).

Evaluation of Antimicrobial Activities

The disk diffusion method was chosen for preliminary evaluation of the antimicrobial activities of crude extracts and exudates of the greenhouse plants and the *in vitro* plantlets of *B. fischeri* var. *palustris*. The strains of American Type Culture Collection were used as reference strains: gram-positive bacteria – *Staphylococcus aureus* ATCC 25923 (SA), *Streptococcus pyogenes* ATCC 12344 (SP) and *Bacillus subtilis* ATCC 6633 (BS); gram-negative bacteria – *Escherichia coli* ATCC 25922 (EC), *Klebsiella pneumonia* ATCC 13883 (KP), *Enterobacter aerogenes* ATCC 35029 (EA), *Pseudomonas aeruginosa* ATCC 27853 (PA) and *Enterococcus faecalis* ATCC 19433 (EF) and fungi – *Candida albicans* ATCC 10231 (CA). Ciprofloxacin (5 μg) served as a standard antibacterial agent and itraconazole (10 μg) was used as a standard antifungal agent (positive control). Ethanol served as a negative control.

Seven reference strains (*S. aureus, S. pyogenes, B. subtilis, E. coli, E. arerogenes, E. faecalis* and *C. albicans*) were selected for the determination of Minimal Inhibitory Concentrations (MICs) of the extracts and the exudates, based on their disc-diffusion susceptibility data. MICs of the leaf extracts of the greenhouse plants (L) and the *in vitro* plantlets (LR) and the acetone (A) and the ethanol (E) exudates together with ciprofloxacin and itraconazole were determined using the broth microdilution assay (Andrews, 2001). The bacteria reference strains and the strain of *C. albicans* were grown overnight on nutritive agar for microorganisms and on mycobiotic agar (respectively) from Laboratorios Conda and diluted equivalent to a 0.5 McFarland standard (Andrews, 2001). Microtiter plate wells (final total volume of 200 μL), each containing 90 μL of Mueller–Hinton (MH) broth were inoculated with 10 μL of cell suspension and serial dilutions of the extracts and the exudates (100, 50, 20, 10 and 5 μg·m L^{-1}) and antimicrobial agents ciprofloxacin (0.025 μg·mL^{-1} to 128 μg·m L^{-1}) and itraconazole (0.008 μg·m L^{-1} to 4 μg·m L^{-1}). Test range of itraconazole was established in accordance with European Committee for Antimicrobial Susceptibility Testing (Arendrup *et al.*, 2017). The plates were incubated at 30°C for 24 h without shaking. The negative control wells contained MH broth only and the positive control wells contained the respective cell suspensions with no plant extract (exudates)/antimicrobial agents added. This was done in triplicate (Andrews, 2001). The MIC was the lowest concentration of antimicrobial agent, which inhibited visible growth of organism.

Statistical Analysis

All the data were expressed as mean ± standard error (SE) of three replicates %. The data were analyzed using one-way analysis of variance (ANOVA) and means were compared using Duncan multiple range test. The values of P<0.05 were considered significant.

Results

B. fischeri var. *palustris* seed germination in the greenhouse conditions generally occurred within 3 weeks and final germination percentage (FG) was in the range of 50-60%.

When evaluating the final germination percentage of the species *in vitro*, significant differences were observed between all the variants tested. The highest percentages of seed germination (92.5%) were recorded in the half strength MS basal medium (½ MS) followed by 1% agar medium (83.3%). In the tissue culture conditions, germination rate of seeds was the highest in Var.1: seed emergence occurred within 11 days (Table 1).

In Var. 2 and 2A the germination rate was poor, it took more than 15 days for seed emergence and seedlings developed more slowly (Fig. 1).

Half strength MS medium produced optimal results for the *B. fischeri* var. *palustris* seedling development suggesting that this medium was the most suitable for the germination of the species.

After 30 days, all the germinated seeds were transferred to a sterilized medium containing full-strength MS salts, 2% sucrose and 0.6% agar. For acclimatization, plantlets with well-developed roots and shoots (Fig. 1c) were removed from the tissue culture, transferred to small plastic pots and exhibited 85% survival in the mixture of sterile garden soil and river sand (1:1). Seedlings were incubated in a room condition at 20°–22°C and 12 h photoperiod under cool-white light (40 $\mu mol\,m^{-2}s^{-1}$) for 40 days. Then the hardened plantlets, as well as the greenhouse plants, were investigated for antimicrobial activity and flavonoid composition (Fig. 2).

Table 1: Germination of *B. fischeri* var. *palustris* in sterile conditions using four different initial medium variants

Variants of media	Medium composition	Mean daily germination	Final germination %
Var.0	1% agar	8.2±0.6 a	83.3±2.0 b
Var.1	½ MS	7.8±0.5 a	92.5±1.5 a
Var.2	MS	4.0±0.1 b	64.5±2.2 c
Var. 2 A	MS+0.5 mg·L^{-1} BAP+0.3 mg·L^{-1} NAA	3.5±0.3 b	38.4±0.7 d

*mean ± SE in columns followed by the same letter do not differ significantly according Duncan test
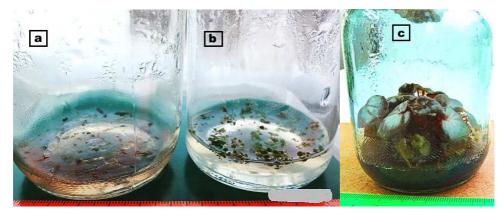

Fig. 1: Developmental stages of *B. fischeri* var. *palustris* seedlings grown on the different variants of germination medium (a) Poor seed germination in Var. 2 A observed on 7th day of seeds inoculation (b) Vigorous seed germination in Var.1 (7th day) (c) Well-developed plantlets before the transplantation in ex vitro conditions
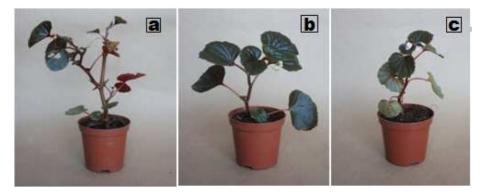

Fig. 2: Greenhouse stock plant (a), hardened *in vitro* plantlet (b) and greenhouse plant (c) of *B. fischeri* var. *palustris* (the age of the plants is 18, 4 and 6 months, respectively)

Table 2: Flavonoid composition of the leaves and the leaf exudates of *B. fischeri* var. *palustris* and yield of total flavonoids per plant

| Parameter | Value | | | |
	La	LR	Exudate A	Exudate E
Total phenolic compounds, number	17	18	16	10
Flavonoids, number	10	9	13	8
Concentration, $\mu g \cdot g^{-1}$ of dry weight				
Orientin	1320.9 ± 144.2	1024.5 ± 88.3	1.0 ± 0.2 (0.10)[b]	30.1 ± 4.2 (2.9)
Vitexin	524.0 ± 62.2	Nd	1.5 ± 0.1 (-)	12.8 ± 1.5 (-)
Isovitexin	1115.4 ± 212.3*	833.0 ± 54.2	1.9 ± 0.2 (0.23)	Nd
Hyperoside	3051.2 ± 199.6*	2345.1 ± 250.2	1.0 ± 0.1 (0.04)	20.2 ± 3.1 (0.86)
Isoquercitrin	6818.3 ± 702.2*	5535.2 ± 408.6	1.6 ± 0.2 (0.03)	90.0 ± 8.4 (1.63)
Rutin	553.6 ± 308.5*	1107.1 ± 118.2	0.2 ± 0.0 (0.02)	10.1 ± 1.2 (0.91)
Astragalin	211.2 ± 15.8	222.4 ± 26.2	0.7 ± 0.0 (0.31)	14.6 ± 1.6 (6.56)
Kaempferol rutinoside	218.0 ± 30.3*	137.0 ± 15.3	0.7 ± 0.1 (0.51)	Nd
Quercetin	112.4 ± 27.5*	43.7 ± 3.9	0.2 ± 0.0 (0.46)	3.5 ± 0.5 (8.01)
Luteolin	15.1 ± 3.2	Nd	Nd	Nd
Kaempferol	Nd	Nd	0.2 ± 0.0 (-)	Nd
Total flavonoids	15459.0 ± 2318.5	13636.3 ± 1295.1	14.3 ± 1.9 (0.10)	438.6 ± 52.1(3.22)
Total phenolic compounds (9.21)	27618.1 ± 2008.3*	22024.3 ± 2234.3	18.7 ± 2.5 (0.08)	2028.9 ± 21.4
Anthocyanins	7211.5 ± 432.0	7835.0 ± 728.4	Nd (-)	214.3 ± 32.2 (2.74)
Yield of total flavonoids per plant, g	174.0 ± 12.2*	189.1 ± 13.4	0.2 ± 0.0	6.1 ± 0.4

*Significant differences between L and LR (p>0.05) [a] the leaves of greenhouse plants (L) and *in vitro* plantlets (LR); acetone (A) and ethanol (E) extracts of exudate compounds of the leaves [b] In parentheses is ratio "concentration of the constituent in exudate A or E/its total concentration in the leaf of *in vitro* plantlets"

In the course of flavonoid composition studying of the leaves of greenhouse plants and *in vitro* plantlets, as well as the leaf exudates of *B. fischeri* var. *palustris*, quercetin glycosides (isoquercitrin, hyperoside), C-glycosyl flavones (vitexin, isovitexin) and free aglycones, quercetin, luteolin and kaempferol (in minute amounts) were found (Table 2). The main constituent of the leaves and the leaf exudates was isoquercitrin.

Numbers of flavonoids in the leaves and leaf exudates slightly varied mainly due to free aglycones and minor C-glycosyl flavones. Minimal number of flavonoids was found in the exudate E, whereas concentrations of the constituents were substantially higher than in exudate A. In exudate A, number of flavonoids was maximal due to the presence of isovitexin, kaempferol rutinoside, free kaempferol and the others minor compounds.

Proportions of the constituents in the leaves and leaf exudates were also equal. Concentrations of total flavonoids in the leaves of *in vitro* plantlets were insignificantly lower in comparison with greenhouse plants. Both values were substantial (above 13 and 15 $mg \cdot g^{-1}$, respectively). Significant differences were found between the concentrations of isovitexin, hyperoside, isoquercitrin, rutin, kaempferol rutinoside and free quercetin. Flavonoids were more than half of the concentration of total phenolic compounds. Isoquercitrin was about 60% of total flavonoids concentration.

Concentrations of phenolic compounds in the exudates A and E were < 0.02 $mg \cdot g^{-1}$ and > 2 $mg \cdot g^{-1}$,

respectively. In the exudates A this concentration was 0.08% and concentration of flavonoids was 0.10% of their concentration in the leaves as a whole. In the exudates E, comparable percentages were 3.22% and 9.21%. Certain constituents were extracted with acetone better, for example, free quercetin and kaempferol glycosides. Thereby, extraction of exudate flavonoids with acetone caused the enrichment of the extract by free quercetin and kaempferol glycosides and extraction with ethanol promoted the increase of total flavonoids in the extract of exudate compounds. Some amount of anthocyanins in exudate E indicates possible extraction of definite amounts of vacuolar constituents.

Despite of some decrease of total flavonoids concentration in the leaves of the *in vitro* plantlets in comparison with greenhouse plants, the yield of total flavonoids per plant in the plantlets was significantly higher (Table 2).

The examination of antimicrobial properties of the leaf extracts showed that crude extracts of the leaves of *B. fischeri* var. *palustris* inhibited growth of seven from nine reference strains (Table 3). Both extracts were active against gram-positive bacteria, *S. aureus* (MICs of 50 μg/mL), *S. pyogenes* (MICs of 20 μg·mL⁻¹) and *B. subtilis* (MICs of 20 and 50 μg·mL⁻¹), as well as E. aerogenes (MICs of 50 μg·mL⁻¹). The extract L inhibited the growth of *E. coli*, *E. faecalis* and *C. albicans* (MICs of 50 μg·m L⁻¹). None of the extracts showed activity against *K. pneumonia* and *P. eaeruginosa*.

Table 3: Antimicrobial properties of crude extracts and exudates of the leaves of *B. fischeri* var. *palustris*

| | Minimal inhibition concentration, $\mu g \cdot mL^{-1}$ | | | | | | | | |
	SA	SP	BS	KP	EA	EC	PA	EF	CA
Extract L[b]	50	20	20	-[a]	50	50	-	50	50
Extract LR	50	20	50	-	50	-	-	-	-
Exudate A	-	-	50	-	-	-	-	-	-
Exudate E	50	-	50	-	-	-	-	-	-

[a]The absence of activity
[b]L - extract from the leaves of greenhouse plants; LR - extract from the leaves of *in vitro* plantlets; exudates A and E– acetone and ethanol extracts of exudate compounds of the leaves, respectively

Therefore, the spectra of antimicrobial activity of two extracts differed. The spectrum of extract L from the leaves of greenhouse plants (7 reference strains) was wider than the spectrum of extract LR from the leaves of *in vitro* plantlets (4 reference strains). Only extract L from the leaves of greenhouse plants was active against *C. albicans*.

Antimicrobial effects of the exudates was reduced. The exudate E exhibited inhibition of the growth of two reference strains of gram-positive bacteria, *S. aureus* and *B. subtilis* (MICs of 50 $\mu g \cdot mL^{-1}$) and the exudate A inhibited the growth of only one reference strain, *B. subtilis* (MIC of 50 $\mu g \cdot m\,L^{-1}$).

Discussion

The result suggests that a low profile of macro- and micronutrients is effective for the species seed germination. This is consistent with the earlier report of Chamandoosti (2016), where among of different basal media that were evaluated for their effects on sesame seed germination, half strength MS was found to be the best medium for germination.

During the plants establishment in greenhouse, we note that plant status and size of the leaves of *in vitro* plantlets were higher in comparison to greenhouse plants (Fig. 2b, a, c). The difference in phenolic and flavonoid contents in the leaves reflected the difference in the morphological traits. Thus, the plantlets with more vigorous growth had lower flavonoid content. This corresponds with many reports about increase of flavonoids in the stress or unfavorable conditions (Karpova *et al.*, 2016).

On account to the significantly higher yield of flavonoids per plant and a shorter (by 2-3 months) growing cycle, the *in vitro* plantlets of *B. fischeri* var. *palustris* can be regarded as a promising source of flavonoids.

We showed that antimicrobial activity of the extracts of *B. fischeri* var. *palustris* varied with various flavonoid composition and content. The spectra of antimicrobial activity of the extracts of *in vitro* plantlets and the exudates were substantially reduced. At the same time, inhibition effect of the extracts on the growth of the strains of *B. subtilis* and *S. aureus* manifested in most cases. Unstable inhibition effects of the extracts of plants of *B. fischeri* var. *palustris* in relation to the strains of *E.*

coli, *E. faecalis* and *C. albicans* indicates that antimicrobial activity against these pathogens requires higher concentrations of flavonoids. Further works should be designed in order to assess the impact of concentration of the main constituents and some varying components of extracts (quercitrin, free aglycons and kaempferol glycosides) on the antimicrobial action.

Another direction of the study can be dealt with the selection of clones with high flavonoid content. Our results are in accordance with the reports on high flavonoid content of micropropagated plants (Rad *et al.*, 2014; Goda *et al.*, 2017), but the level can be increased.

Of great importance are compounds located on the leaf surface. Recently, these compounds are the objectives of new direction of investigations, the metabolic engineering concerning with *in vitro* synthesis and secretion of large amounts of target compounds by leaf surface structures. The works in this field increase, but at this point, they are still quite fragmentary (Huchelmann *et al.*, 2017). We showed that the flavonoid composition of exudates of *B. fischeri* var. *palustris* differs slightly from the composition of the leaves as a whole, but the concentrations of most of components are insignificant. This caused a weak antimicrobial activity of exudates. However, the direct contact of the flavonoids of the leaf surface with the air creates the base for antimicrobial properties sufficient to reduce a colony-forming units in the air. This reduction was previously shown in experiments with intact plants in kindergartens and libraries (Fershalova *et al.*, 2018). One of the further directions of the investigation should be the nutritious medium optimization to get a significant increase of exudative flavonoids amounts for high antimicrobial activity (Kim *et al.*, 2015).

Thus, in our study we established an efficient *in vitro* propagation and regeneration protocol of *B. fischeri* var. *palustris* plants, with rich flavonoid content, obtained from the seeds culture, for the aims of interior design and health benefits.

Conclusion

In our study, we developed the protocol of micropropagation and acclimatization of *in vitro* plantlets of *B. fischeri* var. *palustris* with high flavonoid

content in the leaves (13 mg·g^{-1} of dry weight) insignificantly differed from the leaves of the greenhouse plants and with higher yield of total flavonoids per plant (189.1 g in comparison with 174.0 g). The highest percentages of seed germination were revealed in the half strength MS medium (92.5%) and in 1% agar medium (83.3%). The extract of the leaves inhibited the growth of four from nine reference strains, *S. aureus* (MIC of 20 µg·mL^{-1}), *B. subtilis*, *S. aureus* and E. aerogenes (MICs of 50 µg·mL^{-1}). Concentrations of flavonoids in acetone and ethanol extracts of exudative compounds of the leaves of *in vitro* plantlets were 0.02 and 2.0 mg·g^{-1} of dry weight, respectively. These extracts inhibited the growth of one (*B. subtilis*) and two reference strains (*B. subtilis* and *S. aureus*). The *in vitro* derived *B. fischeri* var. *palustris* plants are promising for indoor design of children institution and public places and for air cleaning. The purpose of further investigation is the improvement of culture conditions for the production of plants with genetically stable high level of flavonoid content.

Funding Information

Authors' Contributions

Evgeniya A. Karpova: Designed the study, performed the HPLC, the statistical analyses and wrote the manuscript.

Alexandra Yu. Nabieva: Performed *in vitro* seed germination and the statistical analysis, maintained seedlings cultures, wrote the protocol and wrote the manuscript.

Tatiana D. Fershalova: Conducted the acclimatization of the regenerants and wrote the manuscript.

Yuliya L. Yakimova and Natalya V. Tsybulya: Performed the evaluation of antimicrobial activity. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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