Biotechnology for the production of *Iris spuria* L. plant material with a specified content of extractives

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**Abstract.** The study was aimed at the establishment of stock plant material of *Iris spuria* L. (Art And Soul) through biotechnological methods and its primary phytochemical analysis. For the first time, a series of studies aimed at multiplying plant materials *Iris spuria* L. (Art And Soul) resulted in the development of culture media and cultivation schemes. In the stage of micropropagation as such, the most optimal content in culture media was found to be 2.5-5.0 µm BAP. To ensure a more complete realization of morphogenetic potentials, it is necessary to alternate phytohormone and hormone-free media. In this case, L-glutamine and adenine sulfate should be added to hormone-free media in the amount of 100 mg/l (MS+100 mg/l L-glutamine+100 mg/l adenine sulfate). Qualitative and quantitative compositions of extractives removed in a Soxhlet apparatus from the technological plant material of *Iris spuria* depends on the solvent polarity. The amount of extractives increases with accelerated solvent polarity (96% ethanol → 60% ethanol → water). In this case, phenols, condensed and hydrolyzable tannins, alkaloids, glycosides were extracted. The study proves the relationship between the accumulation of quercetin and the hormonal composition of culture media, which allows for the regulation of these polyphenols being accumulated in the production of the plant material of *Iris spuria* L. (Art And Soul).

1. Introduction

The genus *Iris* L. is represented by medicinal and ornamental perennials. *Iris spuria* L. (false iris) is a perennial plant 30-90 cm tall. The flowering stem is slightly flattened, powerful, rounded, articulated, slightly branched, bearing from three to eight flowers. The leaves are 30-40 cm long, 1-2 cm wide, erect, slender, sword-shaped, acuminate, glaucous and have an unpleasant smell when rubbed. The bracts are acutely keeled, densely leather-like. The petals are 4-5 cm long and 6-8 cm in diameter, lilac or bluish-violet, with dark veining, without aroma, sessile or nearly sessile. It blooms in May and June. It flowers in July-August. The rhizomes are 1-1.5 cm thick. It is hydrophilous, but drought tolerant. It is native to Central and Eastern Europe, and Asia [1].

A promising ornamental and medicinal perennial plant of *Iris spuria* synthesizes a wide range of biologically active substances, such as isoflavones: tectorigenin, iristectorigenin A, 5,7-dihydroxy-6,2’-dimethoxyisoflavone; isoflavone glycosides: tectoridin, tectorigenin 4’-O-β-D-glucopyranoside, tectorigenin 4’-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside], tectorigenin 7-O-β-D-glucopyranosyl-4’-O-β-D-glucopyranoside, tectorigenin 7-O-β-D-glucopyranosyl-4’-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside], tectorigenin 7-O-β-D-glucopyranosyl-(1→6)-glucopyranoside, iristectorin A, iristectorin B, iristectorigenin B 4’-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside], genistein 7-O-β-D-glucopyranoside, maesopsin 6-O-β-D-glucopyranoside, 1,11-
dihydroxy-9,10-methylenedioxy-12a-dehydrorotenone; flavones and flavone glycosides: 5,7,3′-trihydroxy-6,4′-dimethoxy flvanone and isoscutellarin 6-C-β-D-glucopyranoside, as well as other polyphenols: Vanillic Acid 4-O-β-D-glucopyranoside, Lilac Acid 4-O-β-D-glucopyranoside, E-coniferin, tenuifodion [2-5].

The isoflavonoids tectorigenin and tectorin as well as their glycans have anti-inflammatory, anti-tumor, and hepatoprotective activity. Tectorigenin has antiproliferative effect, and can also be crucial in the prevention and treatment of diabetic complications [6-9].

The study aimed to establish the stock plant material of Iris spuria L. (Art And Soul) through biotechnological methods and its primary phytochemical analysis.

2. Material and methods

Tissue culture
The explants used for releasing were undissolved flower buds of Iris spuria, cultivar Art And Soul, provided by Z.V. Dolganova, Dr.sc.agr., principal researcher of M.A. Lisavenko Scientific-Research Institute of Horticulture, Federal Altai Scientific Center for Agrobiotechnology. The progeny and hydroponic plants were obtained and grown in the Department of Biotechnology of Altai State University in accordance with the developed recommendations [10, 11].

In the stage of in vitro cultivation, the following phytohormones were placed in the culture medium:

- a) cytokinin type of action:
  - 6-benzylaminopurine (BAP) Sigma, USA 1-8 μM,
  - α-benzylaminopurine (BAP) Sigma, USA 1.8 μM,

- b) auxin type of action:
  - α-naphthylacetic acid (NAA) Sigma, USA 1-5 μM,
  - indole-3-butyric acid (IBA) Sigma, USA 0.1 μM.

The phytohormones were placed in the culture medium consisting of MS medium [12] in relevant concentrations.

Sucrose at a concentration of 30 g/l was used as the main carbohydrate for the cultivation of organs and tissues. The pH of the medium was adjusted using 0.1 N. HCl and 0.1 N. KOH up to 5.0-5.8 prior to autoclaving. The conditions for autoclaving the culture media were as follows: pressure – 1.0 atm, temperature – 105-110 °C, and duration – 20 min.

The explants were grown in a culture room, where the temperature was maintained at 26-30 °C, a 16-hour photoperiod, and the illumination intensity was 2000–4000 lux. The shoots were subcultured after 30 days. Each experiment had 10 replications.

Methods of phytochemical analysis
An air-dry plant specimen was ground to a particle size able to pass through a sieve with a hole diameter of 1 mm. The biologically active compounds were sequentially extracted in a Soxhlet apparatus with petroleum ether, 96% ethanol, 60% ethanol, and water.

The extract reactions were performed in accordance with the methodological recommendations of R.A. Muzychkina, et al. (2011) [13].

The content of extractives in the extracts was calculated in accordance with general pharmacopeial monograph of State Pharmacopoeia of Russian Federation XIV ed., Vol. 2, 2018 [14], taking into account the humidity. The latter was determined on an MX-50 moisture analyzer at a temperature of 105 °C. The extractives were evaporated in a porcelain cup (pre-adjusted to constant weight). A technique was used to quantify the content of flavonoids in the extracts, based on their ability to form a colored complex with AlCl₃ solution [15].

3. Results and discussion

Establishment of plant material Iris spuria
In the stage of micropropagation as such, Iris spuria was cultured in the media containing 2.5, 5.0, 7.5, and 10.0 μM BAP, and the media containing the same amount of cytokinin supplemented with auxins
1.0 μM NAA and 0.1 μM IBA. There were 9 options used for the experience. A culture medium containing 1 μM BAP was used as a control. Without phytohormone, plants do not commonly grow in tissue culture. During the cultivation of *Iris spuria*, the number of microprobe shoots formed and the height of the plant were determined. Unlike *Iris sibirica* [10], false iris is less demanding on the content of BAP. The most optimal was the BAP content of 2.5-5.0 μm. To ensure a more complete realization of the morphogenetic potentials of *Iris spuria* in the stage of micropropagation as such, it is necessary to alternate between the phytohormone and hormone-free media. Besides, L-glutamine and adenine sulfate should be added to hormone-free media in the amount of 100 mg/l (MS+100 mg/l L-glutamine+100 mg/l adenine sulfate). This cultivation scheme allows the propagules to produce multiple adventive and axillary buds, and in the next passage, with a decreased hormone burden, the buds have the opportunity to develop into shoots. The content of hormone-free growth stimulants of L-glutamine and adenine sulfate in the culture medium has a positive effect on the quality of developing shoots and their ability to root. It is necessary to strictly observe the alternation of media, otherwise, an excessive amount of BAP is accumulated in the tissues of propagules, which results in a reduced multiplication factor, shoot growth prevention and, ultimately, plant death. To root *Iris spuria*, a medium containing 3 μM NAA was used.

The plants were removed from agar media. The residual agar was rinsed in running water and placed in cassettes to adapt to non-sterile conditions for 30 days in an aeroponic installation. Once adapted and grown up to 30 cm, the plants were transferred to the next layer of the installation with a less dense plant population (Fig. 1). The plants were grown in an aeroponic system in a cultivation room equipped with a climate control unit. Water and mineral nutrition were supplied through periodical injections of a nutrient solution (under a pressure of 3 atm), thus irrigating the root system of the plants. Between the supplies, the roots were aerated.

Plant materials produced on the basis of microclonal propagation and cultivation in aeroponics avoid a number of problems. Biomass in the conditions of aeroponics is not affected by pesticides, heavy metals, and contamination by microorganisms. Deliberate or erroneous species adulteration is impossible. Constant growing conditions do not entail changes in the chemical composition, which makes it possible to standardize this type of plant material.

**Phytochemical analysis**

The content of extractive substances in medicinal plant materials is an important numerical indicator of adequate quality. Resulting from sequential extraction of biotechnological materials of *Iris spuria* in the Soxhlet apparatus with various solvents, the plant materials were found to contain a small amount of substances recovered by petroleum ether (4.8%), much more – by 60% ethanol (27.9%) and water (25.6%). A fraction removed by 96% ethanol (46.5%) was dominant.
Subject to a chemical composition of the medicinal plant material and the solvent used, certain active and related substances were extracted. A qualitative analysis of the multiple fractions for the main groups of biologically active substances resulted in the following features: petroleum ether – phenols, condensed tannins, alkaloids; 96% ethanol – phenols, condensed and hydrolyzable tannins, alkaloids, glycosides; 60% ethanol – phenols, condensed and hydrolyzable tannins, xanthones, alkaloids, glycosides; water – phenols, condensed and hydrolyzable tannins, alkaloids.

The amount of flavonoids was quantitatively determined in terms of quercetin in plant materials of *I. spuria* subject to a hormonal composition of the culture medium.

Quercetin and its rutin glycoside are among the most well-known and well-studied flavonols that are widely distributed in the plant community. Young, growing organs are richest in flavonoids. The flavonoids accumulate due to an environment rich in nitrogen, potassium and phosphorus. In the southern and alpine regions, the impact of light and soils full of trace elements favour the increased content of flavonoids [16]. The MS medium contains a comprehensive and balanced set of macro and micro salts. The plants were grown in containers with increased humidity at the optimum temperature and illumination. The authors evaluated the effect of BAP concentration and the presence of auxins as necessary factors for the successful accumulation of quercetin in the tissues of *I. spuria* offspring. The study established the relationship between the flavonoids accumulated and the amount of BAP content. With an increase in the concentration of phytohormone from 1.0 to 10.0 μM, the content decreased. The introduction of auxins almost had no effect on the accumulation of flavonoids (Table 1).

**Table 1. Quercetin content in biotechnological materials of *I. spuria* vs hormonal composition of culture media**

| Experiment No. | MS-based hormone composition | Quercetin dry mass, % |
|---------------|------------------------------|----------------------|
| Control       | 1.0 μM BAP                   | 2.50±0.06            |
| 2/1           | 2.5 μM BAP                   | 1.20±0.08*           |
| 2/2           | 2.5 μM BAP +1.0 μM NAA + 0.1 μM IBA | 1.50±0.04*         |
| 3/1           | 5.0 μM BAP                   | 1.30±0.03*           |
| 3/2           | 5.0 μM BAP + 1.0 μM NAA + 0.1 μM IBA | 1.10±0.02*         |
| 4/1           | 7.5 μM BAP                   | 1.30±0.06*           |
| 4/2           | 7.5 μM BAP + 1.0 μM NAA + 0.1 μM IBA | 1.20±0.05*         |
| 5/1           | 10.0 μM BAP                  | 0.90±0.04*           |
| 5/2           | 10.0 μM BAP + 1.0 μM NAA + 0.1 μM IBA | 0.80±0.03*         |

Note. * is the difference with control is significant at a 5% significance level

**4. Conclusion**

For the first time, a series of studies aimed at multiplying plant materials of *Iris spuria* L. (Art And Soul) resulted in the development of culture media and cultivation schemes. In the stage of micropropagation as such, the most optimal content in the culture medium was found to be 2.5-5.0 μM BAP. To ensure a more complete realization of morphogenetic potentials, it is necessary to alternate phytohormone and hormone-free media. In this case, L-glutamine and adenine sulfate should be added to hormone-free media in the amount of 100 mg/l (MS+100 mg/l L-glutamine+100 mg/l adenine sulfate). A universal aeroponic installation can be used to adapt offspring to non-sterile conditions and to multiply stock plant material.

Qualitative and quantitative compositions of the extractives removed in a Soxhlet apparatus from the biotechnological plant material of *Iris spuria* depends on the solvent polarity. The amount of extractives increases with accelerated polarity (96% ethanol → 60% ethanol → water). Phenols, condensed and hydrolyzable tannins, alkaloids, glycosides are extracted.
The study proves the relationship between the accumulation of quercetin and the hormonal composition of culture media, which allows for the regulation of these polyphenols being accumulated during the production of the plant material *Iris spuria* L. (Art And Soul).

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