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CHANGES IN ROSMARINIC ACID CONTENT IN *SALVIA ABROTANOIDES* AND *S. YANGII* CALLUS CULTURES UNDER LED LIGHT AND GROWTH REGULATOR TREATMENTS

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Running title: *Białas et al. …… RA in* S. abrotanoides *and* S.yangii *callus cultures*

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Salvia yangii (B.T.Drew) and *Salvia abrotanoides* (Kar.) Sytsma are perennial shrubs native to central-west Asia. Previous studies have highlighted the distinct metabolic profiles of these species, although little is known about the phytochemical profile of their tissue cultures. This study presents the first report on callus cultures of *S. yangii* and *S. abrotanoides* derived from various plant organs such as root, cotyledon, hypocotyl, petiole and leaf. The effect of two types of light and the supplementation of plant growth regulators (PGRs) on growth and metabolic profile was investigated. Specifically, white light-emitting diodes (LEDs) and a mix of blue and red LEDs in a 15:85 ratio were used, alongside kinetin or 6-

benzyloaminopurin (1.0 μM), 1-naphtalenieacetic acid (1.0 μM), and 2,4-dichlorophenoxyacetic acid (0.5 μM). The UHPLC-DAD analysis showed rosmarinic acid (RA) as the predominant compound in extracts. Culture conditions significantly influenced the RA content, as well as the levels of total phenolics, flavonoids, and DPPH radical scavenging activity. The study provides new insights into the tissue cultures of *S. yangii* and *S. abrotanoides*, enriching the current knowledge of these valuable species.

Keywords: rosmarinic acid, *Salvia yangii*, *Salvia abrotanoides*, tissue culture

INTRODUCTION

Plant tissue culture is a substantial tool for studying secondary metabolism and optimizing the production of valuable specialized metabolites. Various environmental factors, including plant growth regulators (PGRs) and light conditions, may significantly influence metabolic pathways, affecting both biomass accumulation and metabolite content (Cavallaro et al., 2022; Espinosa-Leal et al., 2018). Light in particular, as a crucial environmental signal, regulates plant growth and metabolism through complex signaling pathways that interact with hormonal regulation (Batista et al., 2018). These interactions involve key transcription factors such as phytochrome interacting factors (PIFs), which play central roles in photomorphogenesis and metabolic regulation (Cordeiro et al., 2022).

Previous studies have demonstrated that light quality affects the biosynthesis of phenylpropanoids, by modulating enzyme activity within the metabolic pathway (Nazir et al., 2020). While certain wavelengths, such as blue and red light, have been linked to enhanced phenolic and flavonoid accumulation, the precise mechanisms and their interaction with PGRs remain insufficiently understood, particularly in undifferentiated plant tissue cultures.

Tissue cultures serve as a valuable source of specialized metabolites, though not all compounds can be efficiently obtained through this method. Undifferentiated plant cells often accumulate metabolites in quantities that differ significantly from those found in mature plants (Efferth, 2019). Moreover, metabolites produced in tissue cultures are generally easier to extract than those from fully developed organs, simplifying production and isolation processes (Espinoza-Leal et al., 2018). Additionally, high yield cultures maintained under controlled conditions free from seasonal variations, environmental fluctuations, and pollution risks, offer a reliable and sustainable source of bioactive compounds (Cavallaro et al., 2022; Espinosa‑ Leal et al., 2018; Skała et al., 2014). Non-transgenic approaches, such as optimizing light spectra and supplementing growth regulators, provide a cost-effective and efficient strategy to enhance metabolite production, making tissue culture a promising tool for biotechnological applications (Hazrati et al., 2022; Nazir et al., 2020; Anjum et al., 2017).

In the present research, two *Salvia* species (Lamiaceae) viz. *S. yangii* (B.T.Drew) and *S. abrotanoides* (Kar.) Sytsma were used. Both plants are perennial subshrubs native to Central Asia, Pakistan, Afghanistan, and Iran. Until 2017, both species were classified within the small genus *Perovskia*, under the names: *P. atriplicifolia* (Benth.) for *S. yangii* and *P.*

abrotanoides (Karel.) for *S. abrotanoides*. Traditionally, they have been used to treat stomachache, vomiting, skin allergies, rheumatic pain, and fever (Ahmed et al., 2021). Previous studies involving phytochemical analyses of field-grown *S. yangii* and *S. abrotanoides* plants have revealed a rich profile of secondary metabolites, particularly polyphenols, with rosmarinic acid (RA) being the most abundant (Stafiniak et al., 2021). RA, an ester of caffeic and 3,4-dihydroxyphenyllactic acid, is predominantly found in plant species belonging to the Boraginaceae and Lamiaceae families. This bioactive compound possesses strong antioxidant, anti-inflammatory, antimutagenic, and antibacterial properties, making it highly valuable in the pharmaceutical, cosmetic, and food industries (Marchev et al., 2021). RA biosynthesis can occur in undifferentiated plant cells, sometimes yielding even higher concentrations than those found in the mother plants. Non-transgenic approaches have been successfully employed to develop callus cultures with high RA yields in various species, including *Salvia officinalis* (Petersen and Simmonds, 2003; Karam et al., 2003), *Ocimum basilicum* (Nazir et al., 2019), *Agastache rugosa* (Zielińska et al., 2020), and *Coleus blumei*; (Qian et al., 2007).

As mentioned before, light is a fundamental factor of plant growth and development, playing a crucial role in regulating metabolism. With the widespread adoption of LED technology, numerous studies have explored the impact of light spectra on plant metabolism. These investigations have focused on both specific wavelengths and multispectral monochromatic irradiance, highlighting the influence of light quality on metabolic processes (Khurshid et al., 2020; Zielińska et al., 2019). There are several reports regarding the use of LEDs for improving the phytochemical composition of medicinal plants, including both *Salvia* sp. (Ghaffari et al., 2019). However, most of them evaluate how LED light affects plants in pots. Conversely, this study investigated the application of LEDs for *in vitro* improvement of rosmarinic acid in *Salvia abrotanoides* and *S. yangii*.

Therefore, we established tissue cultures of *S. yangii* and *S. abrotanoides* to investigate the effects of PGRs and light conditions on biomass accumulation and specialized metabolite production. The combination of treatments was the most successful in reaching the desired goal (biomass accumulation and specialized metabolite production). By exploring the interplay between light signaling and hormonal regulation, this research provides new insights into optimizing tissue culture conditions for enhanced metabolite accumulation, offering a valuable approach for biotechnological applications in plant-based RA production.

MATERIALS AND METHODS

PLANT MATERIAL

Callus cultures were obtained from explants of aseptically germinated *S. yangii* and *S. abrotanoides* seedlings. Seeds of both *Salvia* species were collected from the Botanical Garden of Medicinal Plants in Wroclaw (17° 04′ 27″ E, 51° 07′ 03″ N, altitude 117 m a.s.l.) (under the approval of the Ministry of Environment, Republic of Poland, Decision No. DOPogiz-4210-26-6024-/05/kl) and used to obtain tissue cultures. Surface sterilized seeds (20 min, 5% sodium hypochlorite) were washed three times in sterile water (5 min) and sowed on an agar-solidified MS medium (6g/L). Four-week-old seedlings were cut, and explants of

root, hypocotyl, cotyledon, leaf blade, and petiole were placed on culture media supplemented with plant growth regulators (PGRs). Two cytokinins were used, 6-benzylaminopurine (BAP) or kinetin (KIN) at the concentration of 1.0 μM, and in a combination with 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at the concentration of 1.0 and 0.5 μM, respectively. Callus cultures were transferred on fresh media every four weeks. Data were collected on callus culture diameter, fresh and dry biomass weights, as well as water content.

Callus cultures were maintained in growth chambers at 25±2°C, under LED light of different wavelengths: white (W:400-700 nm; 8000-10,000K) or mix of blue (B) and red (R) light in 15:85 ratio (photosynthetic radiation range, PAR). The following light lengths were used: blue - 430 nm 5%, and 460 nm 10%; red - 610 nm 10%, 630 nm 35%, 660 nm 35% , and 730 nm 5%. The photoperiod was set at 16/8h day/night.

PHYTOCHEMICAL ANALYSIS

Extraction. Phytochemical analysis was performed for plant material harvested after 6 months of culture. Callus obtained in different culture conditions was dried at 35°C until the constant weight and micronized using a mortar and a pestle. Accurately weighed samples of 50 mg were extracted with 3 mL of methanol 80% (v/v), sonicated (15 min at room temperature), and centrifuged (5000 rpm for 15 min). Supernatants were collected, and the plant material was re-extracted following the same procedure. Combined supernatants were dried at 35°C under the nitrogen flow, and the obtained residues were dissolved in 1 mL of 80% methanol (v/v), filtered (nylon, 0.22 um), and immediately analyzed.

UHPLC analyses. Analysis was performed using UHPLC (Thermo Scientific™ Dionex™ UltiMate™ 3000) equipped with a photodiode array detector (UV, DAD-3000). Separation was carried out using a C-18 column (3 um; 150 x 4.6 mm) (Dr. Maisch GmbH, Germany) at 30°C in a gradient flow rate of 0.4 mL min^{-1}. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% of formic acid in acetonitrile). The program began with 5% B and increased to 60% B (0-9 min), then to 98% B (9-10 min), held isocratic at 98% B for 2 min, returned to the initial composition of 5% B (12-13 min) and held constant to re-equilibrate (2 min).

Identification was performed by comparing retention times (RT) and UV spectra with the rosmarinic acid authentic standard (Sigma-Aldrich). Detection was carried out at 280 nm and 320 nm, while quantitative analysis was performed at 280 nm. Quantification of compounds was based on calibration equation y= 0.0947x+33.2716 with the coefficient of determination R^2=0.994.

Phenolic and flavonoid contents. Total phenolic contents (TPC) were evaluated using the spectrophotometric method with Folin-Ciocalteau (FC) reagent, according to Khurshid et al. (2020). Diluted callus tissue extract (20 μL) was mixed with FC reagent (90 μL) (POCH) and 5% Na_2CO_3 (100 μL) in 96-well microplates. After 10 minutes of incubation at room temperature, the absorbance was measured at 630 nm wavelength (BioTeq, μQuantTM, Microplate Spectrophotometer). Gallic acid was used as a reference standard, and the total phenolic content was expressed as gallic acid equivalents (GAE) per 1g of callus tissue DW.

Total flavonoids content (TFC) was estimated using the aluminium chloride colorimetric method according to Kurshid et al. (2020) with modifications. Callus tissue extract (20 μL)

was mixed with 10% $AlCl_3$ (10 µL), CH_3COONa (1M; 10 µL) and filled with distilled water up to 200 µL. Analysis was performed using 96-well microplates at 415 nm after 30 min of incubation (BioTeq, µQuantTM, Microplate Spectrophotometer). Rutin (Fluka) was used as a reference standard, and flavonoid content was expressed as rutin equivalents (RE/g) per 1g of callus tissue DW.

DPPH assay. The assay was performed using 2,2-diphenyl-1-picrylhydrazyl reagent (DPPH) (Sigma-Aldrich) according to Abbasi et al. (2010). To determine the antioxidant activity of the obtained callus extracts, 20 µL of diluted samples was mixed with 160 µL of DPPH (0.2 mM) in a 96-well microplate and incubated for 1 h at room temperature in the dark. The absorbance was read at 517 nm (BioTeq, µQuantTM, Microplate Spectrophotometer), and the free radical scavenging activity was calculated based on a formula: DPPH % = 100 x (1-AE / AD), where AE is the absorbance of extract mixed with DPPH reagent after 1 h of incubation, whereas AD is the absorbance of DPPH without extract.

Statistical analysis. All results were subjected to one-way analysis of variance (ANOVA). The significant differences between probes were determined using Tukey's test at $p<0.05$ level. STATISTICA 12.0 (StatSoft Inc., Poland) was used to accomplish statistical analyses. The relative contribution analysis was conducted to assess the effect of light, PGR combinations, plant species and the type of explant, as well as their interactions on the content of RA, TPC, TFC and radical activity.

RESULTS

All explant types of *S. yangii* and *S. abrotanoides* developed callus cells (Table 1).

Tissue growth and biomass accumulation of both *Salvia* species were influenced by light spectra, PGRs, and the type of explant (Table 1). All callus cultures were orange-brownish with slight differences in color intensity (Fig. 1). The cultures were friable, highly hydrated, and contained 92.01-97.91% water (Table 1).

The most intensive growth of *S. yangii* was observed with KIN under PAR light, whereas on BAP it grew more efficiently under white light. In turn, *S. abrotanoides* exhibited better growth on BAP-supplemented media under PAR light, while KIN promoted greater growth under white light. Regarding explant type, the highest growth was observed in cultures initiated from the petiole in *S. yangii*, while in *S. abrotanoides* the greatest growth occurred in cotyledon-derived cultures (Table 1).

RA CONTENT

UHPLC-DAD analyses showed that rosmarinic acid was the predominant compound in callus cultures of both species, accounting for the majority of substances detected in the methanolic extract. The RA content ranged from 0.44 to 56.65 mg/g DW in *S. abrotanoides* and from 7.71 to 56.35 mg/g DW in *S. yangii* (Table 2). Higher concentrations were detected in cultures grown on KIN-supplemented media compared to BAP and under PAR light rather than white light. Among the explant types, root-derived cultures produced the highest amount of RA, whereas leaf-derived cultures were less efficient, regardless of the growth conditions.

PHENOLIC AND FLAVONOID ACCUMULATION

The total phenolic content (TPC) and total flavonoid content (TFC) were measured using a colorimetric method with gallic acid and rutin as standard reference substances. The results indicated significant variations in TPC accumulation depending on the species, light conditions, and the type of PGR used (Table 3). For *S. yangii*, the highest TPC levels were observed when cultured on KIN under PAR light conditions, while the lowest were found on BAP-supplemented media under the same lighting. In contrast, for *S. abrotanoides*, the highest polyphenolic content was achieved when grown on KIN-supplemented media under white light.

Similarly, for flavonoid content, *S. yangii* showed the highest accumulation when cultured on KIN under PAR light, whereas the lowest content was observed under white light (Table 3). Relatively high flavonoid levels were found in leaf-derived callus of this species. For *S. abrotanoides*, flavonoid accumulation did not depend on the explant type and varied significantly under different culturing conditions, with the highest content observed in root-derived callus grown on KIN under white light.

DPPH RADICAL SCAVENGING ACTIVITY

Radical scavenging activity (RSA) for both species is shown in Fig. 2. *S. yangii* callus cultures grown on KIN-supplemented media exhibited the highest RSA, while the lowest activity was found in extracts from *S. abrotanoides* tissue cultures grown on BAP-supplemented media under white light. Interestingly, in *S. abrotanoides*, RSA varied significantly between hypocotyl-induced callus, with the lowest activity observed under the PAR light in the presence of KIN and BAP as well as under white light conditions with BAP in the culture media. The highest RSA was observed in cultures grown on KIN-supplemented media under white light.

STATISTICAL ANALYSIS

The relative contribution analysis (Table 3) revealed that PGR treatment had the most significant influence on RSA (42.8%), while the effect of light was comparatively lower (ranging from 5.8-1.5%). However, light conditions had a nearly fourfold greater impact on TPC and RSA than on TFC and DPPH activity. Surprisingly, when examining each factor separately, the type of explant had a greater influence on RA and TPC than the species itself. Overall, the analysis showed that the combination of species, light conditions, and PGR significantly influenced RA, TPC, TFC, and DPPH activity.

DISCUSSION

In the present study, we established tissue cultures from different organs of *S. yangii* and *S. abrotanoides* and investigated the effects of two light conditions (PAR and white) and exogenous PGR supplementation on their growth and metabolite content. Despite the close phylogenetic relationship between the two species, tissue cultures of each grew most efficiently under distinct light regimes and with a different PGR combination (Table 1). Additionally, the metabolite content varied significantly depending on the culturing

conditions (Tables 2, 3). To the best of our knowledge, no studies have specifically explored the impact of light and PGRs on *S. yangi* and *S. abrotanoides* tissue cultures, although a few reports have addressed *S. abrotanoides* callogenesis potential (Ghaderi et al., 2019; Zaker et al., 2013). No data on their metabolic profiles are currently available.

RA was the most abundant compound found in the tissue cultures of the studied species, with its concentration primarily influenced by the type of PGR used. In this study, KIN had more significant effect on secondary metabolite accumulation compared to BAP. The effect of PGRs is species-specific, and can even vary among cultivars of the same species. Among cytokinins, BAP is often noted for its strong influence on plant secondary metabolites. Previous studies have reported its ability to enhance polyphenolic compounds (Weremczuk-Jeżyna et al., 2018), flavonoids (Al-Hawuamdeh et al., 2013), isoflavonoids (Łuczkiewicz et al., 2014), and iridoid glycosides (Piątczak et al., 2015) in shoot or callus cultures grown *in vitro*. In contrast, in suspension cultures of *Agastache rugosa*, the effects of KIN and BAP on RA content were comparable, reaching 9.6-10.4 and 9.7-10.7 mg/g DW, respectively, across a PGR concentration range of 0.01-1mg/L. Moreover, various PGRs may influence the accumulation of different compounds within the same culture. For example, in *Chelidonium majus*, KIN promoted protopine accumulation, while BAP stimulated sanguinarine production (Zielińska et al., 2018).

Light plays a crucial role in regulating metabolic processes in plants. It not only provides the energy necessary for photosynthesis, but also conveys environmental signals. The blue and red-light spectra are particularly important due to their absorption maxima by chlorophyll, which are key to photosynthesis. Blue light has been shown to enhance the synthesis of photosynthetic pigments, increase Rubisco activity, and promote the efficiency of the photosynthetic electron transport chain (Chen et al., 2023; Wang et al., 2015). In contrast, red light has been associated with limited CO_2 fixation and reduced plant biomass (Brown et al., 1995). Although light signals are received by various independent receptors, these receptors interact and form a complex, interdependent system (Demotes-Mainard et al., 2016).

In the presented studies, biomass accumulation was primarily influenced by species, while light conditions had no statistically significant impact (Table 3). However, previous observations in other species suggest that a combination of blue and red light can enhance callus accumulation. For instance, *Hyoscyamus reticulatus* exhibited optimal growth under a blue-red ratio of 25%:75% (Hassanpour, 2021), while *Panax vietnamensis* responded best to a 40:60 blue-red ratio (Nhut et al., 2015). Conversely, *Ocimum basilicum* callus culture showed the most efficient growth under blue light (Nazir et al, 2020), whereas *Rhodiola imbricata* responded best under red light (Kapoor et al, 2018).

The levels of specific metabolites can also be significantly influenced by light. Blue light has been shown to stimulate the expression of phenylalanine-ammonia-lyase (PAL), a key enzyme in the phenylpropanoid biosynthetic pathway. This effect has been demonstrated in several studies on rosmarinic acid biosynthesis (Ahmadi et al., 2019). Similarly, Manivannan et al. (2015) reported an increase in phenolic content under blue light, while flavonoid accumulation was enhanced under red light in *Rehmania glutinosa* shoot cultures. This phenomenon is attributed to phytochrome mediated activation of flavonoid synthesis as protective response to light induced damage (Manivannan et al., 2015).

The combined effects of light and PGRs follow an additive regulatory pattern, with light signalling pathway interacting with hormonal regulation through shared integrators. Key components of this process include phytochrome interacting factors (PIFs), elongated hypocotyl 5 (HY5), ethylene insensitive 3 (EIN3), and DELLA proteins (Liu et al., 2017, Lau et al., 2010). These factors play a crucial role in promoting photomorphogenesis. Among them, HY5 is particularly significant in interacting light and hormonal signalling pathways. Light and cytokinins, by modulating ubiquitin E3 ligase activity, promote HY5, thereby facilitating the transition to photomorphogenesis. However, little is known about how this mechanism interacts with light specific spectra, and even less about its role in undifferentiated tissue cultures. This knowledge gap highlights the need for further research to elucidate the underlying mechanisms.

CONCLUSIONS

In this study, we successfully established tissue cultures of *S. yangii* and *S. abrotanoides* and analyzed the accumulation of specialized metabolites in both species. Both *Salvia* species grown on KIN-supplemented media proved to be a rich source of rosmarinic acid. Their vigorous biomass accumulation and high RA content suggest that they could serve as a valuable source of this compound. To the best of our knowledge, this is the first report to examine specialized metabolite content in tissue cultures of *S. yangii* and *S. abrotanoides*, highlighting the potential of PGR and light treatments as effective strategies for enhancing metabolite production in these species.

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TABLE 1. *S. yangii* and *S. abrotanoides* callus growth parameters ± SD after 42 days of culture on MS medium supplemented with BAP or KIN [1μM] in combination with NAA[1μM] and 2,4-D [0.5 μM] under LED white and PAR illumination. R-root; H-hypocotyl, P-petiole, L-leaf, C-cotyledon.

				Diameter [cm]	FW [g]	Mean biomass growth	DW [g]	Water content in FW [%]
S. yangii	WHITE	KIN	R	1.64±0.28	7.84±3.25	1.25±0.54	0.32±0.09	95.00
			H	1.89±0.36	9.63±2.80	1.84±1.33	0.4±0.14	95.68
			P	2.01±0.14	7.39±2.62	1.37±1.05	0.38±0.1	96.19
			L	1.99±0.22	6.35±1.84	1.24±0.79	0.37±0.08	95.63
			C	2.49±0.25	15.68±1.24	2.6±0.96	0.61±0.04	96.58
		BAP	R	2.91±0.44	12.65±8.38	2.14±0.96	0.58±0.31	96.82
			H	1.80±0.18	3.33±1.27	0.69±0.39	0.22±0.14	94.40
			P	2.34±0.46	8.26±1.92	1.36±0.36	0.35±0.15	95.52
			L	2.24±0.23	10.71±2.70	1.47±0.18	0.44±0.13	95.66
			C	2.66±0.42	10.17±0.19	2.05±0.62	0.4±0.04	96.09
	PAR	KIN	R	2.19±0.43	15.56±30	2.58±1.31	0.48±0.31	96.33
			H	1.83±0.35	9.7±3.12	1.4±0.44	0.36±0.14	95.59
			P	2.23±0.18	15.85±6.78	2.24±1.17	0.44±0.15	95.98
			L	1.90±0.33	9.08±5.57	1.87±1.14	0.35±0.13	95.53
			C	2.34±0.30	9.33±3.36	2.03±0.74	0.58±0.09	94.97
		BAP	R	1.99±0.35	3.94±1.52	0.71±0.18	0.16±0.14	94.57
			H	2.03±0.13	6.55±3.50	1.23±0.12	0.15±0.1	95.35
			P	1.73±0.31	5.47±0.43	0.89±0.31	0.29±0.08	94.93
			L	1.28±0.07	1.83±1.05	0.23±0.09	0.07±0.04	92.01
			C	1.56±0.21	5.56±2.05	0.97±0.69	0.25±0.31	93.24
S. abrotanoides	WHITE	KIN	R	1.96±0.25	7.54±3.56	1.3±0.41	0.37±0.1	95.37
			H	2.59±0.34	15.35±3.77	2.51±1.11	0.48±0.21	96.93
			P	2.01±0.27	7.67±1.89	1.37±0.53	0.38±0.01	95.91
			L	2.46±0.16	15.68±5.51	2.47±0.62	0.52±0.12	97.20
			C	2.14±0.40	8.98±2.97	1.23±0.14	0.37±0.12	95.46
		BAP	R	1.93±0.23	7.11±3.60	0.99±0.15	0.13±0.1	95.73
			H	2.16±0.28	8.95±1.61	1.32±0.29	0.33±0.21	96.91
			P	1.76±0.26	3.1±1.66	0.54±0.06	0.08±0.01	93.16
			L	1.66±0.35	6.62±1.45	0.75±0.2	0.27±0.12	95.01
			C	2.16±0.39	5.88±3.63	1.58±0.12	0.24±0.12	95.52
	PAR	KIN	R	2.60±0.26	14.87±1.97	2.47±0.74	0.49±0.01	96.80
			H	1.73±0.26	5.33±1.18	1.09±0.51	0.28±0.12	94.68
			P	1.80±0.28	12.18±6.76	2.34±2.48	0.24±0.1	94.60
			L	2.31±0.35	16.24±3.96	2.61±1.41	0.51±0.1	96.08

		C	1.88±0.17	8.46±3.85	2.16±2.15	0.27±0.21	94.55
		R	2.46±0.29	13.41±2.68	2.16±0.23	0.52±0.01	96.63
		H	2.18±0.46	10.22±5.06	1.4±0.45	0.48±0.12	96.46
	BAP	P	2.16±0.68	7.26±5.98	1.02±0.69	0.51±0.12	96.38
		L	2.38±0.44	10.27±7.57	1.41±1	0.5±0.15	96.88
		C	2.73±0.51	27.45±7.10	2.85±0.16	0.68±0.2	97.91

TABLE 2. Rosmarinic acid content in *S. yangii* and *S. abrotanoides* callus cultures mg/g DW ±SD. R-root; H-hypocotyl; P- petiole; C-cotyledon; L-leaf. Values presented as means of triplicate measurements ± SD.

	S.yangii				*S.abrotanoides*			
Explant	White		PAR		White		PAR	
	KIN	BAP	KIN	BAP	KIN	BAP	KIN	BAP
R	14.12±0.85	42.49±1.18	56.35±0.97	7.71±0.30	46.68±0.78	1.24±0.12	53.23±1.96	39.05±3.89
H	21.04±1.14	22.01±0.29	41.77±1.54	34.18±0.75	51.22±0.97	0.44±0.26	12.70±0.78	8.94±1.94
P	42.87±0.74	14.60±2.03	50.03±1.50	15.43±0.28	40.16±3.15	5.99±0.03	36.51±1.20	20.50±2.65
L	32.96±0.58	36.01±0.11	38.36±2.72	10.83±0.94	30.84±5.96	4.90±0.93	38.28±1.20	39.60±1.42
C	55.74±0.38	27.46±1.54	50.54±0.95	12.07±0.32	21.19±2.21	26.56±1.96	20.86±3.40	56.65±1.06

TABLE 3. Relative contribution of total variance. Variables: light conditions, PGR and type of explant and their relation to RA, TPC, TFC and DPPH results from *S. yangii* and *S. abrotanoides* callus cultures; p>0.05 was marked by *.

Source of variation	**FW**	**RA**	**TPC**	**TFC**	**DPPH**
Species	4.3%	0.8%	0.3%	2.6%	2.5%
Light conditions	1.2%*	5.4%	5.8%	1.5%	1.6%
PGR	0.3%*	42.8%	10.0%	27.2%	26.3%
Type of explant	1.5%	2.2%	2.3%	2.1%	1.5%
Species × light conditions	10.7%	1.9%	5.5%	2.9%	3.7%
Species × PGR	1.4%*	2.2%	13.5%	1.0%	0.6%
Species × type of explant	4.6%	1.0%	0.8%	1.1%	1.4%
Light conditions × PGR	0.7%*	0.1% *	8.3%	0.6%	0.9%
Light conditions × type of explant	2.5%	0.8%	2.7%	1.8%	1.6%
PGR × type of explant	2.4%	1.5%	3.7%	8.9%	6.8%
Species × light conditions × PGR	58.8%	24.6%	39.1%	37.5%	41.5%
Species × light conditions × type of explant	3.9%	5.4%	2.4%	3.2%	4.8%
Species × PGR × type of explant	2.6%	6.7%	3.1%	4.4%	3.8%
Light conditions × PGR × type of explant	4.8%	2.9%	1.3%	2.8%	1.7%
Species × light conditions × PGR × type of explant	0.4%*	1.6%	1.2%	2.4%	1.3%

FIGURES

Fig. 1. Callus cultures of *S. abrotanoides* (A-D) and *S. yangii* (E-H) after four weeks of culture on: A, E - PAR BAP; B, F - PAR KIN; C, G - white BAP; D, H - white KIN; scale bar: 5 mm.

Fig. 2. UHPLC analysis of *S. yangii* callus tissue extract. A. An UHPLC chromatogram at 280 nm; B. a UV spectrum of the main compound corresponding to rosmarinic acid (RT=8.073 min); C. 2D-DAD chromatogram of extract.

Fig. 3. The total phenolic content (TPC) of *S. yangii* and *S. abrotanoides* callus cultures extracts as GAE mg/g DW. Statistical significance of differences was estimated using Tukey's test at $p < 0.05$. Different lowercase letters indicate significant differences. n=8

Fig. 4. The total flavonoid content (TFC) of *S. yangii* and *S. abrotanoides* callus cultures extracts as RE mg/g DW. Statistical significance of differences was estimated using Tukey's test at $p < 0.05$. Different lowercase letters indicate significant differences. n=8

Fig. 5. DPPH radical scavenging activity of callus cultures derived from different explants of *S. yangii* and *S. abrotanoides*. Statistical significance of differences was estimated using Tukey's test at $p < 0.05$. Different lowercase letters indicate significant differences. n=8

Figure 1

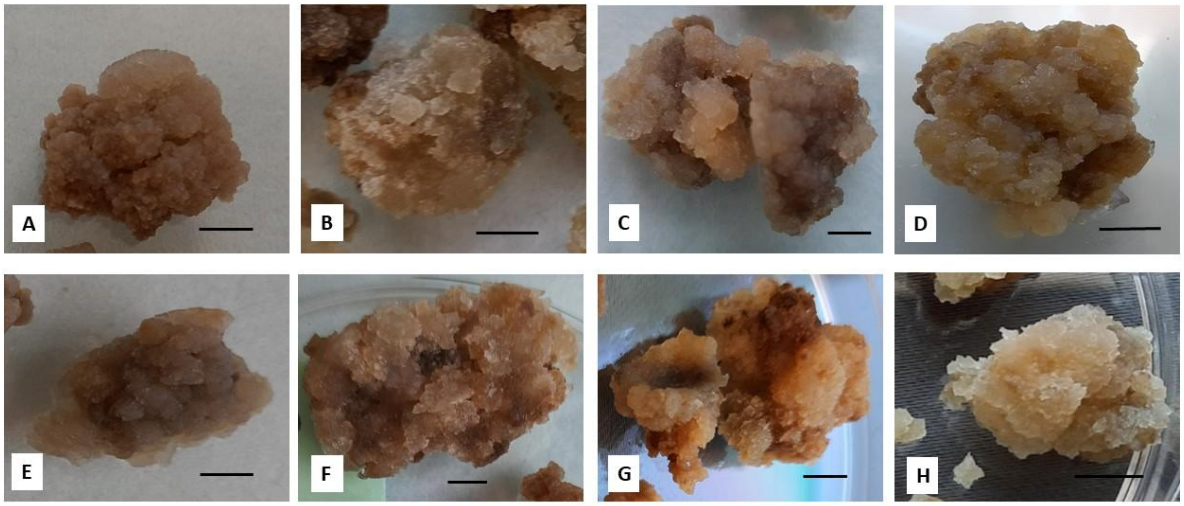


Figure 2

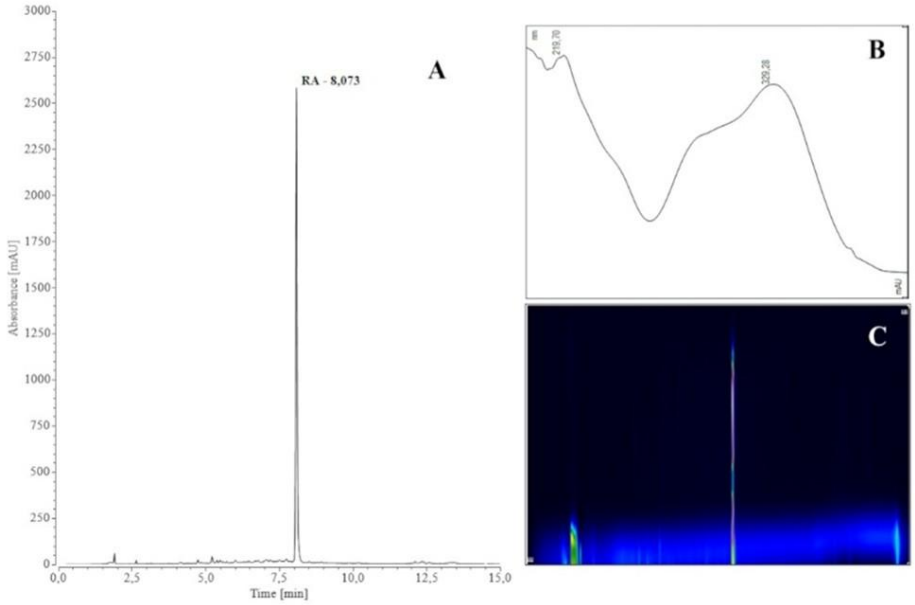


Figure 3

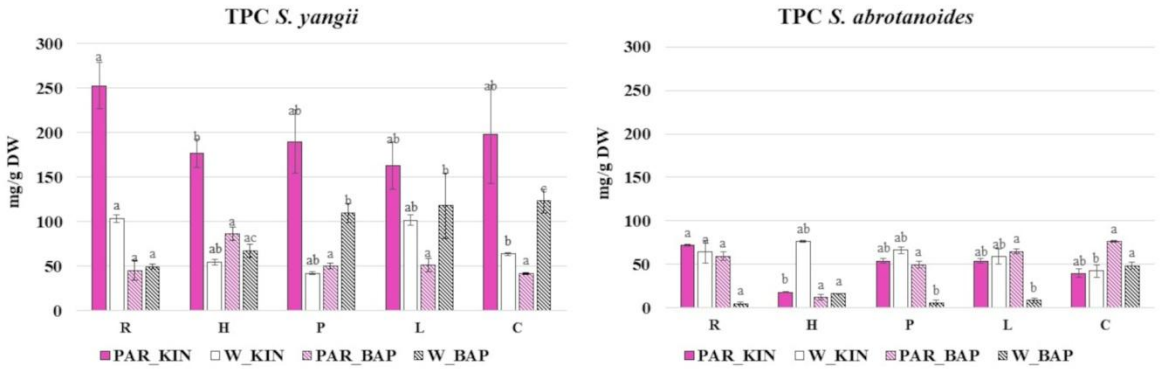


Figure 4

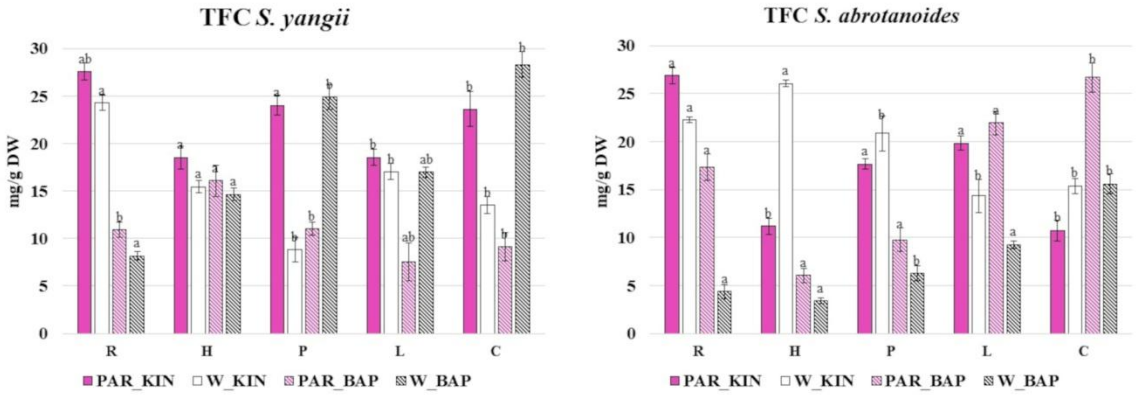
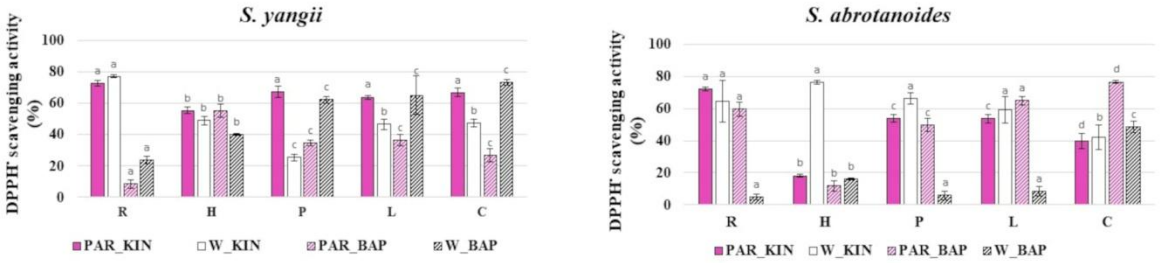


Figure 5


S. yangii
S. abrotanoides
DPPH scavenging activity (%)
100
80
60
40
20
0
R
H
P
L
C
PAR_KIN
W_KIN
PAR_BAP
W_BAP