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INFLUENCE OF SUBSTRATE TYPE ON THE GROWTH OF *OSMUNDA REGALIS* L. SPOROPHYTES UNDER GREENHOUSE CONDITIONS

Florentina ALDEA, Monica-Elena MITOI, Alexandra CIOCAN, Florența-Elena HELEPCIUC

Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, 060031, District 6, Bucharest, Romania

Corresponding author email: monica.mitoi@ibiol.ro

Abstract

Ferns constitute a group of vascular plants relevant for the study of the evolution of terrestrial plants due to their morphological and physiological adaptations, which confer them high ecological versatility. This adaptive capacity explains their distribution in a variety of habitats. In accordance with these characteristics, the present study examined the influence of the cultivation substrate on gravimetric and morphometric growth parameters and on the accumulation of metabolites by Osmunda regalis sporophytes in one-year potted cultures under greenhouse conditions. Six types of substrates (S1-S6), with different characteristics from specific ornamental mixtures for shrubs, cacti, aquatic or acidophilic plants, to natural peat, were tested. The plants developed on natural peat substrate (S6, presented the best morphometric characteristics, with the highest concentrations of metabolites, whereas variant S2 (enriched with microelements) had the best development of the vegetative part, with the lowest content of polyphenols, being the least stressed variant. Overall, the results showed that the optimal substrate should be selected according to the intended objective: vegetative growth or phytochemical potential.

Key words*: ferns, greenhouse, Osmunda regalis, secondary metabolites, substrate.*

INTRODUCTION

Ferns are an ancient lineage of vascular plants, with an evolutionary history spanning more than 360 million years, as documented by the fossil record (Shen et al., 2018; DiMichele & Phillips, 2002). Over time, they have diversified and adapted to an impressive range of habitats and are now represented by approximately 12,000 species (PPG I, 2016). They occur in terrestrial, aquatic, epiphytic, and lithophytic environments, on diverse substrates, from soils to rock surfaces and tree bark, and under climatic conditions ranging from tropical and temperate to semi-arid regions (Anderson, 2021; Dubuisson et al., 2009).

The adaptive potential of ferns derives from the integration of specialized morpho-physiological traits. Rhizomes function both as storage organs and support vegetative growth (Tani & Kudo, 2003; 2005), fine roots facilitate efficient nutrient uptake (Wang et al., 2019), and adaptable leaves enable photosynthesis under varying light conditions (Weng & Wong, 2015). Over time, ferns have developed complex morphophysiological mechanisms that have enabled them to adapt to a wide range of environmental conditions (Alejo-Jacuinde et al., 2022). This adaptation has involved not only structural modifications of vegetative organs, but also physiological and biochemical adjustments, including those related to oxidative metabolism. In this context, the synthesis of secondary metabolites, particularly phenolic compounds and flavonoids, contributes significantly to cellular protection and to plant responses to abiotic stress. The present study represents a continuation of previous research on the *in vitro* propagation and acclimatization of *Osmunda regalis* (Aldea et al., 2025), a species that has disappeared from the spontaneous flora of Romania (Boșcaiu et al., 1994; Dihoru & Dihoru, 1994) and is naturally found mainly in humid and shaded habitats, such as peat bogs, marshes, lake shores, and forests, on peat-rich soils (Birks et al., 1991; Matchutadze, 2014). In the current research, we aimed to analyze the influence of substrate type on the growth and development of sporophytes cultivated in pots under greenhouse conditions. The gravimetric and morphometric parameters, as well as the accumulation of proteins and secondary metabolites belonging to the classes of polyphenols and flavonoids, with antioxidant

activity, were assessed in order to identify the most favourable substrate variant for plant development.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The biological material used in this experiment consisted of sporophytes of the species *Osmunda regalis*, obtained *in vitro* by sexual reproduction according to our previous works (Mitoi et al., 2024; Aldea et al., 2025). Acclimatization was carried out following the protocol described in the previous study (Aldea et al., 2025). The acclimated sporophytes were grown in pots for one year in a greenhouse on six types of substrates, differentiated according to pH and composition:

- S1 - pH 5.5, substrate for planting and propagation, consisting of 80% peat and 20% humus, enriched with N, P, and K;
- S2 - pH 5.5, substrate for ornamental shrubs, with bark, enriched with basic nutrients, microelements (B, Cu, Mn, Mo, Zn, Fe), N, P, and K;
- S3 - pH 6.7, substrate for aquatic plants, with peat, quality compost, sand, and clay, enriched with N, P, and K;
- S4 - pH 3.5- 5, substrate for acidophilic plants, based on natural peat;
- S5 - pH 4.6 substrate for cacti, with a high proportion of porous material, consisting of a mixture of peat, siliceous sand, and expanded granules, supplemented with small amounts of N, P, and K;
- S 6 - pH 4.2, natural peat substrate.

All substrates, except S6, were obtained from Agro CS Romania SRL (https://agrocs.ro/). The S6 substrate was collected from a natural peatland. Each experimental variant was performed in six replicates.

The plants were maintained in plastic pots under greenhouse conditions at a temperature of 22 ± 2°C, with natural photoperiod and relative humidity of 40-45%. Watering was provided manually every two days, keeping the substrate slightly moist.

Gravimetric and morphometric parameters

To assess the response of sporophytes to the different substrates, the following morphometric and gravimetric parameters were assessed for each individual: plant weight, whole plant length, number of fronds per individual, length of the dominant frond, root length, and volume. Growth rates for the whole plant and root parameters were calculated as follows:

$$\text{growth rate} = (\text{final value} - \text{initial value}) / \text{initial value}$$

For dry weight determination, approximately 1 gram of sample represented by fronds, rhizomes, or roots was dried at 70 °C, for 10 h in an Ecocell oven (MMM Group, Germany). The dry weight of each sample was expressed as grams of dry weight (DW) reported to 1 g of fresh material (FW) - g DW/g FW. The moisture content (Mc) was calculated as a percentage from fresh weight using the formula: $Mc\ (\%) = (FW - DW)/FW \times 100$.

All gravimetric measurements were performed using an analytical balance (Radwag AS 220/C/2, Radom, Poland), while whole plant, dominant frond, and root lengths were recorded in cm using a measuring tape. Root volume was determined via the volumetric method, using 10 mL graduated cylinders for initial measurements and 250 mL cylinders for final measurements.

Biochemical Analyses

Protein extraction

Cytosolic soluble proteins were extracted from fronds and roots using 50 mM phosphate buffer, pH 7.0 with 2 mM EDTA, 4% PVP at a ratio of 1:3 (w/v). The homogenates were centrifuged at 15,000 rpm for 10 min, and the resulting supernatants were used for protein quantification.

Protein Content Analysis

Total protein content was determined using the Bradford assay (1976), with Coomassie Brilliant Blue G-250 protein stain. Appropriately diluted samples reacted with the dye solution, and the absorbance was recorded at 590 nm. Protein concentrations were calculated by interpolating the measured absorbances against a bovine serum albumin (BSA) standard curve (100–1000 µg/mL; $R^2 = 0.996$), and results were expressed as µg BSA/g FW.

Secondary Metabolites Extraction

Plant material was separated into root, rhizome, and leaf samples, ground and homogenized with methanol at a ratio of 1:10 (w/v). Extractions were prepared by maceration at room temperature, in the dark, with constant agitation

at 169 rpm for 72 h. Subsequently, the samples were centrifuged at 11,000 × g for 15 min at 4 °C, and the resulting supernatants were used for biochemical analyses.

Total polyphenolic content assay

The total phenolic content of the extracts was determined using the classic Folin-Ciocâlteu method (Stankovic et al., 2012). Appropriately diluted extracts were mixed with the Folin-Ciocâlteu reagent (Sigma Aldrich-Merck, Germany), followed by the addition of a freshly prepared 7.5% Na_2CO_3 solution. After incubition for 30 minutes at room temperature, the absorbance of the samples was measured at 765 nm. The results were quantified by extrapolation from a gallic acid calibration curve (concentrations: 10-100 µg/mL, R^2 = 0.9968) and expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

Flavonoid Content Assay

Flavonoid concentration was assessed using a spectrophotometric method based on the interaction of flavonoid compounds with aluminium ions, according to Cai et al. (2010). The signal intensity was measured at 510 nm and correlated with a calibration curve prepared using a rutin standard (100-1000 µg/mL, R^2 = 0.9996). Results were expressed as mg rutin equivalents per gram of fresh weight (mg RE/g FW).

Determination of Antioxidant Activity Using the DPPH Method

The antioxidant activity was analysed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, following the procedure described by Marxen et al., 2007. The sample was subjected to a reaction with methanol and DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured at 515 nm, and the results were expressed as mM Trolox equivalents per gram of fresh weight (mM TE/g FW), based on a Trolox calibration curve (50-150 µg/mL, R^2 = 0.9873).

Determination of Antioxidant Activity Using the FRAP Method

The reduction of ferric ions ($Fe^{3+} \rightarrow Fe^{2+}$) by the compounds present in the extract was monitored using the FRAP method, according to Simirgiotis et al. (2013), by measuring the absorbance at 593 nm after 30 minutes of incubation in the dark. Results were calculated based on a Trolox calibration curve (0.2-1 mM, R^2 = 0.9979) and expressed as mM Trolox equivalents per gram of fresh weight (mM TE/g FW).

All absorbance measurements were performed using a Spectronic Helios Gamma UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Each experimental variant consisted of three biological replicates for all biochemical analyses.

Statistical analyses

The experimental data were subjected to normality and homogeneity of variance tests, after which a Two-Way Analysis of Variance (ANOVA) was performed to assess the statistical significance of the combined effect of the two independent variables (type of organ and type of substrate). To determine the differences between means, we used the Tukey HSD test. All analyses were performed using MS Excel (Microsoft) and RStudio (packages emmeans, ggplot2 and multcomp). The results were reported as means ± standard deviation (SD) for all analysed parameters. The *p*-values considered statistically significant were $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS AND DISCUSSIONS

The comparative analysis of vegetative development highlights the influence of substrate type on the growth of *Osmunda regalis* sporophytes cultivated in pots for one year after acclimatization (Figure 1).

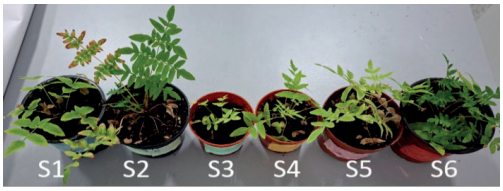

Figure 1. Influence of different substrate types on the growth of *O. regalis* potted cultures under greenhouse conditions

The gravimetric and morphometric analyzed parameters supported the macroscopic observations noted between substrate variants. Thus, the highest gravimetric whole-plant growth rate was recorded in variants S2 (45.24 ± 8.02) and S6 (44.22 ± 7.20), followed by S5 (41.34 ± 3.28) and S1 (39.99 ± 6.70), while the

other two variants had significantly lower values. The variant cultivated on substrate S3 showed the lowest gravimetric growth rate value of 7.07 ± 1.13 (Table 1). The plant growth rate in length displayed maximum values for variants S1 (5.50 ± 1.35) and S5 (5.16 ± 0.59), followed by S2 and S6 (4.77 ± 1.83 and 4.77 ± 1.12), with approximately similar values (Table 1). These results suggest that plant elongation does not always directly reflect total biomass accumulation; in our case, some substrates supported gravimetric growth more efficiently than linear expansion. The significant differences were noted only for plants cultivated on S1 and S5 substrates in comparison with plants with slower growth cultivated on substrate S4 (Table 1).

Table 1. Gravimetric and morphometric parameters in one-year potted cultures of *O. regalis*

Variant	Gravimetric growth rate	Plant growth rate in length	Length of the dominant frond (cm)	Number of fronds/ individuals	Root growth rate in length	Root growth rate in volume
S1	39.99±6.70^{a}	5.50±1.35^{a}	9.90±1.15^{ab}	9.5 ± 2.34^{ab}	7.16±1.88^{a}	23.17±6.21^{a}
S2	45.24±8.02^{a}	4.77±1.83^{ab}	12.30±1.68^{a}	10.33 ± 2.58^{a}	5.74±2.44^{a}	23.86±6.84^{a}
S3	7.07±1.13^{c}	4.16±1.36^{ab}	5.25±0.94^{c}	5.83 ± 0.75^{c}	7.52±4.01^{a}	10.94±4.99^{b}
S4	20.71±1.50^{b}	3.89±0.52^{b}	8.22±0.96^{b}	6.33 ±1.03^{bc}	6.70±1.48^{a}	10.94±2.22^{b}
S5	41.34 ±3.28^{a}	5.16±0.59^{a}	11.72±0.98^{a}	9.83 ± 3.43^{ab}	7.38±1.39^{a}	25.25±5.05^{a}
S6	44.22 ±7.20^{a}	4.77±1.12^{ab}	11.12±1.49^{a}	9.16 ± 1.16^{a}	7.46±2.04^{a}	24.14±6.11^{a}

The mean ± standard deviation (SD) followed by different letters in the column were considered statistically significant with $p < 0.05$.

A similar response was observed regarding fronds' development. The dominant frond length reached the highest values in variants S2 (12.30 ± 1.68 cm), S5 (11.72 ± 0.98 cm), and S6 (11.12 ± 1.49 cm), whereas in S3 and S4 significantly lower values were recorded (Table 1). The number of fronds was also highest in S2, S5 and S6, suggesting that these substrates supported not only the elongation of the fronds but also the initiation of new organs. Also, in S3 and S4 significantly lower values were registered (Table 1), indicating a slower development of the vegetative part.

Compared with frond parameters, roots showed a differentiated response. Surprisingly, root length showed the highest value in variant S3 (7.52 ± 4.01) and the lowest in variant S2, but the differences between variants were not significant due to the high SD values. In contrast, root volume differentiated more clearly between efficient and restrictive substrates. The highest values were observed in S5 (25.25 ± 5.05), S6 (24.14 ± 6.11), S2 (23.86 ± 6.84), and S1 (23.17 ± 6.21), whereas S3 and S4 registered significantly lower root volumes (Table 1). This contrast between root length and root volume suggests that, under unfavourable substrates, the root system may invest in exploratory elongation but without sufficient differentiation of a robust absorptive apparatus capable of sustaining an adequate flow of water and minerals. From a functional perspective, root volume appears to be a better indicator of the capacity to support aerial biomass than root length alone (Figure 2).



Figure 2. The appearance of *O. regalis* potted plants cultivated on different types of substrates

Substrate type significantly influenced water distribution and the degree of dry matter accumulation in organs (fronds, rhizomes, and roots). Thus, following separation (Figure 3) we monitored the differentiated accumulation of dry matter, water content, and metabolites' concentration in these organs.



Figure 3. The appearance of *O. regalis* potted plants: fronds (a), rhizome (b) and roots (c)

In fronds, the highest values of the DW were recorded in S4 (0.407 ± 0.007) and S3 (0.363 ± 0.046), while significantly lower values were obtained in S2, S5 and S6 (Table 2). In contrast, the highest fronds' water content was determined for S6 (78.64 ± 0.45%), followed by S5 (77.60 ± 0.80%) and S2 (76.03± 4.08%), while S4 and S5 variants had the lowest values (Table 2). The interpretation of these data should be coupled with plant growth rates. Thus, the variants with the highest dry matter content had the lowest water content and were the least productive in terms of biomass. This association suggests that the decrease in biomass accumulation in variants S3 and S4 could be due to the reduction of water content, rather than growth inhibition. However, the lowest growth parameters in these variants indicate that the relatively higher dry matter accumulation was not associated with a more active metabolism, but rather with reduced tissue hydration and structural changes induced by substrate-related stress. In these variants, the reduction of water content could be due to nutritional imbalance or inadequate aeration determined by the substrate composition and granulation.

Table 2. Dry weight and water content of *O. regalis* fronds, rhizomes, and roots from one-year potted cultures

Variant	Fronds		Rhizomes		Roots	
	g DW/g FW	% Water Content	g DW/g FW	% Water Content	g DW/g FW	% Water Content
S1	0.266±0.070^{bc}	73.36 ± 7.00^{ab}	0.245±0.037^{b}	75.54 ± 3.74^{a}	0.198±0.021^{b}	80.17 ± 2.06^{a}
S2	0.240±0.041^{c}	76.03± 4.08^{a}	0.288±0.026^{ab}	71.16 ± 2.60^{ab}	0.248±0.019^{a}	75.15 ± 1.92^{b}
S3	0.363±0.046^{ab}	63.70± 4.64^{bc}	0.331±0.022^{a}	66.92 ± 2.17^{b}	0.241±0.020^{ab}	75.90 ± 2.01^{b}
S4	0.407±0.007^{a}	59.29 ± 0.73^{c}	0.263±0.089^{ab}	73.73 ± 8.89^{ab}	0.261±0.025^{a}	73.89 ± 2.47^{b}
S5	0.224±0.008^{c}	77.60 ± 0.80^{a}	0.270±0.023^{b}	73.01 ± 2.29^{a}	0.187±0.028^{b}	81.31 ±2.83^{a}
S6	0.214±0.005^{c}	78.64 ± 0.45^{a}	0.269±0.016^{b}	73.13 ± 1.61^{a}	0.239±0.023^{ab}	76.08 ± 2.27^{b}

The means ± standard deviation (SD) followed by different letters in a column were considered statistically significant $p < 0.05$.

This dry matter accumulation, in association with the decrease in water content in variants S3 and S4 can also be observed at the rhizome and root level (Table 2), but, additionally, dehydration in variant S2 was observed at the rhizome and root level. Overall, the obtained data indicate that the less favourable substrates S3 and S4 modified the water content and the proportion of dry matter in rhizomes and roots, in correlation with the inhibition of plant growth. In the case of variant S2, the substrate produced dehydration in the root system, but in association with promoting the development of the vegetative system (Table 1).

The results obtained in the case of variant S2 suggest that a more aerated substrate structure, associated with an adequate supply of macro- and microelements, may favour long-term pot cultivation more efficiently than a substrate based exclusively on peat and humus. In contrast, substrate S3, intended for aquatic plants, recorded the weakest growth response, although it maintained relatively high values for root elongation and water content. This behaviour may be explained by the substrate composition, which includes clay and compost, as well as by its higher pH, factors that may increase compaction and reduce rhizosphere aeration. For a hygrophilous species such as *O. regalis*, high humidity is not sufficient in the absence of an adequate physical structure of the substrate. Under these conditions, the root system may respond through exploratory elongation, without, however, developing a sufficiently absorptive capacity to support the overall growth of the plant.

A relevant contrast was also observed in the case of S4, the substrate for acidophilous plants. Although its acidity falls within the favourable range for the species, the lower conductivity and the lack of available values for N, P, and K suggest a weaker nutrient supply. Thus, the data indicate that acidity alone does not guarantee optimal growth, as performance depends on the interaction between substrate reaction, physical structure, and nutrient availability. Taken together, these observations support the idea that substrate pH may play an important role in plant growth, mineral nutrition, and physiological response, in agreement with Ferrarezi et al. (2022), who showed that the availability of several nutrients to plants varies depending on substrate pH.

Unlike these results, our experimental variants with the best growth parameters displayed the lowest DW, in association with the highest water content. The substrates that significantly promoted

water accumulation, in association with higher growth parameters, were S6, S5 and S2 in fronds, S1, S6 and S5 in rhizomes, and S5 and S1 in roots (Tables 1 and 2). Interestingly, variant S5 supported a high biomass and a large root volume, suggesting that good drainage and efficient aeration of the root zone may compensate, at least partially, for a less favourable nutritional status, while the root system may respond through exploratory elongation without developing a sufficiently absorptive capacity to support the overall growth of the plant.

The results showed that the substrate is a determining factor in the balance between growth and nutritional requirements in *O. regalis*. The variants that supported the highest biomass accumulation and superior fronds development, namely S2, S1, S5, and S6, probably ensured a more favourable balance between water retention, aeration, and nutrient availability. This interpretation is consistent with our previous studies on the acclimatization of *O. regalis*, which reported a superior performance on substrates rich in peat and organic matter (Aldea et al., 2025), as well as with research on other fern species, where amended organic substrates stimulated growth and the accumulation of secondary metabolites (Pietrak et al. 2022).

The biochemical analyses provided new insights into the growth of the vegetative and root systems, highlighting that the substrate also affects plant metabolism. Thus, protein content varied depending on both the substrate and the analysed organ. In fronds, significantly higher protein concentrations were identified in variants S2, S6, and S5, in comparison to variants S1 and S3 (Figure 4). At root level, the significant values were recorded for S6, S2 and S4 variants compared with S1, S3 and S5 (Figure 4).

Differences between frond and root proteins' accumulation suggest that certain substrates (S5) supported a more efficient vegetative development, with more intense metabolic activity, reflected in a higher protein biosynthesis.

In some variants, roots had a significantly higher protein content than fronds (S6, S4 and S1), which could indicate an accentuated metabolic activity of the root system under pot culture conditions. These data showed a more intense root activity related to nutrient absorption and adaptation to substrate conditions.

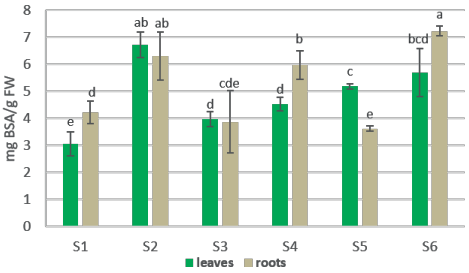

Figure 4. The protein concentrations in frond and root extracts from *O. regalis* potted plants cultivated on different types of substrates. The mean ± SD followed by different letters were considered statistically significant with $p < 0.05$

The literature indicates that ferns represent important sources of phenolic compounds (Mitoi et al., 2024; Langhansová et al., 2021). Our study showed that the properties of the cultivation substrate influenced the differential accumulation of secondary metabolites and antioxidant activity in O. *regalis* fern plants. In all variants, rhizomes presented the highest values for polyphenols, flavonoids and antioxidant activity, suggesting their important role in storage and regeneration (Klimešová et al., 2023). Fronds displayed the lowest values, while roots presented intermediate levels, which indicates a differentiated distribution of phenolic compounds among plant organs. A higher phenolic content in underground organs, compared with leaves, was also reported by other authors in several fern species (Valizadeh et al., 2015; Shin and Lee, 2010; Pietrak et al., 2025).

In our experiments, the significantly higher polyphenol content was recorded in the rhizomes of plants grown on substrate S6, followed by those on substrates S5 and S1, compared to variant S2, which presented the lowest polyphenols' concentration (Figure 5a). In roots and fronds, the variants with higher polyphenol content were S6, S3, S4 and S5, which showed significant differences compared with the other variants, S2 and S1 (Figure 5a).

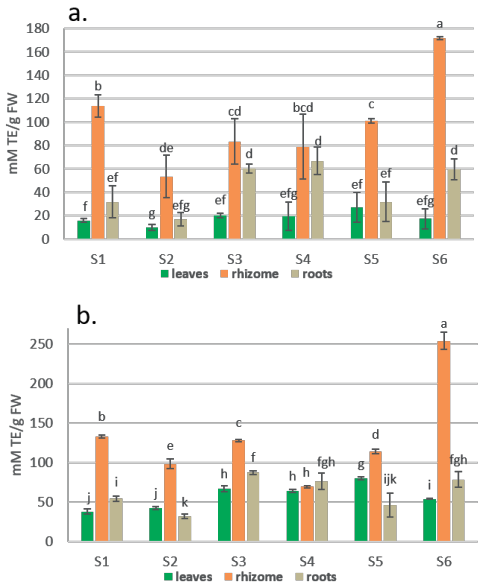

Figure 5. The polyphenols (a) and flavonoids (b) content in frond, rhizome and root extracts from *O. regalis* potted plants cultivated on different types of substrates. The means ± SD followed by different letters were considered statistically significant $p < 0.05$

A relatively similar accumulation pattern was observed for the flavonoids' content. The same variant S6 recorded the highest values in the rhizome, but, also in this case, at the fronds and roots' level, suggesting that the natural peat substrate favoured flavonoid accumulation and, implicitly, the intensification of secondary metabolism compared with the other variants. Interestingly, variant S1 presented higher values for both metabolite classes' accumulation in the rhizome, while the fronds and roots measured significantly lower concentrations (Figure 5a and b). The antioxidant activity determined by the DPPH method was correlated with the polyphenol content, with the same variation pattern noticed between substrate variants. Thus, the most significant value was seen in variant S6, at the rhizome level, followed by variants S1 and S5. Generally, higher antioxidant activity at the rhizome and roots level could suggest an intensification of antioxidant protection mechanisms in the underground organs. The variation pattern of antioxidant activity determined by the FRAP assay was not poorly correlated with the metabolites' determinations. In the case of rhizomes, all obtained values were significantly different, with the maximum value for variant S6 (Figure 6b). By comparison with the DPPH determinations, fronds showed a higher FRAP antioxidant activity (Figure 6a and b), depending on the substrate type, with a significantly increased activity in variant S5. A possible explanation might be that fronds are a reservoir of more Fe^{3+}-compounds that must be protected from oxidation reactions.

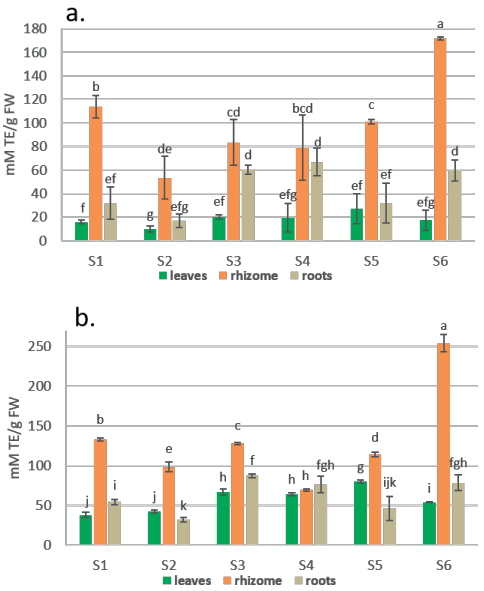

Figure 6. The DPPH (a) and FRAP (b) antioxidant activity in frond, rhizome, and root extracts from *O. regalis* potted plants cultivated on different types of substrates. The means ± SD followed by different letters were considered statistically significant with $p < 0.05$.

Correlated with the growth parameters, our biochemical data indicated that substrate type influenced not only vegetative and root system development but also the metabolic resources targeting secondary metabolism and antioxidant protection. These observations are consistent with other studies conducted on fern species, which have shown that substrate modifications may differentially affect growth, the accumulation of secondary metabolites, and antioxidant activity, depending on the species and the dose of the applied amendment (Pietrak et al., 2022). Substrate variants that did not necessarily support the highest biomass accumulation (S3 and S4) may have favoured the synthesis of phenolic compounds and the intensification of

antioxidant activity, reflecting a reorientation of metabolism toward adaptation and cellular protection. Also, variants with the best development of the root system (S6 and S5) possessed a rich phenolic content and high antioxidant activity. Our results are in agreement with the growth–defence trade-off concept (Sun et al., 2020).
However, the data obtained in the present study also showed that this relationship is not absolute. Certain substrate variants (S6 and S5) simultaneously supported both biomass accumulation and an increase in secondary metabolite content, highlighting a species- or substrate-specific response.

CONCLUSIONS

Substrate type had a direct influence on the plant growth, root development, and biochemical response of *Osmunda regalis* sporophytes in potted cultures.
The variants S1 and especially S2 proved to be the most favourable for vegetative development, while variants S5 and especially S6 had the best root development, in association with higher polyphenol content and antioxidant activity. In contrast, S3 and S4 proved to be less favourable for growth, highlighting the importance of physical structure and nutrient availability in cultivation substrates.
Root volume proved to be more relevant than root length for supporting aerial biomass. The rhizomes showed the highest levels of phenolic compounds and antioxidant activity, as we expected.
The most suitable variants for *ex situ* multiplication, for obtaining plants with optimal development and higher biomass, were substrates S6, natural peat, and S2, enriched with microelements.

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