



Effect of salt stress on growth and phenolic compounds production in callus suspension culture of the dioecious species thyrese sorrel (*Rumex thyrsiflorus* Fingerh.)

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Abstract

The sex-dependent differences in the response to salt stress of the dioecious *Rumex thyrsiflorus* and the influence of different concentrations of sodium chloride (NaCl) on the biosynthesis of phenolic compounds in callus suspension cultures were evaluated. The cultures originated from callus obtained on hypocotyls isolated from male and female seedlings were cultured on Murashige and Skoog medium supplemented with 0.4 mg/L 6-benzylaminopurine and 1 mg/L 2,4-dichlorophenoxyacetic acid. The results showed that the response to salt stress depends on both the sex of the plant and the NaCl concentration in the culture medium. The analysis of the main morphometric parameters showed that callus tissue derived from the hypocotyls of female seedlings was less sensitive to salt stress than that from male seedlings, which was correlated with higher concentrations of phenolic compounds. The optimal NaCl concentration to increase the production of phenolic compounds was 129 mM for females and 43 mM for males. In the methanolic tissue extracts 22 compounds were determined using the HPLC–DAD method. In general, higher amounts of compounds were detected in the extracts from the female tissue. The following dominated: catechin (max. 213.31), cryptochlorogenic acid (max. 76.35) and epicatechin (max. 54.84) (mg/100 g DW). This comprehensive phytochemical analysis of the sex-related aspects of the response to salt stress was performed for the first time in this dioecious model species. The results revealed potential application of NaCl as the ecological friendly and inexpensive elicitor to increase the production of pharmaceutically valuable compounds and highlight the importance of dioecy in phytochemistry.

Key message

The influence of salt stress on the phenolic compounds production as well as the sex-dependent callus tissue response was documented for the first time for the dioecious model species *Rumex thyrsiflorus*.

Keywords Thyrese sorrel · Salinity Stress · Elicitation · Callus Suspension Culture · Secondary Metabolites · HPLC–DAD

Introduction

Phenolic compounds (PCs) are naturally occurring bioactive molecules demonstrating a wide range of biological activities. They impart aroma, flavor and color and act as antioxidants against reactive oxygen species (ROS). PCs include groups such as phenolic acids, coumarins, flavonoids and tannins, and moreover stilbenes, quinones and lignans. They have found the greatest use in the pharmaceutical industry, due to their wide range of pharmacological activities, i.e., anti-inflammatory, antimicrobial, anticancer or antioxidant potential (Al Mamari 2021). The synthesis and accumulation of secondary metabolites (SMs), including

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PCs, is dependent on environmental conditions, i.e., light, temperature, or salinity (Yang et al. 2018; Mera et al. 2019). This phenomenon is frequently used in plant biotechnology. In tissue culture, the synthesis and accumulation of SMs under highly controlled conditions is known (Pan et al. 2020). Elicitation is one of the most promising techniques to increase SMs production. The known elicitors used under in vitro conditions are physical factors, such as UV radiation, osmotic pressure, or temperature changes, as well as chemical factors, such as heavy metals, antibiotics, or salinity (Datta et al. 2021).

According to Van Zelm et al. (2020), salt tolerance of plants is a complex trait that can be assessed in different ways, e. g. by ion accumulation, tissue-specific growth rates, biomass production, survival, and seed production. Halophytes are plants that have adapted to saline environments and use specialised strategies to cope with salinity. One of the most important salt tolerance mechanisms of halophytes is the accumulation of osmolytes in the cytoplasm for osmotic adjustment. These osmolytes are low-molecular-weight compounds such as proline, sugar alcohols, sorbitol, quaternary ammonium compounds, and α -amino nitrogen. Maintaining a high cytosolic K^+/Na^+ ratio, especially in the shoots, is crucial for the salt tolerance of glycophytes (Horie et al. 2012). Salt stress can not only have a negative effect on germination or the morphology of developing plants but can also lead to changes at the physiological or molecular level. It can affect the stability of many key proteins involved in photosynthesis, e.g., ribulose biphosphate carboxylase/oxygenase (RuBisCO), and even alter the levels of sugars. Such a negative effect can lead to an inhibition of photosynthesis, which leads to stunted plant growth and development. Plants sensing the accumulation of Na^+ ions accumulate large amounts of Ca^{2+} , thus activating ROS and changing the composition of phospholipids. Several processes are then activated to maintain ionic balance and osmotic homeostasis and, as a result, plant growth is slowed down, but metabolism is more activated (Zhao et al. 2021). Salt stress can lead to a reduction in water potential, resulting in physiological drought. Drought can affect not only the growth of individual plants but also the stability of the population structure and even the sex ratio in dioecious plants (Liao et al. 2019 and literature therein). Understanding the tolerance of male and female individuals to environmental stress factors is important to understand the evolution of dioecious plants under changing climatic conditions (Juvany and Munné-Bosch 2015).

Rumex thyrsiflorus, also known as thyrse sorrel, belongs to the Polygonaceae family. It is a dioecious plant with heteromorphic sex chromosomes. In female individuals: $2n = 12A + XX$, in males: $2n = 12A + XY_1Y_2$ (Žuk 1963). It is a synanthropic species that grows along roadsides and in ruderal areas, but also occurs in meadows and pastures.

Its distribution area includes Asia, Europe and Canada (Zarzycki and Rychlewski 1982). Phenolic acids, flavonoids, tannins and anthraquinones, e. g. emodin, chrysophanol or rhein, have been detected in extracts of *R. thyrsiflorus* (Litvinenko and Muzychkina 2008). They have also shown an antiproliferative effect on the A431 and MCF7 lines, and an antimicrobial effect on various bacterial strains, including *Bacillus subtilis* and *Staphylococcus epidermidis* (Orbán-Gyapai et al. 2017; Lajter et al. 2013).

Studies on natural populations of *R. thyrsiflorus*, in which female-biased sex ratio occurred, have shown a sex-dependent content of certain PCs. Female plants of this species were found to have a statistically significantly higher content of eleven natural compounds compared to male individuals. Among the free phenolic acids, chlorogenic acid, *p*-coumaric acid, gallic acid and vanillic acid as well as flavonoids such as rutin and catechin were detected (Dziedzic et al. 2020).

The aim of the study was to determine whether the response or tolerance to salt stress is sex-related and whether NaCl can act as an elicitor to increase the production of pharmaceutically valuable phenolic compounds.

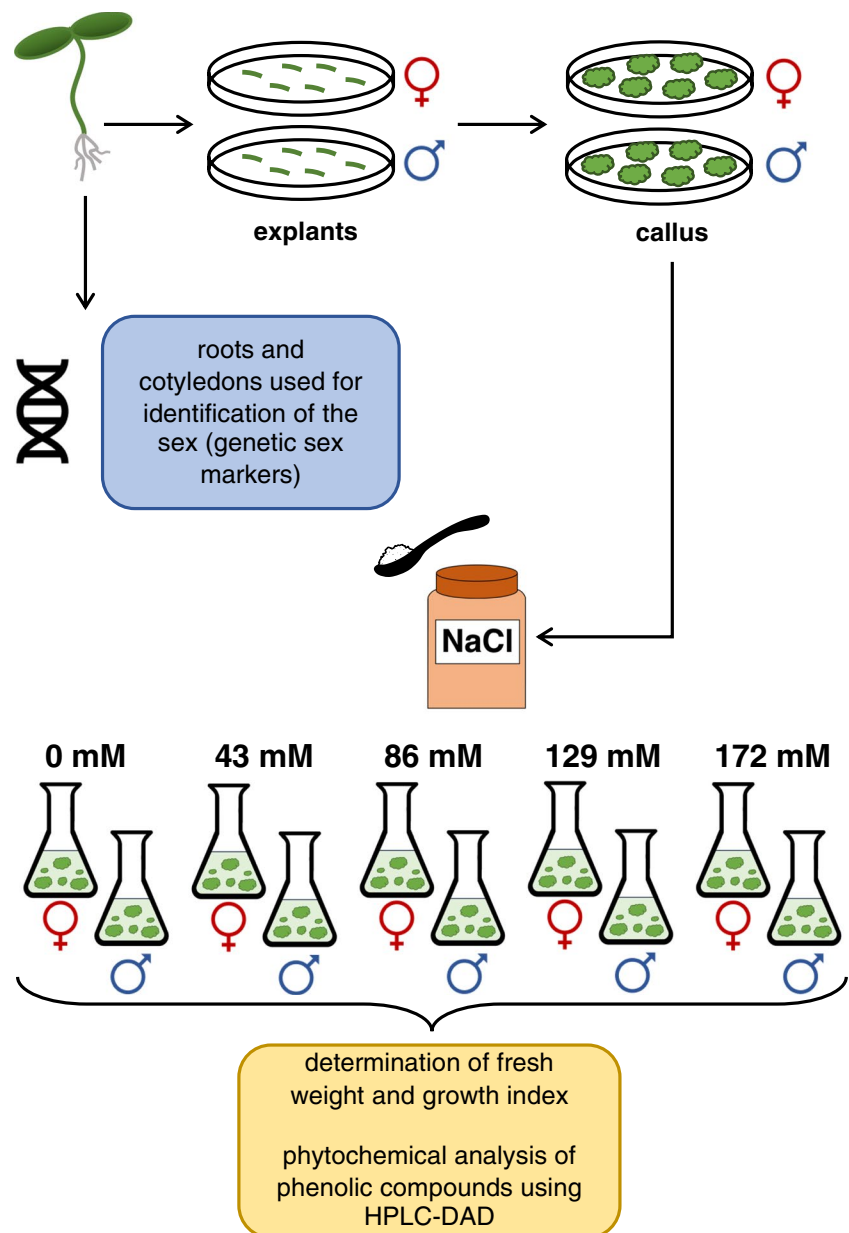
Materials and methods

Induction of callus tissue

Seeds of *Rumex thyrsiflorus* Fingerh. (POLAN, Poland) were surface sterilized in 70% EtOH for 1 min, and then in 50% (v/v) commercial bleach (< 5% sodium hypochlorite) for 12 min. The sterilized seeds were rinsed three times in sterile, deionized water. The prepared seeds were sown on Petri dishes with sterile, moist filter paper. The Petri dishes were placed in a culture room for 11 days at $25 \pm 3^\circ C$, 16 h/8 h photoperiod (day/night) and fluorescent white light (intensity: $60 - 90 \mu mol \cdot m^{-2} s^{-1}$).

In the next step, hypocotyls (approx. 5 mm) were isolated from seedlings and placed on MS medium (Murashige and Skoog 1962) containing 3% (w/v) sucrose, 0.8% (w/v) agar, and two plant growth regulators: 0.4 mg/L BAP (6-benzylaminopurine) and 1 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid) callus induction medium (CIM) as described by El-Shafey et al. (2019). Five explants were inoculated onto one Petri dish (90 mm) (185 explants/37 Petri dishes in total). The experiment was repeated four times. Passage of cultures into fresh medium was done every 4 weeks to multiply callus tissue. Two lines of callus cultures were performed from hypocotyls isolated from male and female seedlings, as soon as their sex was determined (see below). The scheme of the experiment is shown in Fig. 1.

Fig. 1 Scheme of the experiment: hypocotyls that were derived from 11-day-old male and female seedlings were used as the explants, non-morphogenic callus tissue induced on explants was used for initiation of suspension culture



Identification of the sex

To determine the sex of the hypocotyls used as explants for callus induction, the cotyledons and roots isolated from the same germinated seedling from which the hypocotyl was excised, were used for DNA isolation. Extraction of genomic DNA was performed using the hexadecyltrimethylammonium bromide (CTAB) method, based on the protocol of Gawel and Jarret (1991) with some modifications (Kwolek and Joachimiak 2011). A PCR-based approach employing DNA markers specific to Y chromosomes was used. Primers developed by Korpelainen (2002) targeting the male-specific RAYSI sequence found on the Y chromosomes of *R. acetosa* and its closely related species, as described by Navajas-Pérez

et al. (2006), were used. Specifically, the primers RAY-F (5'-ACTCGAATGTAAGCATTGTTGTCCTCCATAAA GTGGA-3') and RAY-R (5'-ACTACACGATTGTCCATA AAGTGGA-3') were used. In addition, primers URG08-F (5'-CCAATTGGTCTCAACTAGAACA-3') and URG08-R (5'-TGTTATAGGTTGGACTGCCA-3'), designed for the amplification of the male-specific repetitive sequence RAY-SII in *R. acetosa* L., were employed, following the methodology described by Mariotti et al. (2009). To verify the integrity of the template DNA, primers targeting the autosomal sequence RAE730: R730-A (5'-CTCGGACCAATTATC TCAT-3') and R730-B (5'-CATTATTGGGAGCCGAT-3') were employed (Navajas-Pérez et al. 2005). The amplification process was conducted using a T100 Thermal Cycler

(BioRad), as described by Ślesak et al. (2015). PCR products were then separated in a 1% agarose gel in $1 \times$ TBE buffer with Simply Safe (EURx) for 60 min at a constant voltage of 120 V, with Perfect Plus 100 bp DNA Ladder (EURx) as a molecular standard.

Initiation of suspension culture

The 500 mg of highly viable and friable callus induced on female (F) (Fig. 2a) or male (M) (Fig. 2b) explants (referred to as female or male callus) were weighed under sterile conditions and then transferred to Erlenmeyer flasks (100 mL) containing 40 mL of liquid MS medium (same composition as CIM but without agar) (see above) (Fig. 2c). An appropriate amount of NaCl was dissolved in the medium to obtain the required NaCl concentration as follows: 0 mM (control), 43 mM, 86 mM, 129 mM and 172 mM (Fig. 1). Three replicates of each concentration were prepared for each sex. The flasks were maintained for 4 weeks on a rotary shaker (Elpin+, type 358S, Poland) at a constant 120 c.p.m. (cycles per minute) with an amplitude of 3 mm in the culture room under the conditions described above.

Determination of growth indices of callus

The fresh weight (FW) of the suspension culture was determined by removing the culture medium after 4 weeks. The callus suspension from each flask (Fig. 2d) was separated from the liquid medium by filtration through a Buchner funnel and washed with distilled water under a vacuum pump. The fresh weight of the callus (FW) was then weighed (Fig. 2e). The dry weight (DW) was determined after drying at 65 °C for 48 h in a hot air oven (Fig. 2f). The following parameters were calculated:

Percentage of callus water content (PCWC) (Salehi et al. 2017):

$$\text{PCWC} = \frac{\text{FW} - \text{DW}}{\text{FW}} \times 100$$

Relative fresh weight growth (RFWG) (Salehi et al. 2017):

$$\text{RFWG} = \frac{W1 - W0}{W0}$$

where:

W0 – initial fresh callus mass,

W1 – final fresh callus mass.

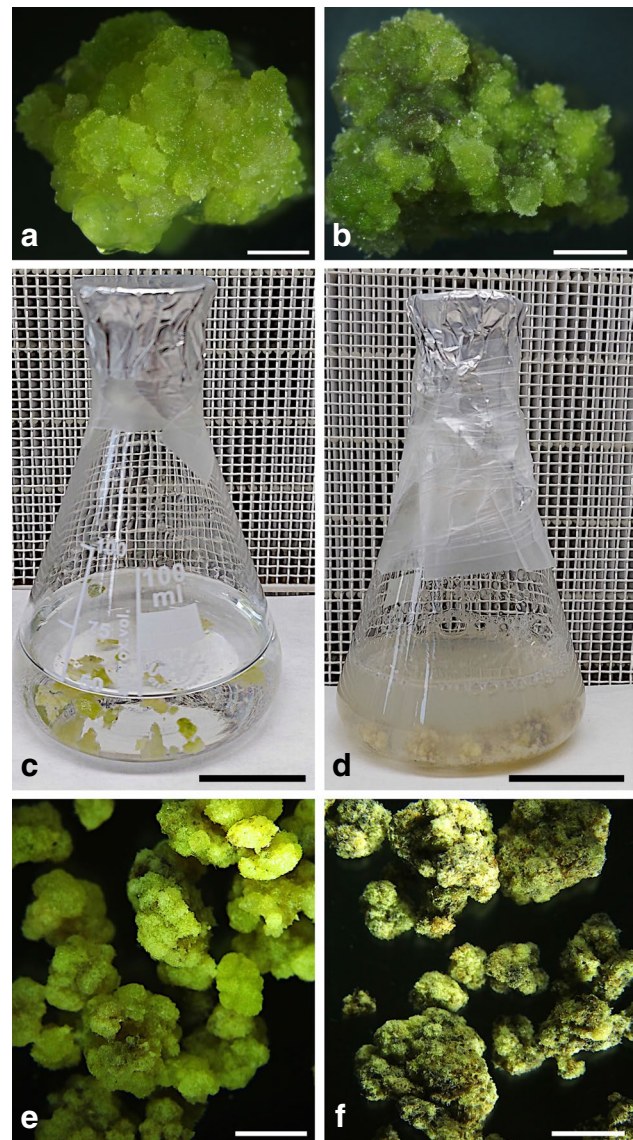


Fig. 2 Callus used to initiate suspension culture on MS medium with BAP and 2,4-D: female **a** and male **b** (after 12 weeks from the beginning of the culture). Callus suspension culture before **c** and after 28 days of treatment with NaCl **d**. Fresh callus drained from suspension culture medium **e** and dried callus after 48 h at 65 °C **f**. Bars 3 mm **a-b**, 2 cm **c-d**, 7 mm **e-f**

Relative growth rate per day RGR (day^{-1}) (Salehi et al. 2017):

$$\text{RGR} (\text{d}^{-1}) = \frac{\ln(W1) - \ln(W0)}{\text{duration of culture in days}} \times 100$$

Tolerance index (TI) (Soheilikhah et al. 2013):

$$TI = \frac{RGR1}{RGR0}$$

where:

RGR1 – relative growth rate of the treatment,

RGR0 – relative growth rate of the control.

Extraction and HPLC–DAD analyses

Lyophilized callus tissue 200 mg each sample, were extracted with methanol (3 mL, HPLC-grade pure, Merck, Germany) in falcons by sonification (Sonic 2, POLSONIC Palczyński ultrasonic bath, Warszawa, Poland) 2 times over 30 min at room temperature. The extracts were centrifuged for 8 min at 4000 rpm (MPW-223E, MPW, Warsaw, Poland). The supernatant was passed through syringe filters (pore diameter=0.2 µm Millex® GP, Filter Unit, Millipore, Bedford, MA, USA). Analysis of PCs was carried out using the HPLC–DAD method (Ellnain-Wojtaszek and Zgórk 1999; Sułkowska-Ziaja et al. 2017; Szopa et al. 2017a, b). A liquid chromatograph apparatus (Merck-Hitachi, Japan) was used, which is equipped with a DAD detector (L – 245) in the UV light range (200 – 400 nm). The analytical column, Purospher® RP – 18e (4× 250 mm, 5 µm; Merck, Germany), which was used utilizes a gradient program with a flow rate of 1 mL/min. at 25 °C, a detection wavelength 254 nm and an injection volume of 10 µL. The mobile phase consisted of: A – methanol: 0.5% acetic acid in a ratio of 1:4 (v/v), B – methanol, and the gradient elution occurred according to the scheme: 100% A 0 – 20 min; 100 – 80% A 20 – 35 min; 80 – 60% A 35 – 55 min; 60 – 0% A 55 – 70 min; 0% A 70 – 75 min; 0 – 100% A 75 – 80 min; 100% A 80 – 90 min at a flow rate of 1 mL/min. All samples were injected in three replications. Identification of PCs was based on comparison of their spectra with UV spectra and retention times of reference substances acquired in Sigma-Aldrich (Germany) and Chemfaces (China): phenolic acids: gallic, neochlorogenic, protocatechuic, chlorogenic, cryptochlorogenic, vanillic, caffeic, *p*-coumaric, ferulic acids; flavonoids: catechin, epicatechin, vitexin, rutoside, hyperoside, isoquercetin, myricetin, quercitrin, quercetin, apigenin, rhamnetin; anthraquinones: emodin, chrysophanol. Quantitative analysis of the compounds was performed using the calibration curve method.

Statistical analysis

The results of FW, DW, RFWG, RGR, PCWC, TI measurements for each sample from the 0, 43, 86, 129 and 172 mM NaCl treatment of each sex were expressed as mean values ± SEM. Differences between means were analyzed using two-way ANOVA and Tukey's Multiple Comparison Test. The results of PCs measurements were expressed as mean values ± SD. Differences between means within the same sex were analyzed using one-way ANOVA and Tukey's Multiple Comparison Test. In addition, differences between the diverse

sexes within the NaCl concentration were analyzed using the unpaired Student's *t*-test. Statistical analysis was performed using GraphPad Prism software (v. 8.01).

Results

Identification of the sex

In males, the PCR reaction with primers RAY-R and RAY-F targeting the RAYSI sequence resulted in a product of approximately 930 bp (Fig. 3a). In addition, the use of primers URG08-R and URG08-F led to a strong amplification of the RAYSII sequence, which resulted in two different products of approximately 700 and 600 bp in males (Fig. 3b). The integrity of the DNA template was confirmed by the successful amplification of the autosomal sequence RAE730 with the primers R730-A and R730-B (Fig. 3c).

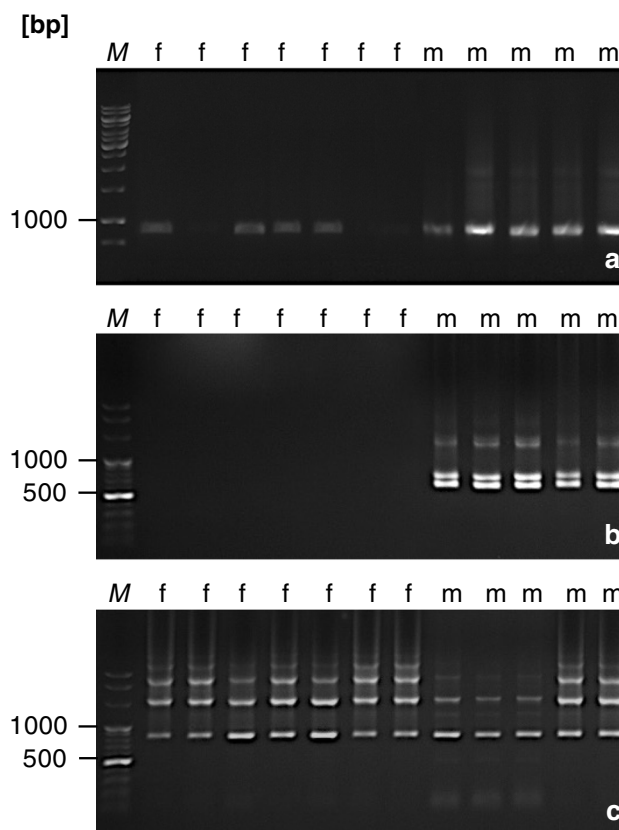


Fig. 3 PCR products of *Rumex thyrsiflorus* explants cultured in vitro: RAY-F and RAY-R primers **a**, URG08-F and URG08-R primers **b**, R730-A and R730-B primers **c**; f – female, m – male, M – 100 bp molecular weight marker

Effect of salt stress on callus growth

The analysis of variance (ANOVA) showed that the NaCl concentration had a significant sex-specific effect on callus growth. After 4 weeks of suspension culture, the weight of the female callus increased sevenfold at 0 mM NaCl, while that of the male callus increased threefold (Fig. 4a, Table S1 and S2). For the female callus, the medium with the addition of 172 mM NaCl was best suited for callus growth (4.10 ± 0.25 g), for the male callus the medium without NaCl (1.92 ± 0.2 g). The fresh weight values (Fig. 4a) showed significant differences between the sexes on each medium tested and between the female callus on 129 mM and 172 mM NaCl medium.

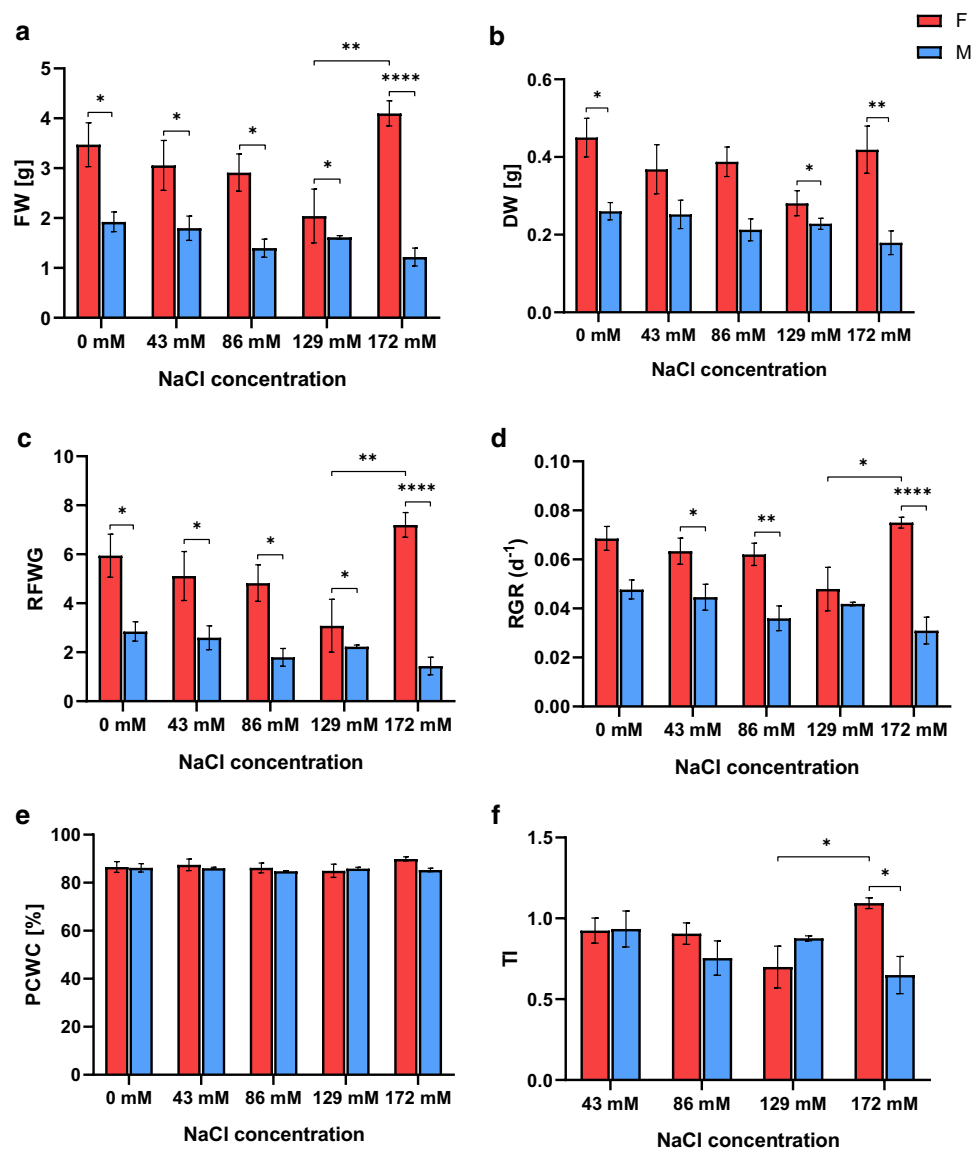
The values of RFWG (Fig. 4c) and RGR (d^{-1}) (Fig. 4d) decreased with increasing NaCl concentration, but on the medium with 172 mM NaCl they increased above the control

value in the female callus. The statistical analysis of the parameter RFWG (Fig. 4c) showed the same statistically significant difference trend as for FW (Fig. 4a). The values of RGR (d^{-1}) (Fig. 4d) showed statistically significant differences between the sexes on 43 mM, 86 mM and 172 mM as well as between female individuals on medium with 129 mM and 172 mM NaCl.

The values of PCWC (Fig. 4e) varied between 89.88 ± 0.87 (female callus on medium with 172 mM NaCl) and 84.78 ± 0.25 (male callus on medium with 86 mM NaCl) (Table S1 and S2). The results of the statistical analysis of this parameter showed no statistical differences between the sexes and the media.

The values of the tolerance index (TI) (Fig. 4f) show that the female callus was more tolerant to salinity than the male callus. The values ranged from 0.70 to 1.09 for the female callus, with the highest values on the

Fig. 4 Growth indices of callus: callus fresh weight (FW) [g] **a**, callus dry weight (DW) [g] **b**, relative fresh weight growth (RFGW) **c**, relative growth rate per day (RGR (d^{-1})) **d**, percentage of callus water content (PCWC) **e** and tolerance index (TI) **f** on day 28 after treatment with 0, 43, 86, 129 and 172 mM NaCl; *F* female callus, *M* male callus; data are mean $\pm$ SEM ($n=3-4$). Asterisks indicate significant differences between means at the level of $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.0001$ (***) according to Tukey's Multiple Comparison Test



172 mM NaCl medium (Table S1 and S2) and were statistically significant compared to the male callus on the same medium and the female callus on 129 mM NaCl.

Influence of the NaCl stress on the phenolic compounds production

In the analyzed methanol extracts of the biomass from suspension cultures, 9 phenolic acids, 11 flavonoids and 2 hydroxyanthraquinone derivatives were determined (Fig. S1). The phenolic acids included: gallic acid, neochlorogenic acid, protocatechuic acid, chlorogenic acid, cryptochlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid. Among flavonoids, vitexin, rutin, hyperoside, isoquercetin, myricetin, quercitrin, quercetin, apigenin, rhamnetin, catechin and epicatechin were identified. In addition, the 2 hydroxyanthraquinone derivatives emodin and chrysophanol were quantified. There were observed differences in the content of PCs related to sex and the studied NaCl concentration in the culture medium (Tables 1 and 2). For phenolic acids, the

average content ranged from 1.02 mg/100 g DW (*p*-coumaric acid) to 76.35 mg/100 g DW (cryptochlorogenic acid), for flavonoids from 0.19 mg/100 g DW (vitexin and isoquercetin) to 213.31 mg/100 g DW (catechin), and for hydroxyanthraquinone derivatives from 1.60 mg/100 g DW (emodin) to 5.58 mg/100 g DW (chrysophanol). The highest PCs content was observed in female callus, with the exception of hyperoside and myricetin, whose content was higher in male callus grown exclusively on a medium containing 43 mM NaCl. The following trend was observed: male callus exhibited the highest concentrations of phenolic compounds when cultured on medium containing 43 mM NaCl, but in the case of chlorogenic acid, apigenin and catechin, the peak levels also occurred in male callus cultivated in 86 mM NaCl medium. In contrast, in most cases, female callus showed the highest concentrations of phenolic compounds when grown on medium supplemented with 129 mM NaCl, with the exception of neochlorogenic acid (172 mM NaCl). In most cases, the lowest content of phenolic compounds was found in male callus cultured on medium with 0 mM NaCl and in female

Table 1 Average content of phenolic compounds [mg/100 g DW $\pm$ SD] in extracts of *Rumex thyrsiflorus* from female callus tissue cultured on media with different concentrations of NaCl (0 mM, 43 mM, 86 mM, 129 mM and 172 mM)

Phenolic compounds	NaCl concentration				
	0 mM (control)	43 mM	86 mM	129 mM	172 mM
Gallic acid	9.21 $\pm$ 0.03 ^a	14.01 $\pm$ 0.12 ^b	12.85 $\pm$ 0.08 ^c	26.25 $\pm$ 0.07 ^d	22.44 $\pm$ 0.18 ^e
*Neochlorogenic acid	3.05 $\pm$ 0.13 ^a	4.73 $\pm$ 0.16 ^{ab}	9.08 $\pm$ 0.02 ^{ab}	5.67 $\pm$ 0.32 ^{ab}	10.96 $\pm$ 2.88 ^b
Protocatechuic acid	3.9 $\pm$ 0.02 ^a	6.37 $\pm$ 0.18 ^b	6.72 $\pm$ 0.12 ^{bc}	9.89 $\pm$ 0.22 ^d	9.19 $\pm$ 0.39 ^c
Chlorogenic acid	5.06 $\pm$ 0.23 ^a	7.5 $\pm$ 0.53 ^b	5.8 $\pm$ 0.09 ^{ab}	12.66 $\pm$ 0.51 ^d	7.06 $\pm$ 1.06 ^b
*Cryptochlorogenic acid	8.7 $\pm$ 0.98 ^a	34.22 $\pm$ 1.58 ^b	15.76 $\pm$ 0.06 ^{ab}	76.35 $\pm$ 2.06 ^{ab}	17.66 $\pm$ 3.64 ^{ab}
Vanillic acid	1.63 $\pm$ 0.25 ^a	2.11 $\pm$ 0.35 ^{ab}	1.74 $\pm$ 0.04 ^{ab}	2.78 $\pm$ 0.33 ^b	1.25 $\pm$ 0.53 ^a
Caffeic acid	8.38 $\pm$ 0.97 ^a	8.1 $\pm$ 1.07 ^a	4.73 $\pm$ 0.1 ^b	7.99 $\pm$ 0.63 ^a	5.56 $\pm$ 1.06 ^b
<i>p</i> -Coumaric acid	4.89 $\pm$ 3.28 ^a	1.02 $\pm$ 0.22 ^a	2.79 $\pm$ 1.15 ^a	5.22 $\pm$ 4.98 ^a	3.2 $\pm$ 1.33 ^a
Ferulic acid	5.31 $\pm$ 3.37 ^a	1.65 $\pm$ 0.02 ^a	2.07 $\pm$ 0.9 ^a	6.06 $\pm$ 3.12 ^a	2.32 $\pm$ 0.48 ^a
Vitexin	2.34 $\pm$ 1.66 ^a	0.19 $\pm$ 0.05 ^a	1.22 $\pm$ 0.69 ^a	3.34 $\pm$ 1.32 ^a	1.65 $\pm$ 0.14 ^a
Rutin	6.36 $\pm$ 3.55 ^a	1.36 $\pm$ 0.44 ^a	1.75 $\pm$ 0.91 ^a	7.14 $\pm$ 3.22 ^a	1.98 $\pm$ 0.25 ^a
Hyperoside	6.23 $\pm$ 3.08 ^a	1.38 $\pm$ 0.22 ^a	1.73 $\pm$ 0.8 ^a	3.53 $\pm$ 1.66 ^a	1.73 $\pm$ 0.13 ^a
*Isoquercetin	2.47 $\pm$ 2.04 ^a	0.19 $\pm$ 0.02 ^a	0.98 $\pm$ 0.59 ^a	4.21 $\pm$ 2.02 ^a	1.01 $\pm$ 0.12 ^a
*Myricetin	9.91 $\pm$ 5.91 ^a	1.4 $\pm$ 0.37 ^a	1.25 $\pm$ 0.67 ^a	5.69 $\pm$ 3.34 ^a	0.55 $\pm$ 0.13 ^a
*Quercitrin	5.79 $\pm$ 3.92 ^a	0.95 $\pm$ 0.27 ^a	0.88 $\pm$ 0.6 ^a	3.92 $\pm$ 2.06 ^a	0.37 $\pm$ 0.06 ^a
*Quercetin	7.6 $\pm$ 3.76 ^{ab}	6.87 $\pm$ 0.14 ^{ab}	2.05 $\pm$ 0.77 ^{ab}	24.41 $\pm$ 4.23 ^b	0.78 $\pm$ 0.06 ^a
Apigenin	20.19 $\pm$ 7.74 ^{ab}	24.18 $\pm$ 0.59 ^{ab}	15.23 $\pm$ 0.94 ^{ab}	29.06 $\pm$ 7.6 ^a	9.11 $\pm$ 2.52 ^b
Rhamnetin	9.55 $\pm$ 3.11 ^a	9.36 $\pm$ 0.13 ^a	8.61 $\pm$ 0.59 ^a	10.76 $\pm$ 1.63 ^a	6.65 $\pm$ 1.17 ^a
Catechin	89.83 $\pm$ 2.31 ^a	110.76 $\pm$ 3.36 ^b	117.93 $\pm$ 0.31 ^b	213.31 $\pm$ 4.02 ^c	205.71 $\pm$ 7.64 ^c
*Epicatechin	50.2 $\pm$ 18.12 ^{ab}	35.43 $\pm$ 0.83 ^{ab}	17.09 $\pm$ 1.05 ^{ab}	54.84 $\pm$ 19.53 ^a	5.28 $\pm$ 2.31 ^b
Emodin	2.42 $\pm$ 0.49 ^a	2.25 $\pm$ 0.04 ^a	2.19 $\pm$ 0.05 ^a	2.48 $\pm$ 0.29 ^a	1.96 $\pm$ 0.18 ^a
Chrysophanol	5.58 $\pm$ 0.8 ^a	5.29 $\pm$ 0.1 ^a	5.05 $\pm$ 0.07 ^a	5.36 $\pm$ 0.42 ^a	4.55 $\pm$ 0.34 ^a

n = 3; DW – dry weight; the same letters (a-e) next to values indicate no significant differences between means, at least at the level of *p* $\leq$ 0.05. One-way ANOVA and Tukey's Multiple Comparison Test as *post-hoc* for the statistical analysis of data were used (with the exception of compounds next to which there is an *, Kruskal-Wallis test with Dunn's Multiple Comparison Test as *post-hoc* were used)

Table 2 Average content of phenolic compounds [mg/100 g DW $\pm$ SD] in extracts of *Rumex thyrsiflorus* from male callus tissue cultured on media with different concentrations of NaCl (0 mM, 43 mM, 86 mM, 129 mM and 172 mM)

Phenolic compounds	NaCl concentration				
	0 mM (control)	43 mM	86 mM	129 mM	172 mM
Gallic acid	11.13 $\pm$ 0.03 ^a	22.13 $\pm$ 0.09 ^b	18.43 $\pm$ 0.08 ^c	15.83 $\pm$ 0.04 ^d	16.45 $\pm$ 0.01 ^e
*Neochlorogenic acid	5.51 $\pm$ 0.07 ^a	7.99 $\pm$ 0.03 ^a	7.08 $\pm$ 0.11 ^a	5.37 $\pm$ 0.04 ^a	5.27 $\pm$ 0.35 ^a
Protocatechuic acid	5.54 $\pm$ 0.09 ^a	9.08 $\pm$ 0.03 ^b	7.68 $\pm$ 0.08 ^c	7.19 $\pm$ 0.02 ^d	4.48 $\pm$ 0.14 ^e
Chlorogenic acid	5.37 $\pm$ 0.14 ^a	8.43 $\pm$ 0.06 ^b	8.79 $\pm$ 0.05 ^b	6.99 $\pm$ 0.03 ^c	5.8 $\pm$ 0.63 ^a
*Cryptochlorogenic acid	19.66 $\pm$ 0.46 ^a	41.45 $\pm$ 0.1 ^b	30.54 $\pm$ 0.14 ^{ab}	26.59 $\pm$ 0.11 ^{ab}	23.38 $\pm$ 1.99 ^{ab}
*Vanillic acid	1.7 $\pm$ 0.09 ^{ab}	2.84 $\pm$ 0.04 ^a	2.64 $\pm$ 0.02 ^{ab}	2.33 $\pm$ 0.03 ^{ab}	1.53 $\pm$ 0.36 ^b
*Caffeic acid	4.79 $\pm$ 0.31 ^a	7.04 $\pm$ 0.05 ^a	5.98 $\pm$ 0.04 ^a	7.01 $\pm$ 0.08 ^a	6.08 $\pm$ 0.88 ^a
<i>p</i> -Coumaric acid	5.13 $\pm$ 3.6 ^a	7.95 $\pm$ 1.27 ^a	2.62 $\pm$ 1.71 ^a	3.77 $\pm$ 0.4 ^a	5.87 $\pm$ 3.65 ^a
Ferulic acid	4.29 $\pm$ 2.47 ^a	6.34 $\pm$ 0.96 ^a	3.14 $\pm$ 1.94 ^a	3.05 $\pm$ 0.45 ^a	4.67 $\pm$ 2.44 ^a
Vitexin	1.88 $\pm$ 1.07 ^a	2.45 $\pm$ 0.59 ^a	1.36 $\pm$ 1.44 ^a	1.7 $\pm$ 0.19 ^a	2.05 $\pm$ 1.09 ^a
Rutin	4.44 $\pm$ 1.95 ^a	4.71 $\pm$ 1.41 ^a	2.93 $\pm$ 2.35 ^a	3.16 $\pm$ 0.82 ^a	4.5 $\pm$ 2.08 ^a
Hyperoside	8.06 $\pm$ 2.64 ^a	10.35 $\pm$ 2.18 ^a	5.55 $\pm$ 3.12 ^a	5.99 $\pm$ 1.25 ^a	8.54 $\pm$ 2.71 ^a
Isoquercetin	3.23 $\pm$ 1.66 ^a	3.76 $\pm$ 1.35 ^a	2.12 $\pm$ 2.04 ^a	2.12 $\pm$ 0.84 ^a	2.92 $\pm$ 1.77 ^a
Myricetin	10.13 $\pm$ 4.58 ^a	11.83 $\pm$ 4.05 ^a	5.83 $\pm$ 4.6 ^a	4.59 $\pm$ 1.32 ^a	5.78 $\pm$ 3.16 ^a
Quercitrin	5.13 $\pm$ 2.47 ^a	5.34 $\pm$ 2.13 ^a	3.39 $\pm$ 2.48 ^a	2.44 $\pm$ 1.36 ^a	4.41 $\pm$ 2.73 ^a
Quercetin	7.94 $\pm$ 3.42 ^a	12.07 $\pm$ 3.52 ^a	8.31 $\pm$ 3.26 ^a	7.46 $\pm$ 2.54 ^a	9.08 $\pm$ 4.18 ^a
Apigenin	16.7 $\pm$ 8.07 ^a	18.21 $\pm$ 7.32 ^a	18.33 $\pm$ 3.51 ^a	14.89 $\pm$ 3.59 ^a	10.99 $\pm$ 9.24 ^a
Rhamnetin	8.56 $\pm$ 4.81 ^a	9.53 $\pm$ 2.81 ^a	9.27 $\pm$ 1.18 ^a	8.43 $\pm$ 1.69 ^a	5.18 $\pm$ 4.58 ^a
Catechin	64.46 $\pm$ 0.99 ^a	117.2 $\pm$ 0.41 ^{bc}	123.34 $\pm$ 1.52 ^b	113.95 $\pm$ 0.74 ^c	90.48 $\pm$ 4.51 ^d
Epicatechin	26.06 $\pm$ 1.35 ^a	41.5 $\pm$ 1.04 ^b	26.27 $\pm$ 4.13 ^a	26.25 $\pm$ 4.06 ^a	22.27 $\pm$ 6.73 ^a
Emodin	2.13 $\pm$ 0.77 ^a	2.4 $\pm$ 0.43 ^a	2.39 $\pm$ 0.2 ^a	2.2 $\pm$ 0.19 ^a	1.6 $\pm$ 0.74 ^a
Chrysophanol	5.1 $\pm$ 1.15 ^a	5.39 $\pm$ 0.68 ^a	5.18 $\pm$ 0.31 ^a	4.92 $\pm$ 0.39 ^a	3.97 $\pm$ 1.09 ^a

$n=3$; DW – dry weight; the same letters (a-e) next to values indicate no significant differences between means, at least at the level of $p \leq 0.05$. One-way ANOVA and Tukey's Multiple Comparison Test as *post-hoc* for the statistical analysis of data were used (with the exception of compounds next to which there is an *, Kruskal-Wallis test with Dunn's Multiple Comparison Test as *post-hoc* were used)

callus with 86 mM NaCl. Flavonoids were the most abundant group. Catechin was the predominant metabolite in each gender on all media tested, with its content in callus tissue ranging from 213.31 mg/100 g DW (female callus on 129 mM NaCl) to 64.46 mg/100 g DW (male callus on 0 mM NaCl).

Influence of the NaCl stress on the phenolic compounds production within the same sex

One-way ANOVA and Tukey's Multiple Comparison Test as *post-hoc* or Kruskal-Wallis test with Dunn's Multiple Comparison Test as *post-hoc* analysis revealed statistically significant differences in eleven phenolic compound contents in females (Table 1) and seven in males (Table 2) among 22 identified compounds. No statistically significant differences were found for the following PCs in both sexes regardless of NaCl concentration: *p*-coumaric acid, ferulic acid, vitexin, rutin, hyperoside, isoquercetin, myricetin, quercitrin, rhamnetin, emodin, chrysophanol and

additionally in males for neochlorogenic acid, caffeic acid, quercetin and apigenin.

In both the female and male callus cultures, only the content of gallic acid showed significant differences on each medium. In this case, the highest concentration was significant in the female callus at 129 mM and in the male at 43 mM NaCl. Significantly higher PCs concentrations in the female callus compared to the control culture (0 mM NaCl) were found for neochlorogenic acid on 172 mM NaCl, for protocatechuic acid and catechin on all substrates compared to the control medium, for chlorogenic acid on 43 mM, 129 mM and 172 mM NaCl, for cryptochlorogenic acid on 43 mM, for vanillic acid on 129 mM NaCl and for caffeic acid on 86 mM, and 172 mM. In the male callus, on the other hand, such differences were found in the content of protocatechuic acid and catechin on all substrates compared to the control medium, chlorogenic acid on media with 43 mM, 86 mM and 129 mM NaCl, cryptochlorogenic acid and epicatechin on 43 mM NaCl. The catechin content was statistically higher in all media analyzed than in the control culture (0 mM NaCl).

Influence of the NaCl stress on the phenolic compounds production between the diverse sexes

The unpaired Student's *t*-test was used to reveal statistically significant differences in PCs content between the male and female callus suspension cultures. The highest statistically significant differences ($p \leq 0.0001$) were found between female and male calli in each medium for gallic acid content (Table 3). In addition, there were statistically significant differences in protocatechuic acid content, but with different levels of significance (Table 3). Statistically significant differences ($p \leq 0.0001$) were also found between female and male calli in the content of: neochlorogenic acid on 0 mM, 43 mM and 86 mM NaCl; chlorogenic and cryptochlorogenic acids on 86 mM and 129 mM NaCl (two-fold in male and almost three-fold higher content in the female calli, respectively); vanillic and caffeic acids on 86 mM NaCl; catechin on 129 mM NaCl (almost two-fold higher content in the female calli), with an additional significant difference ($p \leq 0.001$) between the callus of the different sexes on 0 mM NaCl medium (Table 3). Statistically significant differences ($p \leq 0.001$) between female and male calli were also found on the control medium in the content of cryptochlorogenic acid, with additional difference occurring at

the level $p \leq 0.01$ appeared on 43 mM NaCl. A significant difference ($p \leq 0.01$) was also observed in the content of: caffeic acid between female calli (two-fold higher content) and male calli on the control medium, without salt stress; *p*-coumaric acid, ferulic acid, vitexin, hyperoside and epicatechin at 43 mM NaCl and catechin on 86 mM NaCl and quercetin on 129 mM NaCl, with additional difference occurring at the level $p \leq 0.05$ on 172 mM NaCl. A significant difference ($p \leq 0.05$) was also observed in the content of: neochlorogenic acid and epicatechin on 172 mM NaCl (over 4 times higher content in male calli); vanillic acid, rutin, isoquercetin, myricetin, quercitrin on 43 mM and epicatechin on 86 mM NaCl (Table 3).

Discussion

Salinity is one of the most important abiotic stress factors that significantly affects the growth and productivity of crops worldwide. It leads to various detrimental effects, such as osmotic stress, nutrient imbalance and oxidative stress (Munns and Tester 2008; Arzani and Ashraf 2016). Salt stress increases osmotic pressure in plant cells and can lead to an accumulation of toxic sodium concentration

Table 3 Results of the unpaired Student's *t*-test within the content of phenolic compounds between sexes on media with different concentrations of NaCl (0 mM, 43 mM, 86 mM, 129 mM and 172 mM)

Phenolic compounds	NaCl concentration				
	0 mM (control)	43 mM	86 mM	129 mM	172 mM
Gallic acid	****	****	****	****	****
Neochlorogenic acid	****	****	****	ns	*
Protocatechuic acid	****	****	****	****	****
Chlorogenic acid	ns	ns	****	****	ns
Cryptochlorogenic acid	***	**	****	****	ns
Vanillic acid	ns	*	****	ns	ns
Caffeic acid	**	ns	****	ns	ns
<i>p</i> -Coumaric acid	ns	**	ns	ns	ns
Ferulic acid	ns	**	ns	ns	ns
Vitexin	ns	**	ns	ns	ns
Rutin	ns	*	ns	ns	ns
Hyperoside	ns	**	ns	ns	*
Isoquercetin	ns	*	ns	ns	ns
Myricetin	ns	*	ns	ns	ns
Quercitrin	ns	*	ns	ns	ns
Quercetin	ns	ns	ns	**	*
Apigenin	ns	ns	ns	ns	ns
Rhamnetin	ns	ns	ns	ns	ns
Catechin	***	ns	**	****	ns
Epicatechin	ns	**	*	ns	*
Emodin	ns	ns	ns	ns	ns
Chrysophanol	ns	ns	ns	ns	ns

$n=3$; ns – not significant, $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), $p \leq 0.0001$ (****)

(Zhao et al. 2021). Plants have to develop different strategies to adapt to saline environments. These strategies include a number of signaling transduction pathways involved in activities ranging from salt stress sensing to the expression of many salt-stress responsive genes that regulate processes such as ion transport, osmotic homeostasis, and detoxification. These mechanisms rely on numerous regulatory elements, such as phytohormones, lipids, the cell wall, and the cytoskeleton (Van Zelm et al. 2020; Zhao et al. 2021).

To counteract the harmful effects of salt stress, plant cells produce phenolic compounds (PCs), which are non-enzymatic antioxidants that help to remove reactive oxygen species (ROS) (Santos-Sánchez et al. 2019). An important function of these compounds is also their effect on human health. Studies have shown an inverse relationship between the consumption of foods rich in antioxidants and the occurrence of diseases in humans (Saoudi et al. 2021). The use of synthetic antioxidants is associated with negative health effects. Therefore, there is growing interest in the study of natural antioxidants (Aiyegoro and Okoh 2009) and their identification in different plant species (Saoudi et al. 2021).

Modern biotechnology enables the use of numerous plant breeding methods and techniques for various purposes. In addition to generating high-quality plant material, plant cell and tissue culture techniques are now also used to synthesize secondary metabolites (SMs) (Baiyin et al. 2021; Ozyigit et al. 2023). Considering the fact that female-biased sex ratio has been observed in populations of *Rumex thyrsiflorus*, we hypothesise that the response of this plant to abiotic stress is the result of sex-specific stress adaptation mechanisms based on physiological and biochemical processes, including the production of SMs as non-enzymatic antioxidants. In general, sexual dimorphism has been found in some physiological traits, such as photochemical capacity, antioxidant enzyme activity, stress tolerance or secondary metabolite content (Juvany and Munné-Bosch 2015; Robakowski et al. 2018). Our previous studies have shown that photosynthetic and antioxidant activity in the response of *R. thyrsiflorus* to heat stress is sex-specific (Gozdur et al. 2023). The results of the present experiment with callus suspension cultures show that the combination of auxin 2,4-D and cytokinin BAP, at concentrations of 1 mg/L and 0.4 mg/L, respectively, caused a significant increase in callus weight. Specifically, the weight of female callus increased almost sevenfold, while the weight of male callus increased more than threefold after 4 weeks of culture. The results of the experiment on the effect of salt stress clearly show that on the medium with 172 mM NaCl the highest increase in fresh weight (4.10 g), dry weight (0.42 g), RFWG (7.20) and RGR (0.08 d⁻¹) values were obtained for female callus. The values of these parameters were significantly higher than at 129 mM NaCl (with average values of 2.04 g, 3.08 and 0.05 d⁻¹,

respectively). It is noteworthy that the difference between female and male callus on a medium containing 172 mM NaCl was significant for the above parameters, and that the values of TI index were significantly higher for female callus, suggesting that female callus is more tolerant to salinity than male callus. However, with the exception of the female callus on 172 mM NaCl, the growth of callus tissue of both sexes was significantly reduced under the influence of increasing NaCl concentration in the medium. This decrease in growth could be attributed to nutrient imbalance due to disturbance by salt ions, i.e., Na⁺ and Cl⁻ (Chamoli 2021).

The fluctuations in the activity of PCs at different salt contents are species-specific. For example, *Glaux maritima* showed peaks in different groups of phenolic compounds at concentrations of 50–300 mM NaCl, while *Spergularia marina* showed the highest content of phenolic compounds when cultured without NaCl or with 500 mM NaCl (Pungin et al. 2023). Remarkable studies have shown a direct correlation between salinity and the concentration of phenolic compounds in different plant species such as *Thymus vulgaris* and *Thymus daenensis* (Bistgani et al. 2019), buckwheat (Lim et al. 2012) or even in cotton (Wang et al. 2015). Conversely, the opposite findings have been documented, showing a decrease in the content of phenolic compounds when exposed to NaCl in certain plant species such as broccoli (López-Berenguer et al. 2009) or sugarcane (Wahid and Ghazanfar 2006). Previous studies have confirmed the presence of PCs in extracts from *R. thyrsiflorus* roots, including phenolic acids (caffeic acid, gallic acid, *p*-hydroxybenzoic acid), flavonoids (isorhamnetin, myricetin, rutoside, quercetin), catechins (catechin, epicatechin gallate) and other phenols, e. g. hydroquinone or pyrogallol (Litvinenko and Muzychkina 2008). In 2017, Orbán-Gyapai confirmed the presence of epicatechin, palmitoyl glycerol, β -sitosterol and procyanidin B5 in extracts from the underground parts of *R. thyrsiflorus*. The author also found a significant inhibitory effect of these compounds against human cancer cell lines and bacterial strains.

In the study by Ma et al. (2018) on germinated hullless barley, a peak in total phenolic content was observed after treatment with 60 mM NaCl. However, in our study, we observed the highest phenolic compound content at 129 mM for females and 43 mM NaCl for males. In this experiment, gallic acid content was found to increase significantly in callus tissue of both sexes cultured on NaCl-containing media compared to the control. Gallic acid has been attributed to anti-HIV activity, as it inhibits HIV reverse transcriptase and protease activity (Nutan et al. 2013). The supply of this acid increases the salt tolerance of plants by increasing growth and chlorophyll content (Babaei et al. 2022). A significant increase in the concentration of neochlorogenic acid was only observed in the female callus growing on medium with 172 mM NaCl in this experiment. Neochlorogenic

acid influences lipid and glucose metabolism and has hypoglycaemic and hypolipidemic effects in particular (Yu et al. 2021). The content of protocatechuic acid was significantly increased in the callus tissue of both sexes in the test sample. Protocatechuic acid exhibits antiatherosclerotic, anti-inflammatory, anticancer, analgesic, antimicrobial, hepatoprotective and antiviral properties (Zhang et al. 2021). Chlorogenic acid has properties that can fight cancer, inflammation, oxidation, bacterial infections, and viral infections (Ma et al. 2022). A significant increase in this acid concentration was observed in female callus (except 86 mM NaCl) and male callus (except 172 mM NaCl). Structurally like the former, cryptochlorogenic acid can alleviate inflammatory symptoms caused by the LPS endotoxin of gram-negative bacteria by modulating signaling cascades (Zhao et al. 2020). Its concentration was the highest among the phenolic acids analyzed in *R. thyrsoiflorus*, in female callus tissue at 129 mM NaCl and in male tissue at 43 mM NaCl (76.35 mg/100 g DW and 41.45 mg/100 g DW, respectively). Of the flavonoids analyzed, the most notable compound was catechin, the concentration of which increased significantly when the tissue was treated with salt stress. Significant gender differences were found in the concentrations at 0 mM, 129 mM and 172 mM NaCl, with a predominance of female callus tissue at 129 mM NaCl, similar to epicatechin. Catechin and epicatechin are flavonoids with an isomeric structure relative to each other. These molecules help prevent cell damage and combat certain chronic diseases such as cardiovascular and neurodegenerative diseases. They also have properties that can inhibit tumor growth, reduce inflammation and fight viruses (Eugène et al. 2022). Interestingly, the catechin content of *R. thyrsoiflorus* was 1.5-fold higher in female callus suspension cultures on a medium with 129 mM and 172 mM NaCl and 2.5-fold higher in male callus cultures on 86 mM NaCl than in leaf extracts from natural populations. Similarly, the content of gallic acid was fivefold higher in callus tissue compared to leaves from natural populations (Dziedzic et al. 2020).

Conclusion

For the first time, a comprehensive phytochemical analysis of gender-related aspects of the salinity response was performed in suspension cultures of the dioecious model species *Rumex thyrsoiflorus*. It was shown that the response of *R. thyrsoiflorus* to salt stress is dependent on sex and NaCl concentration in the medium. The analysis of the main morphometric parameters showed that the callus tissue derived from the hypocotyls isolated from female seedlings was

less sensitive to salt stress compared to male, which correlated with higher concentrations of PCs which may serve as non-enzymatic antioxidants in the female callus. The most suitable concentration to increase PCs production in female callus was 129 mM NaCl, while in male callus was 43 mM NaCl in the culture medium. Based on these results, it will be possible to obtain lines of female callus tissue that exhibit increased synthesis of 11 secondary metabolites, with the dominance of catechin. These results also have important implications for the study of tolerance of dioecious plants to environmental stress in the context of climate change. Furthermore, they have shown that NaCl can be used as an elicitor to increase the production of pharmaceutically valuable compounds and highlight the importance of dioecy in phytochemistry.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

AS is one of the Associate Editors of the journal at the time of submission. This had no impact on the peer review process and the final decision.

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References

- Aiyegoro O, Okoh A (2009) Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysum pedunculatum*. *Int J Mol Sci* 10(11):4990–5001. <https://doi.org/10.3390/ijms10114990>
- Al Mamari HH (2021) Phenolic compounds: classification, chemistry, and updated techniques of analysis and synthesis. In: Badria FA (ed) Phenolic compounds – chemistry, synthesis, diversity, non-conventional industrial, pharmaceutical and therapeutic applications. IntechOpen. <https://doi.org/10.5772/intechopen.98958>
- Arzani A, Ashraf M (2016) Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. *Crit Rev Plant Sci* 35(3):146–189. <https://doi.org/10.1080/07352689.2016.1245056>
- Babaei M, Shabani L, Hashemi-Shahraki S (2022) Improving the effects of salt stress by β -carotene and gallic acid using increasing antioxidant activity and regulating ion uptake in *Lepidium sativum* L. *Bot Stud* 63:22. <https://doi.org/10.1186/s40529-022-00352-x>
- Baiyin B, Tagawa K, Yamada M, Wang X, Yamada S, Shao Y, An P, Yamamoto S, Ibaraki Y (2021) Effect of nutrient solution flow rate on hydroponic plant growth and root morphology. *Plants* 10(9):1840. <https://doi.org/10.3390/plants10091840>
- Bistgani ZE, Hashemi M, DaCosta M, Craker L, Maggi F, Morshedloo MR (2019) Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenesis* Celak. *Ind Crop Prod* 135:311–320. <https://doi.org/10.1016/j.indcrop.2019.04.055>
- Chamoli A (2021) Effect of salinity on growth of callus culture in *Dalbergia Sissoo*. *J Plant Biochem Physiol* 9:271
- Datta S, Katna J, Shrivastav D, Narayan SB, Umar S, Sastry JL, Mitra R, Ahmad S (2021) Effect of salinity stress on growth, water content, and guggulsterone production in callus cultures of *Commiphora wightii* (Arnott.) Bhandari. *Phcog Mag* 17(6):225. https://doi.org/10.4103/pm.pm_514_20
- Dziedzic K, Szopa A, Waligórski P, Ekiert H, Ślesak H (2020) Sex-related differences in the dioecious species *Rumex thyrsiflorus* Fingerh. – analysis of the content of phenolic constituents in leaf extracts. *Acta Biol Crac Ser Bot* 62(1):43–50. <https://doi.org/10.24425/abscb.2020.131663>
- Ellnain-Wojtaszek M, Zgórk G (1999) High-performance liquid chromatography and thin-layer chromatography of phenolic acids from *Ginkgo biloba* L. leaves collected within vegetative period. *J Liq Chromatogr Relat* 22(10):1457–1471. <https://doi.org/10.1081/JLC-100101744>
- El-Shafey NM, Sayed M, Ahmed ES, Hammouda O, Khodary SEA (2019) Effect of growth regulators on micropropagation, callus induction and callus flavonoid content of *Rumex pictus* Forssk. *Egypt J Bot* 59(2):293–302. <https://doi.org/10.21608/EJBO.2019.4873.1202>
- Eugène EA, Robert NB, Ganiyou A, Denis YK, Ané A, Sawaliho BEH (2022) Catechin and epicatechin. What's the more reactive? *Comput Chem* 10:53–70. <https://doi.org/10.4236/cc.2022.102003>
- Gawel NJ, Jarret RL (1991) A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Plant Mol Biol Rep* 9(3):262–266. <https://doi.org/10.1007/BF02672076>
- Gozdur K, Ślesak I, Ślesak H (2023) Photosynthetic and antioxidant activity in the sex-related heat stress response of the dioecious species *Rumex thyrsiflorus* Fingerh. *Plant Cell Tiss Organ Cult* 152:151–165. <https://doi.org/10.1007/s11240-022-02397-z>
- Horie T, Karahara I, Katsuhara M (2012) Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice (n y)* 5(1):11. <https://doi.org/10.1186/1939-8433-5-11>
- Juvany M, Munné-Bosch S (2015) Sex-related differences in stress tolerance in dioecious plants: a critical appraisal in a physiological context. *J Exp Bot* 66(20):6083–6092. <https://doi.org/10.1093/jxb/erv343>
- Korpeläinen H (2002) A genetic method to resolve gender complements investigations on sex ratios in *Rumex acetosa*. *Mol Ecol* 11(10):2151–2156. <https://doi.org/10.1046/j.1365-294X.2002.01593.x>
- Kwolek D, Joachimiak AJ (2011) Seed sexing revealed female bias in two *Rumex* species. *Acta Soc Bot Pol* 80(2):93–97. <https://doi.org/10.5586/asbp.2011.028>
- Lajter I, Zupko I, Molnár J, Jakab G, Balogh L, Vasas A, Hohmann J (2013) Antiproliferative activity of Polygonaceae species from the Carpathian Basin against human cancer cell lines. *Phytother Res* 27(1):77–85. <https://doi.org/10.1002/ptr.4690>
- Liao J, Song H, Tang D, Zhang S (2019) Sexually differential tolerance to water deficiency of *Salix paraplesia* A Female-Biased Alpine Willow. *Ecol Evol* 9:8450–8464. <https://doi.org/10.1002/ece3.5175>
- Lim J-H, Park K-J, Kim B-K, Jeong J-W, Kim H-J (2012) Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. *Food Chem* 135(3):1065–1070. <https://doi.org/10.1016/j.foodchem.2012.05.068>
- Litvinenko YA, Muzychikina RA (2008) New antioxidant phyto-preparation from *Rumex thyrsiflorus* roots. *III Chem Nat Compd* 44(2):239–240. <https://doi.org/10.1007/s10600-008-9026-y>
- López-Berenguer C, Martínez-Ballesta MC, Moreno DA, Carvajal M, García-Viguera C (2009) Growing hardier crops for better health: Salinity tolerance and the nutritional value of broccoli. *J Agric Food Chem* 57(2):572–578. <https://doi.org/10.1021/jf802994p>
- Ma Y, Wang P, Chen Z, Gu Z, Yang R (2018) NaCl stress on physio-biochemical metabolism and antioxidant capacity in germinated hulless barley (*Hordeum vulgare* L.). *J Sci Food Agric* 99(4):1755–1764. <https://doi.org/10.1002/jsfa.9365>
- Ma X, Okyere SK, Hu L, Wen J, Ren Z, Deng J, Hu Y (2022) Anti-inflammatory activity and mechanism of cryptochlorogenic acid from *Ageratina adenophora*. *Nutrients* 14(3):439. <https://doi.org/10.3390/nu14030439>
- Mariotti B, Manzano S, Kejnovsky E, Vyskot B, Jamilena M (2009) Accumulation of Y-specific satellite DNAs during the evolution *Rumex acetosa* sex chromosomes. *Mol Genet Genomics* 281(3):149–259. <https://doi.org/10.1007/s00438-008-0405-7>
- Mera IFG, Falconí DEG, Córdova VM (2019) Secondary metabolites in plants: main classes, phytochemical analysis and pharmacological activities. *Bionatura* 4:1000–1009. <https://doi.org/10.21931/RB/2019.04.04.11>
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:437–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Navajas-Pérez R, De La Herrán R, Jamilena M, Lozano R, Rejón CR, Rejón MR, Garrido-Ramos MA (2005) Reduced rates of sequence evolution of Y-linked satellite DNA in *Rumex* (Polygonaceae). *J Mol Evol* 60:391–399. <https://doi.org/10.1007/s00239-004-0199-0>
- Navajas-Pérez R, Schwarzacher T, De La Herrán R, Ruiz Rejón C, Ruiz Rejón M, Garrido-Ramos MA (2006) The origin and evolution of the variability in a Y-specific satellite-DNA of *Rumex acetosa* and its relatives. *Gene* 368:61–71. <https://doi.org/10.1016/j.gene.2005.10.013>
- Nutan, Modi M, Goel T, Das T, Malik S, Suri S, Rawat AK, Srivastava SK, Tuli R, Malhotra S, Gupta SK (2013) Ellagic acid & gallic acid from *Lagerstroemia speciosa* L. inhibit HIV-1 infection through inhibition of HIV-1 protease & reverse transcriptase activity. *Indian J Med Res* 137(3):540–548

- Orbán-Gyapai O, Forgo P, Hohmann J, Vasas A (2017) Phytochemical investigation of *Rumex thyrsiflorus* Fingerh. Acta Biol Hung 68(2):232–236. <https://doi.org/10.1556/018.68.2017.2.10>
- Ozyigit II, Dogan I, Hocaoglu-Ozyigit A, Yalcin B, Erdogan A, Yalcin IE, Cabi E, Kaya Y (2023) Production of secondary metabolites using tissue culture-based biotechnological applications. Front Plant Sci 14:1132555. <https://doi.org/10.3389/fpls.2023.1132555>
- Pan Y, Li L, Xiao S, Chen Z, Sarsaiya S, Zhang S, ShangGuan Y, Liu H, Xu D (2020) Callus growth kinetics and accumulation of secondary metabolites of *Bletilla striata* Rchb.f. using a callus suspension culture. PLoS One 15(2):e0220084. <https://doi.org/10.1371/journal.pone.0220084>
- Pungin A, Lartseva L, Loskutnikova V, Shakhov V, Popova E, Skrypnik L, Krol O (2023) Effect of salinity stress on phenolic compounds and antioxidant activity in halophytes *Spergularia marina* (L.) Griseb. and *Glaux maritima* L. cultured in vitro. Plants 12(9):1905. <https://doi.org/10.3390/plants12091905>
- Robakowski P, Pers-Kamczyc E, Ratajczak E, Thomas PA, Ye Z-P, Rabska M, Iszkuło G (2018) Photochemistry and antioxidative capacity of female and male *Taxus baccata* L. acclimated to different nutritional environments. Front Plant Sci 9:742. <https://doi.org/10.3389/fpls.2018.00742>
- Salehi M, Moieni A, Safaie N (2017) A novel medium for enhancing callus growth of hazel (*Corylus avellana* L.). Sci Rep 7(1):15598. <https://doi.org/10.1038/s41598-017-15703-z>
- Santos-Sánchez NF, Salas-Coronado R, Hernández-Carlos B, Villanueva-Cañongo C (2019) Shikimic acid pathway in biosynthesis of phenolic compounds. In: Soto-Hernández M, García-Mateos R, Palma-Tenango M (eds) Plant physiological aspects of phenolic compounds. IntechOpen. <https://doi.org/10.5772/intechopen.83815>
- Saoudi MM, Bouajila J, Rahmani R, Alouani K (2021) Phytochemical composition, antioxidant, antiacetylcholinesterase, and cytotoxic activities of *Rumex crispus* L. Int J Anal Chem 2021:6675436. <https://doi.org/10.1155/2021/6675436>
- Ślesak H, Góralski G, Kwolek D, Dziedzic K, Grabowska-Joachimiak A (2015) Male adventitious roots of *Rumex thyrsiflorus* Fingerh. as a source of genetically stable micropropagated plantlets. Plant Cell Tiss Organ Cult 123(1):193–203. <https://doi.org/10.1007/s11240-015-0826-z>
- Soheilikhah Z, Karimi N, Ghasmpour HR, Zebarjadi AR (2013) Effects of saline and mannitol induced stress on some biochemical and physiological parameters of *Carthamus tinctorius* L. varieties callus cultures. Aust J Crop Sci 7(12):1866–1874
- Sułkowska-Ziaja K, Maślanka A, Szewczyk A, Muszyńska B (2017) Physiologically active compounds in four species of *Phellinus*. Nat Prod Commun 12(3):363–366. <https://doi.org/10.1177/1934578X1701200313>
- Szopa A, Kokotkiewicz A, Bednarz M, Luczkiewicz M, Ekiert H (2017a) Studies on the accumulation of phenolic acids and flavonoids in different in vitro culture systems of *Schisandra chinensis* (Turcz.) Baill. using a DAD-HPLC method. Phytochem Lett 20:462–469. <https://doi.org/10.1016/j.phytol.2016.10.016>
- Szopa A, Kokotkiewicz A, Kubica P, Banaszcak P, Wojtanowska-Krośniak A, Krośniak M, Marzec-Wróblewska U, Badura A, Zagrodzki P, Bucinski A, Luczkiewicz M, Ekiert H (2017) Comparative analysis of different groups of phenolic compounds in fruit and leaf extracts of *Aronia* sp.: *A. melanocarpa*, *A. arbutifolia*, and *A. xprunifolia* and their antioxidant activities. Eur Food Res Technol 243:1645–1657. <https://doi.org/10.1007/s00217-017-2872-8>
- Van Zelm E, Zhang Y, Testerink C (2020) Salt Tolerance Mechanisms of Plants. Annu Rev Plant Biol 29(71):403–433. <https://doi.org/10.1146/annurev-arplant-050718-100005>
- Wahid A, Ghazanfar A (2006) Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J Plant Physiol 163:723–730. <https://doi.org/10.1016/j.jplph.2005.07.007>
- Wang Q, Eneji AE, Kong X, Wang K, Dong H (2015) Salt stress effects on secondary metabolites of cotton in relation to gene expression responsible for aphid development. PLoS ONE 10(6):e0129541. <https://doi.org/10.1371/journal.pone.0129541>
- Yang L, Wen K-S, Ruan X, Zhao Y-X, Wei F, Wang Q (2018) Response of plant secondary metabolites to environmental factors. Molecules 23(4):762. <https://doi.org/10.3390/molecules23040762>
- Yu M-H, Hung T-W, Wang C-C, Wu S-W, Yang T-W, Yang C-Y, Tseng T-H, Wang C-J (2021) Neochlorogenic acid attenuates hepatic lipid accumulation and inflammation via regulating miR-34a in vitro. Int J Mol Sci 22(23):13163. <https://doi.org/10.3390/ijms222313163>
- Zarzycki K, Rychlewski J (1982) Ekologiczne i genetyczne mechanizmy regulujące proporcje płci w populacjach dwupiennego szczawiu rozpierzchłego (*Rumex thyrsiflorus* Fingerh.). Wiadomości Ekologiczne XXVIII(3/4):199–208
- Zhang S, Zhibo G, Juanli C, Qingfa C, Yunlun L (2021) Antioxidant effects of protocatechuic acid and protocatechuic aldehyde: old wine in a new bottle. Evid Based Complement Alternat Med 2021:6139308. <https://doi.org/10.1155/2021/6139308>
- Zhao XL, Yu L, Zhang SD, Ping K, Ni HY, Qin XY, Zhao CJ, Wang W, Efferth T, Fu YJ (2020) Cryptochlorogenic acid attenuates LPS-induced inflammatory response and oxidative stress via upregulation of the Nrf2/HO-1 signaling pathway in RAW 264.7 macrophages. Int Immunopharmacol 83:106436. <https://doi.org/10.1016/j.intimp.2020.106436>
- Zhao S, Zhang Q, Liu M, Zhou H, Ma C, Wang P (2021) Regulation of plant responses to salt stress. Int J Mol Sci 22(9):4609. <https://doi.org/10.3390/ijms22094609>
- Žuk J (1963) An investigation on polyploidy and sex-determination within the genus *Rumex*. Acta Soc Bot Pol 32(1):5–72. <https://doi.org/10.5586/asbp.1963.001>

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