



Sumac (*Rhus coriaria* L.) sprouts: from in vitro seed germination to phenolic content and antioxidant activity for biotechnological application

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Abstract

The sumac plant (*Rhus coriaria* L.), native to the Middle East, but also growing in Italy, is used for the medicinal properties of its fruits and leaves. Recently, sumac plant have been characterized in terms of their bioactive compound content and biological activity. Regrettably, there is a lack of information on the chemical composition of sumac sprouts, which, on the contrary, could be a rich source of interesting compounds, as demonstrated for other plant species. To make the production of sumac sprouts faster and independent of environmental conditions, in vitro tissue culture can be an efficient solution. This study first established a valid protocol for in vitro sumac seed germination; secondly, the obtained sprouts were characterized, in terms of phenolic content and antioxidant activity. The results showed that mechanical scarification is mandatory to trigger in vitro germination of sumac seeds. Moreover, a significant final germination percentage and a reasonable mean germination time was obtained combining scarification, cold stratification and gibberellic acid-enriched culture media. Chemical characterization of sumac sprouts has shown that they are a source of potentially bioactive compounds, as they are rich in polyphenols and have appreciable antioxidant activity.

Key message

Sprouts are a wealth source of bioactive compounds: an in vitro sumac germination protocol has been set up and sprouts have been physicochemically characterized.

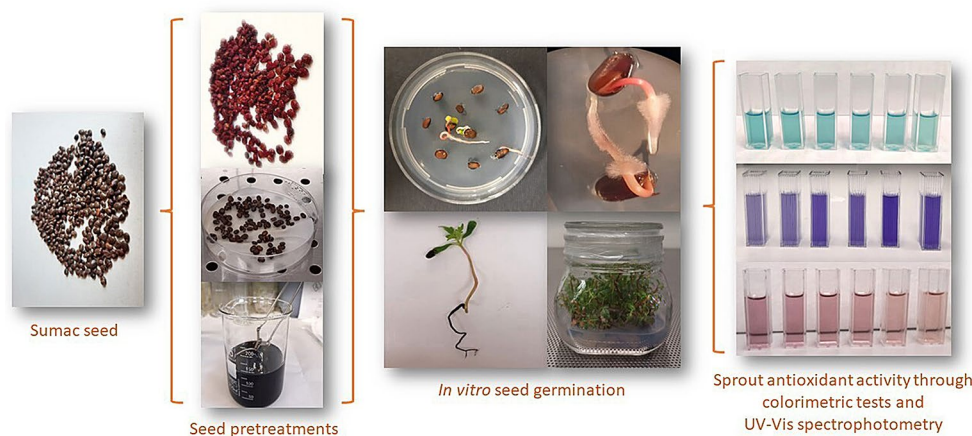
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Graphical Abstract



Keywords Antioxidant activity · Polyphenols · Seed pretreatments · Sumac · Vitro-derived sprouts

Introduction

Sumac (*Rhus coriaria* L.), belonging to the *Anacardiaceae* family, is an evergreen plant, widespread in temperate and tropical regions. In the Middle East, such as in Iraq and in Iran, where it grows both wild and cultivated (Wetherilt and Pala 1994), sumac is used, mainly, as flavoring spice with a specific color, feature from which it derives its name; indeed, in Arabic, the term “summaq” means “dark red”. Sumac is widespread, also, in the Mediterranean area, and specifically in Sicily (an island in Southern Italy), so much that is commonly known as Sicilian sumac. In Sicily, sumac was cultivated back to sixty years ago for leather processing, mainly because of its tannin content and their coloring properties (Giovannelli et al. 2017; Inzenga 1875; Prigioniero et al. 2020). On the other hand, in the Asian traditional medicines, the healing properties of sumac were very well known; several parts of the sumac plant were reported to possess a wide biological activity, including antimicrobial, antioxidant, hypoglycemic, hypolipidemic, antimutagenic, antimigratory, and anti-ischemic activities (Sakhr and El Khatib 2020). In the last years, research has been carried out to individuate the compounds responsible for these important sumac properties; in particular, these chemical constituents can be assigned to various classes of the hydrolysable tannins, phenolic acids, conjugated phenolic acids, anthocyanins, flavonoids, organic acids, coumarins, xanthenes, terpenoids, steroids, essential oils, and other groups of constituents have been reported in literature (Isgrò et al. 2022). Until now, more than 211 phytoconstituents have been extracted and identified from different parts of sumac plant, and most of them are physiologically active (Abu-Reidah et al. 2015; Arena et al. 2022), but there is

insufficient information available regarding the properties of sumac sprouts. In fact, the germination process increases the bioavailability of several nutrients, through the activity of enzymes that convert complex molecules, such as proteins, carbohydrates, and lipids, into forms easier to be metabolized. Several studies, carried out on a plethora of plant species, report as sprouts are a precious source of phytonutrients, coupled with reduced antinutritional factors (Aloo et al. 2021), no data are available for sprouts from sumac. These premises make worth to start investigating on the sumac sprout properties, and, to this aim, the first step should be the set-up of a protocol that would guarantee a high germination percentage. This step is crucial because sumac seeds are characterized by physical and physiological dormancy that needs to be broken in order to start the germination process (Salih et al. 2016). Researches have been carried out evaluating several treatments aimed at speeding sumac seed germination up, such as scarification with sulfuric acid, alone or in combination with cold stratification (Olmez et al. 2007; Tilki and Bayraktar 2013), seed imbibition with gibberellic acid (Pipinis et al. 2017), dry (Ne’eman et al. 1999) or moist (Doussi and Thanos 1994) heat, but, to the best of our knowledge, these treatments have never been used in in vitro germination studies. In vitro culture represents a valuable framework of research, presenting several advantages over the traditional propagation methods, among which the independence from environmental conditions, the necessity of limited growth space and the more rapid growth of sprouts. Moreover, since germination conditions, such as light, temperature and culture medium composition, play a key role in the sprout composition, vitro-derived sumac sprouts could likely present a secondary metabolite profile different from that of those obtained in vivo, as observed

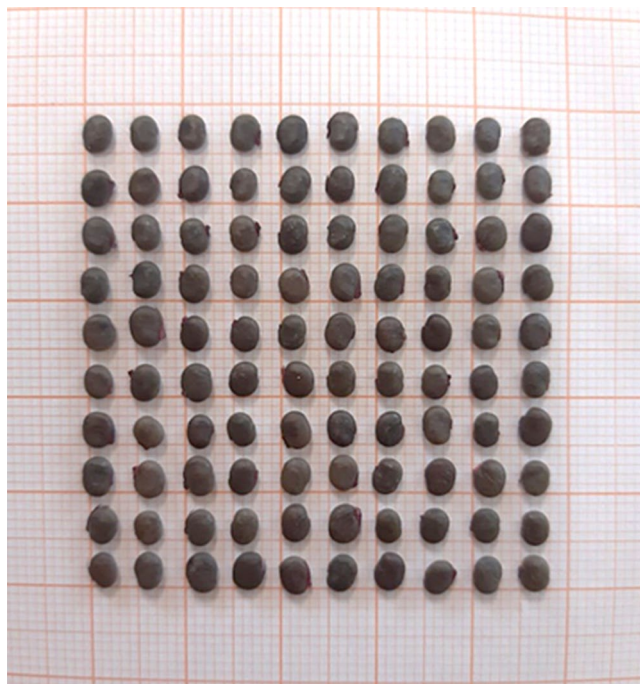


Fig. 1 Sumac seeds before in vitro sowing

in other plant species (Cirlini et al. 2022); acquiring this knowledge represents a turning point in the perspective of using the vitro-derived sprouts as biotechnological matrix for bioactive compound extraction or as novel food.

Therefore, the aim of this study was to fill the knowledge gap on the chemical composition of vitro-derived sumac sprouts, in terms of total phenolic content (TPC) and antioxidant activity (AO), with particular emphasis on exploring their potential for application in various biotechnological

fields such as nutrition, medicine, phytochemistry and pharmacology.

Materials and methods

Plant material

Sumac seeds were isolated from sun dried drupes, harvested full ripen from wild plants grown in Sicily (southern Italy). Each seed was 4 × 3 mm and the weight of 100 seeds used for further experiments was 1.43 g (Fig. 1).

In vitro sumac seed germination

Germination inductive treatments

Prior to implementing treatments, viable sumac seeds were isolated from non-viable ones through flotation. Subsequently, viable seeds underwent mechanical, chemical, and physical scarification methods employing sulfuric acid or hot water to break physical dormancy, along with gibberellic acid (GA_3) and/or cold stratification to overcome physiological dormancy. The details of the treatments applied are summarized in Table 1. *Scarification test*: viable seeds were, first, subjected to mechanical scarification (MeSC) by a blending treatment (30s at 10.000 rpm), afterwards, MeSC seeds were subjected to chemical scarification treatments with 96% sulfuric acid (H_2SO_4 purchased by Sigma-Aldrich®) for 60 min (ChSC), or to physical scarification with distilled water at 100 °C for 1 min (WaSC). MeSC was performed as pre-treatment for the subsequent tests.

Table 1 Different inductive treatments for the germination of *Rhus coriaria* L. seeds

C.: Control (no treatment); MeSC: mechanically scarified seeds; ChSC: chemically scarified seeds; WaSC: physically scarified seeds; T0: no cold stratification; T0.5: cold stratification for 2 weeks; T1: cold stratification for 4 weeks; T1.5: cold stratification for 6 weeks; T2: cold stratification for 8 weeks IGA3:imbibition in solution with 500 mg/l gibberellic acid for 24h at room T in the dark;; MS0:basic culture medium; MS0.5: MS0 with 0.5 mg/ml GA_3 ; MS1: MS0 with 1 mg/ml GA_3 .

Test	Pretreatment	Treatment	Cold stratification	Culture Media
<i>Scarification</i>	C	-	-	MS0; MS0.5; MS1
	C	ChSC	-	MS0; MS0.5; MS1
	C	WaSC	-	MS0; MS0.5; MS1
	MeSC	-	-	MS0; MS0.5; MS1
	MeSC	ChSC	-	MS0; MS0.5; MS1
	MeSC	WaSC	-	MS0; MS0.5; MS1
<i>Cold Stratification</i>	MeSC	ChSC	T0	MS0; MS0.5; MS1
	MeSC	WaSC	T0	MS0; MS0.5; MS1
	MeSC	ChSC	T0.5	MS0; MS0.5; MS1
	MeSC	WaSC	T0.5	MS0; MS0.5; MS1
	MeSC	ChSC	T1	MS0; MS0.5; MS1
	MeSC	WaSC	T1	MS0; MS0.5; MS1
	MeSC	ChSC	T1.5	MS0; MS0.5; MS1
	MeSC	WaSC	T1.5	MS0; MS0.5; MS1
	MeSC	ChSC	T2	MS0; MS0.5; MS1
	MeSC	WaSC	T2	MS0; MS0.5; MS1
	MeSC	-	-	MS0; MS0.5; MS1
	MeSC	IH ₂ O	-	MS0; MS0.5; MS1
<i>Imbibition</i>	MeSC	IGA ₃	-	MS0; MS0.5; MS1

Cold stratification test: MeSC seeds were, previously, treated with 50% commercial bleach to avoid contamination, then, placed in Petri dishes on sterile absorbent paper, and stored at 4 °C for 2 (“T0.5”), 4 (“T1”), 6 (“T1.5”) o 8 (“T2”) weeks.

Imbibition test: MeSC seeds were treated soaking in sterile distilled water (IH₂O) or in a 500 mg/l of gibberellic acid (GA₃) solution (IGA₃) for 24 h in the dark at 25 ± 1 °C.

In vitro establishment of culture

To obtain in vitro cultures, free from contaminations, sumac seeds were subjected, under a flow cabinet, to a sterilization treatment. This process consisted in immersing the seeds, first, in 70% ethanol (v/v) for 5 min, then in 25% commercial bleach (v/v) for 20 min, and finally in sterile distilled water for three times (Liberatore et al. 2018). Sterilized seeds were placed in Petri dishes (60 × 15 mm) (ten seeds for each Petri dish, ten Petri dishes per treatment) with 10 ml of culture medium. To further promote sumac seed germination, various concentrations of GA₃ were added to the culture media. The culture media utilized for all experiments consisted of MS0 salt and vitamin mixture (1×) (Murashige and Skoog 1962), and 30 g/L of sucrose; supplemented with MS0.5 (MS0 with 0.5 mg/L GA₃) and MS1 (MS0 with 1.0 mg/L GA₃). The pH was adjusted to 5.8, and 8 g/L of agar was added to the culture media before autoclaving at 121 °C for 21 min. Petri dishes (60 × 15 mm) with cultured seeds were then sealed and placed in a growth-room in the dark at 25 ± 1 °C and, after 30 days, at 25 ± 1 °C, 20 μmol m⁻² s⁻¹ of light intensity and 16 h of photoperiod, until the end of experiments.

Germinated seeds, with evident rootlets and cotyledons, were transferred from Petri dishes to 500 ml glass jars containing MS0 culture medium to stimulate sprout growing.

Total phenolic content (TPC) and antioxidant activity (AO) of sumac vitro-derived plant material

To evaluate the TPC and the AO, vitro-derived sprouts were selected from those grown on MS0 medium; two stages of development were chosen: sprouts with 2–4 true leaves, about two weeks from in vitro germination (Fig. 2a), and sprouts with 6–8 leaves, about four weeks from in vitro germination (Fig. 2b). Moreover, leaves and roots (Fig. 2c and d), isolated from 6 weeks old plantlets, were subjected to the same chemical analysis of sprouts.

Sample extraction

Prior to the extraction procedure, sprouts and plantlets were extracted from jars, and culture medium residues were eliminated by washing roots with distilled water, according to the protocol proposed by Chiancone et al. (2023). To perform the polyphenols extraction, a protocol reported by Martelli et al. (2020), with slight modifications was used. Plant material was subjected to a lyophilisation step for 48 h, prior the analyses, using a Lio-5P Freeze dryer (5 Pa, Milan, Italy). Then, 5 g of lyophilized and ground sample were extracted with 5 ml of aqueous methanol solution (70%), using a shaker (HS 501 digital shaker, IKA-Werke GmbH & Co, Staufen, Germany), at 200 strokes/min for 30 min, and then extracts were centrifuged using a Centrifuge 4206 centrifuge (ALCI International, Milan, Italy) at room temperature, for 10 min at 5000 rpm. Two dilution tests (1:10 and 1:50) were carried out on the supernatant to determine the ideal concentration range for spectrophotometric analysis.

To performed tannins extraction the protocol proposed by Zalacain et al. (2003) was followed. Briefly, 100 mg of dry samples were diluted with 3 ml of distilled H₂O at 45 °C, for two hours, and then were centrifugated for 10 min at 10,000 rpm. In order to understand the ideal concentration,

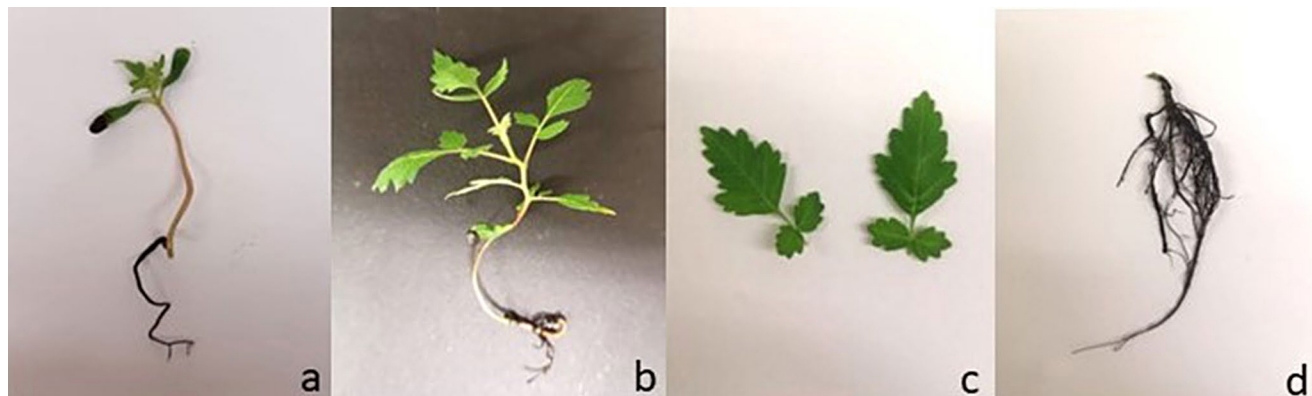


Fig. 2 Plant material tested for polyphenols content and antioxidant activity: (a) 2 weeks old and (b) 4 weeks old vitro-derived sumac sprouts; (c) leaves and (d) roots from vitro-derived sumac plantlets

three dilution tests (1:10, 1:50 and 1:100) were performed. TPC and AO were performed both on methanol and water extracts. For each sprout developmental stage, two replicates were prepared.

Folin-Ciocalteu assay

The TPC was evaluated according to the protocol of Martin-Diana et al. (2017) with few modifications. Briefly, a mixture of 250 μl of extract, 1 ml of Folin-Ciocalteu's phenol reagent, previously diluted ($1/10 \text{ v v}^{-1}$) in bi-distilled water, and 2 ml of a sodium carbonate aqueous solution ($10\% \text{ w v}^{-1}$) were mixed and kept in the dark for 30 min. A spectrophotometer (JASCO V-530 spectrophotometer, Easton, MD, USA) was used to detect the absorbance at 760 nm. In order to calculate the TPC, a calibration curve with gallic acid in a concentration range of 10–100 mg/kg (5 points), was constructed. All the analyses were repeated twice. Results obtained in analyzed samples were then expressed as mg GAE/g DM (Gallic Acid Equivalent; Dry Matter).

DPPH assay

To determine the radical scavenging capacity of leaves and root extracts, the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was applied according to Abram et al. (2015), with slight modifications. In brief, 2.9 ml of an ethanolic DPPH solution (0.05 mM) and 100 μl of sample extract were mixed and kept in the dark for 30 min. The samples' absorbance was then measured using a JASCO V-530 spectrophotometer in triplicate at 517 nm. In addition, a blank constituted of 100 μl of extraction solution was also analyzed under the same experimental conditions applied to samples. Trolox was used as the reference standard to create a calibration curve, a concentration ranges from 0.1 mM to 1 mM (5 points), in order to quantify the antioxidant capacity of the extracts. The radical scavenging capability (I%) was determined using the radical's percentage of inhibition. Results were then expressed as mM TEAC (Trolox Equivalent Antioxidant Capacity). All the analyses were conducted twice.

ABTS assay

ABTS [2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] assay was carried out following the method reported by Wu et al. (2021). ABTS radical solution was prepared by mixing ABTS solution (7mM) and potassium persulfate (2.45 mM), keeping it in the dark for about 16 h. Then, the solution was diluted with ethanol ($1/70 \text{ v v}^{-1}$), in order to obtain an $\text{ABTS}^{+\cdot}$ radical working solution with an absorbance of 0.7 ± 0.2 at 734 nm (JASCO V-530 spectrophotometer, Easton, MD, USA). 100 μl of sample extract (or

a blank or standard solution) were treated with 1900 μl of the ABTS + radical working solution. The reaction was carried out at room temperature in the dark, and after that, the absorbance of each sample was measured at 734 nm. The quantification was performed based on a Trolox as already described in the DPPH determination procedure. All the analyses were repeated twice.

Determination of ferric reducing power

The antioxidant capacity of the tested samples was, also, evaluated by FRAP assay, according to protocol proposed by Keskin et al. (2019). Briefly, the FRAP reagent solution was prepared by mixing 25 ml of acetate buffer solution 300 mM, prepared starting from acetate and acetic acid ($\text{pH}=3.6$), 2.5 ml of an aqueous $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM) and 2.5 ml of an aqueous solution of 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM) acidified with hydrochloric acid (40 mM). Before use, the solution was heated at 37°C for 30 min. Sample extracts, blank sample, and Trolox standard solutions (150 μL) were submitted to the reaction with FRAP solution (1.85 ml) and kept in the dark room temperature for 30 min. The absorbance of all the samples was measured at 593 nm (JASCO V-530 spectrophotometer, Easton, MD, USA), and the ferric reducing power was obtained based on a Trolox calibration curve, in the same concentration range considered for the previous tests, and results were expressed as mM TEAC. All the analyses were repeated twice.

Estimation of gallotannin content

Gallotannin content was estimated by acid hydrolysis according to Zalacain et al. (2003), with some modifications. Briefly, in vials, the aqueous extracts (1 ml) were mixed with 1.2 ml of HCl 4.4 M and heated for 24 h at 90°C . After that, a netutralization step with sodium hydroxide (20 N) was done. The hydrolysed and neutralized samples was centrifuged for 5 min (at 5000 rpm). The supernatant, rich in tannins, was subjected to Folin-Ciocalteu method and results were calculated as difference between hydrolysed and non-hydrolysed extract and expressed as mg GAE/g DM.

Statistical data analysis

In vitro sumac seed germination

For each test, it was monitored the germination process, using a stereomicroscope (Leica Microsystems, Germany), every week for ten weeks; seeds were considered germinated when the protrusion of the radicle embryo was visible (Tilki and Bayraktar 2013).

At the end of the experiments the collected data were used to calculate: the final germination percentage (FGP), the mean germination time (MGT), the germination rate (GR) and the germination value (GV). *Formulae* used were the following: $FGP = (n^\circ \text{ of germinated seeds} \times 100) / \text{total } n^\circ \text{ of cultured seeds}$; $MGT = \sum f_x \times / \sum f$ (f =seeds germinated at day x) (Kader 2005); $GR = n_1/d_1 + n_2/d_2 + \dots + n_N/d_N$, dove (n =seeds germinated at day “d”) (Maguire 1962; Sadeghianfar et al. 2019); $PV = \text{cum. of } \Sigma \text{ germ/days}$ (Czabator 1962) and $GV = PV \times \text{Mean Daily Germination (MDG)}$ (Czabator 1962).

Data recovered from the different tests on sumac seeds were used to calculate means and the influence of pre-treatments, treatments and culture medium composition on the parameters tested was evaluated as follows: for the *Scarification experiment*, three-way ANOVA was carried out considering the influence of the factors “Pretreatment”, “Treatment” and “Culture Medium Composition” on FGP; moreover, since no germination episodes were observed within the not-mechanically scarified seeds, for the parameters MGT, GR, GV, a two-way ANOVA was carried out considering the influence of the factors, “Treatment” and “Culture Medium Composition”; for the *Cold stratification experiment*, three-way ANOVA was carried out considering the influence of the factors “Duration of Cold Stratification”, “Treatment”, and “Culture Medium Composition”; for the *Imbibition experiment*, two-way ANOVA was carried out considering the influence of the factors “Treatment” and “Culture Medium Composition”. Tukey’s test ($p \leq 0.05$) was used for mean separation (SYSTAT 13.1, Systat Software, Inc; Pint Richmond, CA). When data were not normally distributed non-parametric tests were used.

Total phenolic content (TPC) and antioxidant activity (AO) of vitro-derived sumac plant material

To verify the influence of the plant matrix used on the polyphenol content and antioxidant activity, a t test for independent samples ($p = 0.05$) was applied. In addition, Pearson’s two-tailed correlation test was performed on these data, in

order to verify a possible relation between polyphenolic content and the antioxidant capacity. The t test was applied also to the results obtained for tannin evaluation, in order to evidence differences between the two developmental stages as between leaves and roots. These evaluations were carried out using IBM SPSS software, version 28.

Results

In vitro sumac seed germination

Seeds, monitored for 10 weeks after their germination, showed normal development: at first there was the protrusion of the rootlet, followed by the appearance of the hypocotyl and of the cotyledons; finally, the epicotyl and the first “true” leaflets were shown (Fig. 3).

Influence of mechanical, chemical and physical scarification on in vitro sumac seed germination

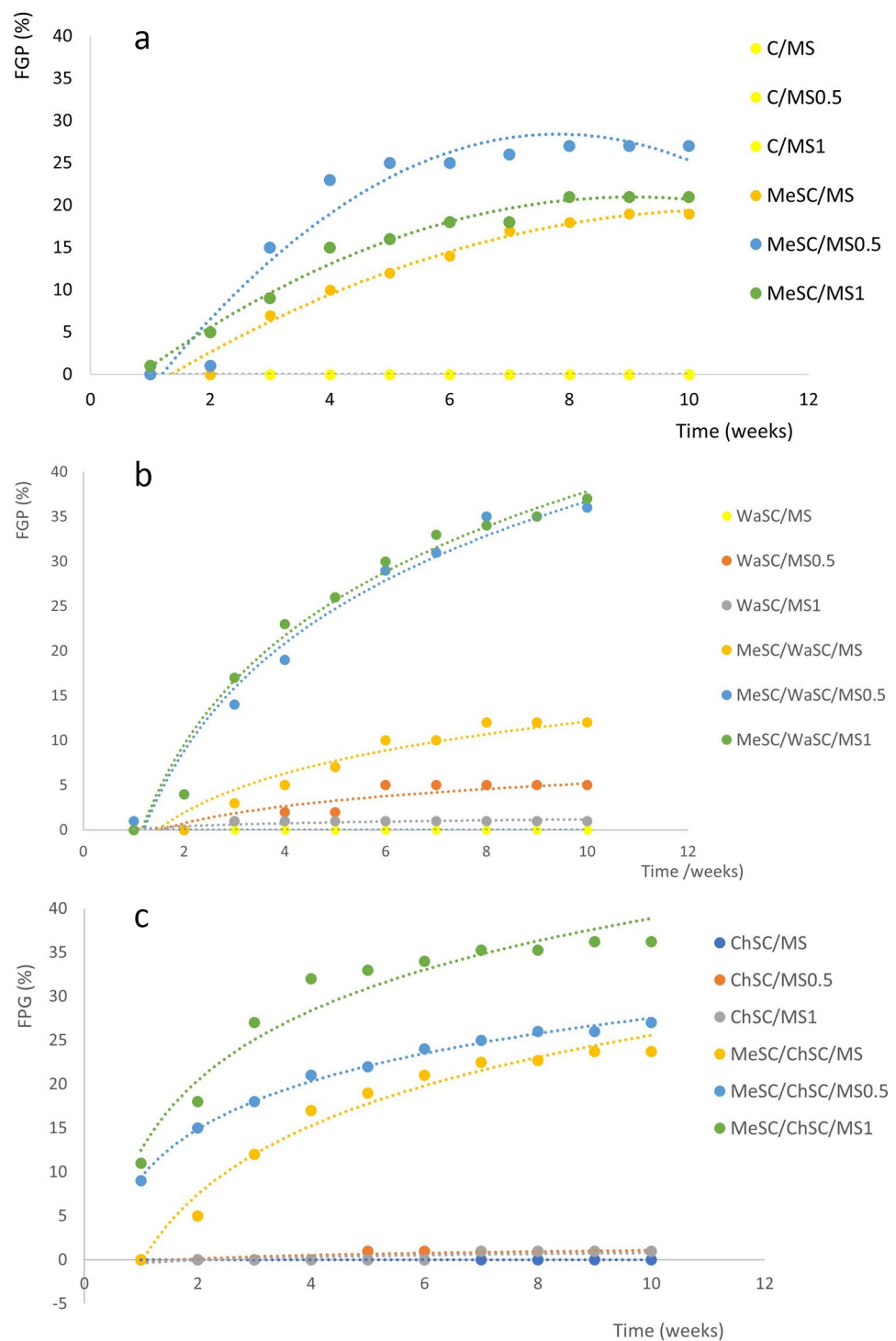
In the “*Scarification experiment*”, seeds were first treated with a blender for mechanical scarification, and then with water for physical scarification or with 96% of sulfuric acid for chemical scarification. In the Fig. 4, the germination trend of sumac seeds, after the MeSC, the WaSC and the ChSC, are shown. Observing the germination trend of MeSC seeds, in comparison with that of not treated seeds (C), the mechanical scarification is a key step to induce sumac seed germination, which would, otherwise, be zero (Fig. 4a). The same trend was observed for physically and chemically scarified seeds (WaSC and ChSC), their germination resulted lower and slower when the mechanical scarification has not been applied (Fig. 4b and c).

The analysis of data collected after 10 weeks of culture evidenced a significant interaction between the factors “Pretreatment” and “Culture medium composition”, as between “Treatment” and “Culture medium composition” (Table 2); in both cases, the presence of GA₃ in the culture media, independently of its concentration, significantly stimulated

Fig. 3 Germinative process of in vitro cultured sumac seeds



Fig. 4 Germination trend of in vitro cultured sumac seeds: (a) mechanical scarification; (b) physical scarification; (c) chemical scarification. FGP: Final Germination Percentage. C: non scarified seeds; MeSC: mechanically scarified seeds; WaSC: physically scarified seeds, treated with water at 100 °C for 1 min; ChSC: chemically scarified seeds, treated with H₂SO₄ for 60 min; MS0= basic culture medium; MS0.5= MS0 with 0.5 mg/ml GA₃; MS1= MS0 with 1 mg/ml GA₃



the seeds to germinate, only if seeds were mechanically, chemically or physically scarified. Since in this first experiment (*Scarification test*), the FGP of non-pre-treated seed was very low, the other parameters were not considered (Table 2).

To evidence the influence of WaSC and ChSC on the evaluated parameters, data of MeSC seeds were analyzed

through two-way ANOVA. Also in this case, FGP was strongly influenced by the factor “Culture medium composition”, confirming that the presence of GA₃ in the culture medium is a key factor to stimulate sumac seed in vitro germination, aside from the scarification method applied (Table 3). “Treatment”, instead, is the factor that significantly influenced the “Mean Germination Time” (MGT):

Table 2 Influence of scarification on sumac seeds Final Germination Percentage (FGP)

Pretreatment	Treatment	Culture medium	FGP	
			%	±SD
-	C	MS0	0.00	0.0
		MS0.5	0.00	0.0
		MS1	0.00	0.0
-	WaSC	MS0	0.00	0.0
		MS0.5	5.00	9.7
		MS1	1.00	3.2
-	ChSC	MS0	0.00	0.0
		MS0.5	1.00	3.2
		MS1	1.00	3.2
MeSC	C	MS0	19.00	7.4
		MS0.5	27.00	14.9
		MS1	21.00	13.7
MeSC	WaSC	MS0	12.00	9.2
		MS0.5	36.00	20.7
		MS1	37.00	14.2
MeSC	ChSC	MS0	23.72	24.1
		MS0.5	27.00	14.9
		MS1	36.25	17.1

Statistical analysis of data

Factor	p-value
Pretreatment (P)	0.000*
Treatment (T)	0.289
Culture medium composition (CMC)	0.000*
P x T	0.603
T x CMC	0.036
P x CMC	0.000
P x T x CMC	0.064

Three-way Anova ($p \leq 0.05$), Tukey's Test (*Kruskal–Wallis's test)

MeSC: Mechanical scarified seeds; C: Control (no treatment); WaSC: physically scarified seeds; ChSC: chemically scarified seeds; MS0: basic culture medium; MS0.5: MS0 with 0.5 mg/ml GA_3 ; MS1: MS0 with 1 mg/ml GA_3

MeSC/ChSC seeds needed indeed a significantly shorter germination time (21.8 days) than MeSC (29.4 days) and MeSC/WaSC (32.8 days) seeds. Other than the factor “Treatment”, also “Culture medium composition” parameter exerted a significant influence on the “germination rate” (GR) and “germination value” (GV); significant GR and GV were detected if seeds were chemically scarified and sown on a culture medium enriched with GA_3 (Table 3).

In conclusion, the mechanical scarification proved to be absolutely essential in initiating the *in vitro* sumac seed germination. Furthermore, a subsequent chemical scarification, coupled with the addition of GA_3 to the culture medium, significantly enhanced germination rates, as well as the average duration and synchronization of the germination process.

Influence of cold stratification on in vitro sumac seed germination In the “Cold stratification experiment”, sumac seeds, after being scarified and sterilized, were kept at

4 °C for different periods of time, from 2 to 8 weeks, after which they were sown *in vitro*, on agarized culture medium enriched with GA_3 . Observing the germination trends, it is clear that when seeds were stored at 4 °C, after the treatment with boiling water (WaSC), the germination process was favored; especially when cold stratification time increases (> 4 weeks), physically scarified seeds germinated faster and in higher number; opposite trend was displayed by chemically scarified seeds (ChSC); indeed, for all the cold stratification treated samples, a lower and more prolonged germination was observed (Fig. 5).

Statistical analysis of data recorded at the end of the cold stratification experiment evidenced a significant interaction between the factors “Treatment” and “Duration of Cold Stratification” for the parameter FGP (Table 4); the statistically highest germinability (39%) was observed within seeds physically scarified and stratified for 8 weeks. So, the combination of chemical scarification with cold stratification seems to inhibit *in vitro* sumac seed germination, reducing significantly their FGP, from 29–16%, while scarification experiment led to obtaining good performances for ChSC seeds.

Evaluating the parameter MGT, the statistical analysis evidenced that the only factor exerting a significant influence on it was the “Treatment” (Table 4); ChSC seeds seemed to spend significantly less time to germinate than the differently treated seeds (21.8 days), results totally in line with that obtained in the absence of stratification. Thus, it is conceivable that the cold stratification treatment does not help in shortening the time needed by sumac seeds to germinate.

Finally, for the parameter GR that describes the germinative vigor, and GV that combine the germinability with the germination velocity, a significant interaction between the factors “Duration of Cold Stratification” and “Treatment” was recorded (Table 4), with a higher response from 8 weeks stratified seeds, physically scarified with boiling water (WaSC); as observed for previous parameters, the combination of cold stratification and chemical scarification (ChSC) is detrimental, also, for these parameters.

In conclusion, the cold stratification treatment appears to lead to an improvement of the germination process, only if it is carried out for a period of 8 weeks (T2) and preceded by a treatment of scarification with water at 100 °C (WaSC).

Influence of imbibition on *in vitro* sumac seed germination

In the “Imbibition experiment”, after being mechanically scarified (MeSC), sumac seeds were soaked for 24 h in the dark at 25 ± 1 °C in water or in 500 mg/l GA_3 aqueous solution. Analyzing the seed germination trend, it is visible that

Table 3 Influence of physical and chemical scarification treatments on different parameters descriptive of the in vitro germination of previously mechanically scarified sumac seed

Treatment	Culture medium composition	FGP		MGT		GR		GV	
		%	±SD	dd	±SD	-	±SD	-	±SD
-	MS0	19.00	7.4	34.48	8.5	0.21	0.1	0.11	0.1
	MS0.5	27.00	15.0	24.69	4.8	0.37	0.1	0.40	0.3
	MS1	21.00	22.3	29.17	2.0	0.32	0.2	0.22	0.3
WaSC	MS0	12.00	9.2	34.03	9.6	0.13	0.1	0.06	0.1
	MS0.5	36.00	20.7	32.89	5.0	0.42	0.1	0.51	0.5
	MS1	37.00	14.2	31.45	3.0	0.47	0.1	0.44	0.3
ChSC	MS0	23.72	24.1	25.08	5.6	0.34	0.2	0.35	0.5
	MS0.5	27.00	14.9	21.90	8.8	0.70	0.3	0.65	0.9
	MS1	36.25	17.1	18.49	5.3	0.92	0.1	0.88	1.0
<i>Statistical analysis of data</i>									
Factor		<i>p</i> -value		<i>p</i> -value		<i>p</i> -value		<i>p</i> -value	
Treatment (T)		0.193		0.005		*0.000		0.019	
Culture medium composition		0.003		0.211		*0.001		0.019	
T x CM		0.093		0.725		*0.214		0.693	

Two-way Anova ($p \leq 0.05$), Tukey's test (*Kruskal-Wallis's test) FGP: Final Germination Percentage; MGT: Mean Germination Time; GR: Germination Rate; GV: Germination Value; C: Control (no treatment); WaSC: physically scarified seeds; ChSC: chemically scarified seeds; MS0: basic culture medium; MS0.5: MS0 with 0.5 mg/ml GA₃; MS1: MS0 with 1 mg/ml GA₃

seeds imbibed in solution containing only water (IH₂O) germinated less and slower than the others (Fig. 6).

Statistical analysis of FGP and GV values evidenced significant differences for the factor "Treatment", indeed it seems that imbibing seed in water significantly reduce the germination percentage and its synchronicity (Table 5). Since the average FGP of seeds imbibed, in water and in gibberellin solution, was 17% and the MGT was 27.5dd, it was deduced that the imbibition treatment did not contribute to improve the germination process of sumac seeds.

Total phenolic content (TPC) and antioxidant activity (AO) of sumac vitro-derived plant material

The methanol and aqueous extracts from sprouts at I and II developmental stage, and from leaves and roots, isolated from 6 weeks old plantlets were evaluate in terms of both polyphenol content and antioxidant activity. As reported in Table 6, the TPC and AO of sprouts at II stage were significantly higher than those from stage I sprouts, both for methanolic and aqueous extracts. If considering the different plant organs, leaves showed a significantly higher amount of polyphenols and antioxidant activity than roots. Regarding the methanol extracts, the TPC were found to be 78.42 mg GAE/g DM and 25.48 mg GAE/g DM for leaves and root, respectively.

Similarly, from what observed for TPC, the leaf extract has an antioxidant activity significantly higher than root. As concerning DPPH and ABTS assay, three times higher activity was measured in leaves (1980.06 ± 33.30 mM and 2206.37 ± 154.22 mM) than in roots (618.27 ± 0.56 mM and 722.60 ± 44.01 mM).

Overall, methanol was found to be more efficient polyphenols and antioxidant substance extraction from plant matrices than water.

To assess the relationship among the activities of four different assays, Pearson's correlation coefficient was calculated. The Pearson correlation test (two-tailed) showed a significant positive correlation ($r > 0.8$) among all four tests (Folin-Ciocalteu, DPPH, ABTS and FRAP), in the case of aqueous extracts, and among three of the four tests performed (Folin-Ciocalteu, DPPH and ABTS), for the methanol extracts; this last result confirmed that the antioxidant properties of the tested samples are directly related to the polyphenolic fraction.

Evaluation of tannin content in vitro-derived sumac seedlings

In order to evaluate gallotannin content, acidic hydrolysis was carried out on the aqueous extract of the seedlings, leaves and roots respectively.

Tannin extractability at 90 °C for 24 h was expressed as mg GAE/g (Folin-Ciocalteu assay) on dry matter and results were calculates as difference between hydrolysed and non-hydrolysed extract analyses. As reported in Fig. 7, the tannin content in sprouts at the stage II was significantly higher than that found in sprouts at stage I (18.36 vs. 2.44 mg GAE/g DM). In addition, in the leaves, a content significantly higher than that observed in the roots (6.50 vs. 3.02 mg GAE/g DM) was registered. These results suggest that tannins accumulate mainly in the leaves, and increase in quantity with the development of plantlets.

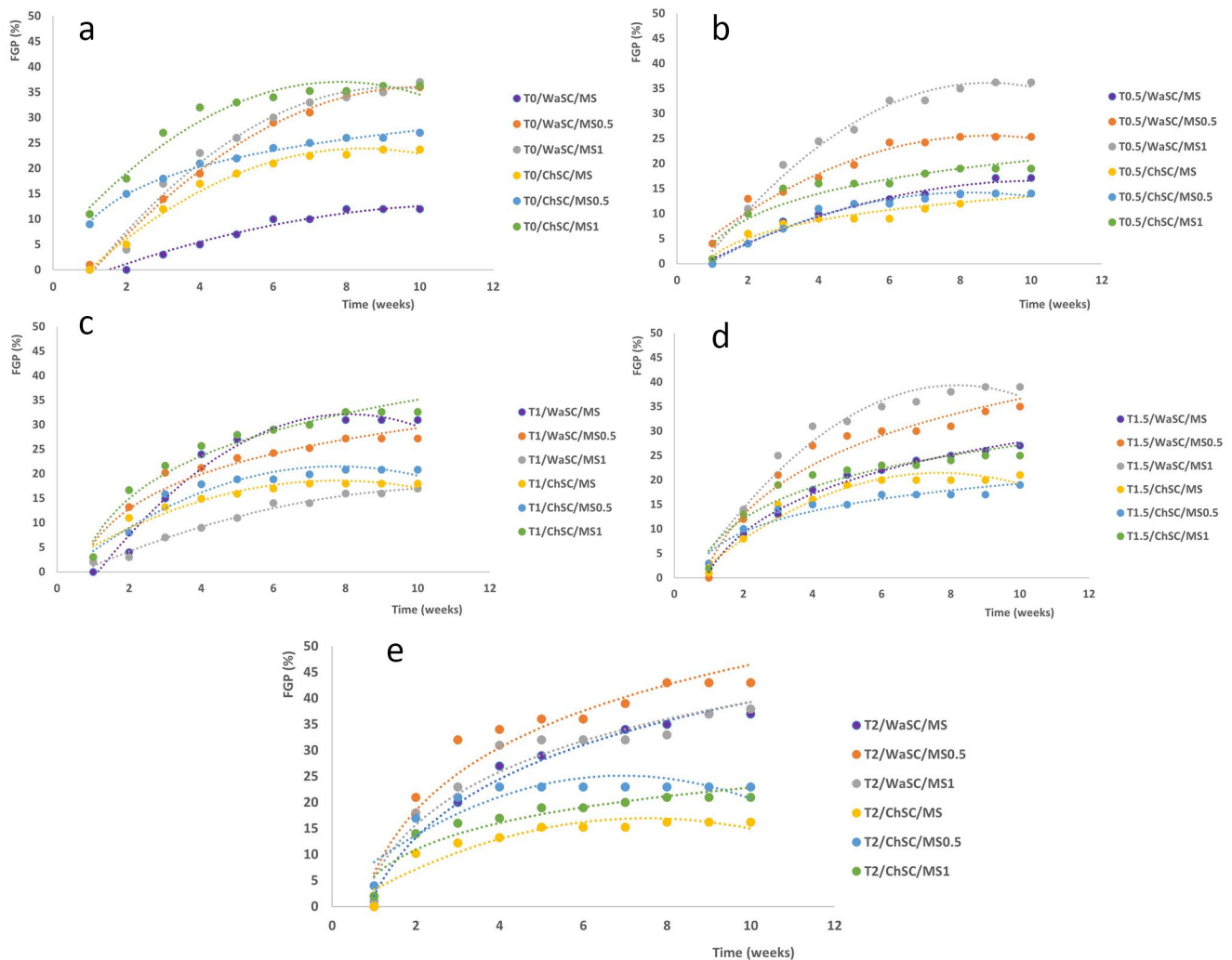


Fig. 5 Germination trend of scarified and stratified seeds at 4 °C for: (a) 0 weeks (T0); (b) 2 weeks (T0.5); (c) 4 weeks (T1); (d) 6 weeks (T1.5); (e) 8 weeks (T2). FGP: Final Germination Percentage. WaSC: physically scarified seeds, treated with water at 100 °C for 1 min;

ChSC: chemically scarified seeds, treated with H_2SO_4 for 60 min; MS0= basic culture medium; MS0.5=MS0 with 0.5 mg/ml GA_3 ; MS1=MS0 with 1 mg/ml GA_3

Discussion

Sumac sprouts are of particular biotechnological interest due to their potentially wealth in bioactive compounds; to exploit this plant material, it is necessary to overcome sumac seed physical and physiological dormancy (Pipinis et al., 2017; Olmez et al. 2007). In fact, untreated sumac seeds have shown either no germination or extremely low germination percentages when exposed to conditions such as water-soaked filter paper (Ne'eman et al., 1999), seed-beds (Olmez et al. 2007), or wet sand (Pipinis et al. 2017; Tilki and Bayraktar 2013). It is indeed well recognized that it is necessary to resort to some treatments to break sumac dormancy. Many authors use sulfuric acid scarification to overcome physical dormancy and cold stratification to overcome physiological dormancy (Olmez et al. 2007; Tilki and

Bayraktar 2013). Tissue culture techniques, allowing a complete control of the growing conditions, could be of great help to evaluate several factors that control the sumac seed behavior and, consequently, their germination process. Setting up a valid protocol for producing sumac sprouts in vitro would set the bases to exploit this plant material as matrix for bioactive compound extraction; moreover, information acquired from in vitro experiments could be transferred to ex vitro conditions, to obtain sumac sprouts to be used as novel food. Sumac seed germination has been studied by several authors (Ne'eman et al. 1999; Olmez et al. 2007; Pipinis et al. 2017; Tilki and Bayraktar 2013), but, at the best of the authors' knowledge, it was never studied in vitro conditions.

In this study, for the first time, *Rhus coriaria* L. seed germination was studied in vitro, applying different

Table 4 Influence of cold stratification treatment on different parameters descriptive of the sumac in vitro seed germination

Duration of cold stratification	Treatment	Culture medium composition	FGP		MGT		GR		GV	
			%	±SD	dd	±SD	-	±SD	-	±SD
T0	WaSC	MS0	12.00	9.2	34.03	9.6	0.13	0.1	0.06	0.1
		MS0.5	36.00	20.7	32.89	5.0	0.42	0.1	0.51	0.5
		MS1	37.00	14.2	31.45	3.0	0.47	0.1	0.44	0.3
	ChSC	MS0	23.72	24.1	25.08	5.6	0.34	0.2	0.35	0.5
		MS0.5	27.00	14.9	21.90	8.8	0.70	0.3	0.65	0.9
		MS1	36.25	17.1	18.49	5.3	0.92	0.1	0.88	1.0
T0.5	WaSC	MS0	17.15	9.9	30.80	8.7	0.30	0.1	0.12	0.1
		MS0.5	25.33	14.4	23.08	7.1	0.44	0.2	0.47	0.5
		MS1	36.25	21.2	29.57	10.3	0.54	0.4	0.65	0.7
	ChSC	MS0	14.00	12.6	26.83	14.6	0.24	0.1	0.15	0.3
		MS0.5	14.00	15.1	26.78	3.0	0.21	0.2	0.16	0.3
		MS1	19.00	14.5	25.23	8.6	0.37	0.3	0.33	0.4
T1	WaSC	MS0	31.00	19.1	27.30	4.6	0.43	0.2	0.52	0.5
		MS0.5	31.22	15.6	22.56	6.1	0.55	0.2	0.45	0.3
		MS1	17.00	9.5	29.33	3.6	0.27	0.1	0.10	0.1
	ChSC	MS0	18.00	10.8	17.27	2.9	0.35	0.0	0.25	0.3
		MS0.5	20.86	9.8	21.50	7.4	0.42	0.1	0.27	0.2
		MS1	32.64	16.9	23.82	1.1	0.58	0.1	0.74	0.8
T1.5	WaSC	MS0	28.80	13.4	28.80	2.1	0.43	0.0	0.28	0.2
		MS0.5	26.56	15.1	26.56	3.9	0.54	0.0	0.53	0.4
		MS1	24.81	18.5	24.81	5.3	0.71	0.2	0.78	0.7
	ChSC	MS0	21.00	8.8	23.96	5.3	0.38	0.1	0.25	0.2
		MS0.5	19.00	12.9	22.43	8.1	0.41	0.2	0.27	0.3
		MS1	25.00	13.5	23.46	5.4	0.50	0.3	0.39	0.4
T2	WaSC	MS0	37.00	9.5	26.90	2.7	0.58	0.1	0.58	0.5
		MS0.5	43.00	13.4	23.81	2.1	0.76	0.1	0.99	0.5
		MS1	38.00	18.7	25.19	4.4	0.69	0.1	0.78	0.6
	ChSC	MS0	16.22	12.7	19.50	6.7	0.31	0.1	0.22	0.2
		MS0.5	23.00	13.4	15.23	1.6	0.59	0.2	0.53	0.6
		MS1	21.00	13.7	20.67	2.1	0.45	0.0	0.47	0.7

Statistical analysis of data

Factor	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
Duration of Cold Stratification (SF)	0.011	0.000	0.350	0.029
Treatment (T)	0.000	0.058	0.004	0.121
Culture Media Composition (CMC)	0.000	0.338	0.000	0.000
SF x CMC	0.148	0.685	0.406	0.475
T x SF	0.002	0.222	0.000	0.002
CMC x T	0.252	0.824	0.063	0.264
T x SF x CMC	0.042	0.913	0.428	0.165

Three-way Anova ($p \leq 0.05$), Tukey's test

FGP: Final Germination Percentage; MGT: Mean Germination Time; GR: Germination Rate; GV: Germination Value; T0: no stratification; T0.5: cold stratification for 2 weeks; T1: cold stratification for 4 weeks; T1.5: cold stratification for 6 weeks; T2: cold stratification for 8 weeks; WaSC: physically scarified seeds; ChSC: chemically scarified seeds; MS0: basic culture medium; MS0.5: MS0 with 0.5 mg/ml GA₃; MS1: MS0 with 1 mg/ml GA₃

pre-treatments, alone or in combination: a mechanical (with a blender for 30 s), a chemical (with H₂SO₄ at room temperature for 60 min) and a physical (with water at 100 °C for 1 min) scarification. Due to the complete lack of literature on in vitro sumac seed germination, the results obtained in this research were compared with the few papers available on in vivo germination of seeds belonging to *Rhus coriaria* L. and other related species to *genus Rhus*.

All the treatments explored, before sowing in vitro, confirmed what was reported by other authors (Ne'eman et al. 1999; Olmez et al. 2007; Pipinis et al. 2017; Tilki and Bayraktar 2013): treating the seeds of sumac before sowing them is a fundamental step to induce germination; indeed, after scarification, the germination percentages of sumac seeds experienced a notable increase, rising from 0 to 22%.

Fig. 6 Germination trend of sumac seeds soaked for 24 h in H₂O or GA₃ solution, pre-treated with mechanical scarification. FGP: Final Germination Percentage IH₂O=imbibition in water for 24 h; IGA₃=imbibition in solution with 500 mg/l GA₃ for 24 h; MS0= basic culture medium; MS0.5=MS0 with 0.5 mg/ml GA₃; MS1=MS0 with 1 mg/ml GA₃

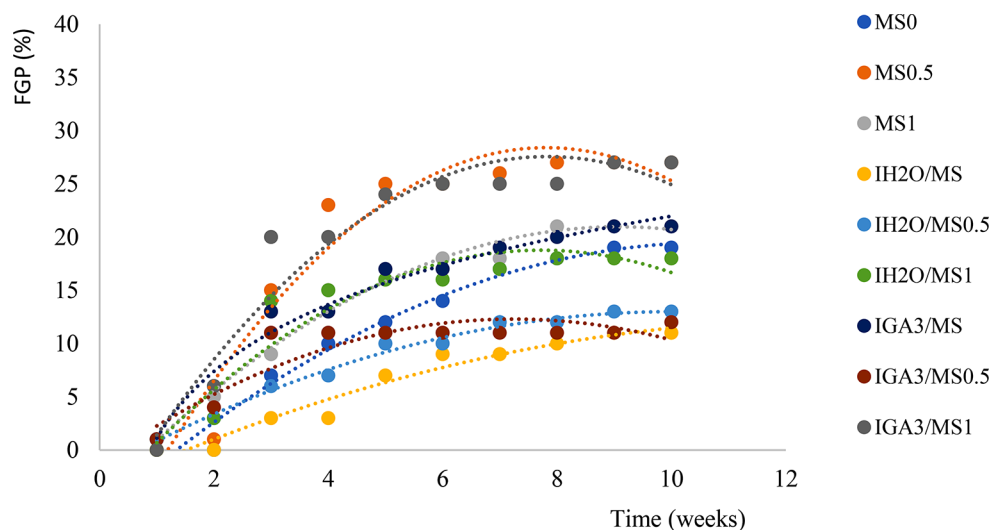


Table 5 Influence of imbibition on different parameters descriptive of the sumac in vitro seed germination

Treatment	Culture medium	FGP		MGT		GR		GV	
		%	±SD	dd	±SD	-	±SD	-	±SD
C	MS0	19.00	7.4	34.48	8.5	0.21	0.1	0.11	0.1
	MS0.5	27.00	14.9	24.69	4.8	0.37	0.1	0.40	0.3
	MS1	21.00	13.7	29.17	2.0	0.32	0.2	0.22	0.3
IH ₂ O	MS0	11.00	7.4	39.20	8.5	0.11	0.1	0.05	0.1
	MS0.5	13.00	8.2	26.67	14.5	0.20	0.1	0.07	0.1
	MS1	18.00	10.3	22.60	5.6	0.28	0.1	0.21	0.2
IGA ₃	MS0	21.00	16.0	24.42	9.2	0.34	0.0	0.31	0.4
	MS0.5	12.00	9.2	22.13	9.6	0.23	0.1	0.14	0.2
	MS1	27.00	17.7	24.21	4.7	0.42	0.1	0.45	0.5
<i>Statistical analysis of data</i>									
Factor		<i>p</i> -value		<i>p</i> -value		<i>p</i> -value		<i>p</i> -value	
Treatment (T)		0.029		0.751		0.064		0.036	
Culture Medium Composition (CMC)		0.215		0.467		0.127		0.165	
T x CMC		0.084		0.297		0.317		0.060	

Two-way ANOVA ($p \leq 0.05$), Tukey's test

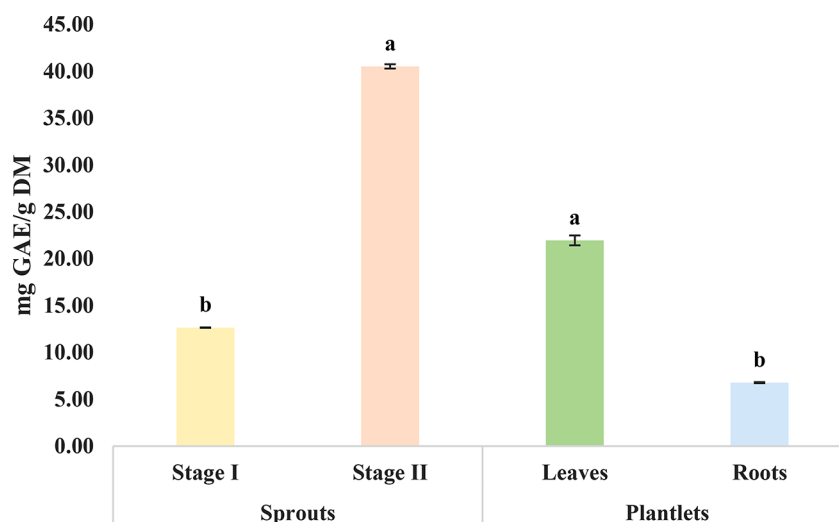
FGP: Final Germination Percentage; MGT: Mean Germination Time; GR: Germination Rate; GV: Germination Value; C: no imbibition; IH₂O: imbibition in water for 24h; IGA₃: imbibition in solution with 500 mg/l GA₃ for 24h. MS0: basic culture medium; MS0.5:MS0 with 0.5 mg/ml GA₃; MS1:MS0 with 1 mg/ml GA₃

Table 6 Total polyphenol content (TPC) and antioxidant capacity (DPPH, ABTS and FRAP) of plant material determined at two growth stages, as on leaves and roots

Plant Material	TPC (mg GAE/g DM)	DPPH (mM TEAC)	ABTS (mM TEAC)	FRAP (mM TEAC)
<i>Methanolic extracts</i>				
Stage I	48.36 ± 0.15 b	603.53 ± 0.38 b	897.34 ± 0.92 b	250.93 ± 4.85 b
Stage II	71.14 ± 0.58 a	1844.44 ± 27.42 a	2100.90 ± 86.81 a	666.11 ± 2.47 a
Leaves	78.42 ± 2.95 a	1980.06 ± 33.30 a	2206.37 ± 154.22 a	902.07 ± 7.01 a
Roots	25.48 ± 2.94 b	618.27 ± 0.56 b	722.60 ± 44.01 b	488.59 ± 8.66 b
<i>Aqueous extracts</i>				
Stage I	10.20 ± 0.03 b	136.37 ± 0.11 b	180.06 ± 0.13 b	79.57 ± 0.63 b
Stage II	22.14 ± 0.23 a	491.89 ± 16.97 a	642.46 ± 40.05 a	264.67 ± 11.71 a
Leaves	15.45 ± 0.53 a	436.65 ± 18.32 a	515.49 ± 39.31 a	255.84 ± 2.94 a
Roots	3.74 ± 0.06 b	56.17 ± 1.33 b	59.07 ± 11.47 b	42.17 ± 4.01 b

Results are expressed as mg GAE/g and mM TEAC on dry weight. Per each parameter and per each type of extraction method, values followed by different letters are statistically different ($p < 0.05$). Stage I: 2 weeks old sprouts; Stage II: 4 weeks old sprouts; Leaves and roots are plant material obtained from 6 weeks old plantlets

Fig. 7 Gallotannin obtained as difference between hydrolysed and non-hydrolysed aqueous extracts by Folin-Ciocalteu assay. Per each type of plant material, different letters indicate values statistically different (t test, $p < 0.05$). Stage I: 2 weeks old vitro-derived sumac sprouts; Stage II: 4 weeks old vitro-derived sumac sprouts; Leaves and roots are plant material obtained from 6 weeks old vitro-derived sumac plantlets



This important result has been reached resorting to mechanical scarification, that, for the best of authors' knowledge, was never used to break tegumental dormancy of sumac seeds, not even in vivo, but it is commonly used for other species, such as carob (Aliero 2004), lupine (Jones et al. 2016), and date palm seeds (Al Zoubi 2020). Probably the key factor for breaking the dormancy of the sumac seeds is, therefore, the mechanical scarification as it creates small cracks on the seed integument that allow the beginning of germination process.

When the mechanical scarification treatment was followed by the chemical one, a further increase of germination percentage up to 29% was obtained. This study achieved a higher germination percentage compared to the findings of Pipinis et al. (2017), who reported a germination rate of 10%, using sand as sowing substrate.

An average germination rate of up to 28% was achieved by combining mechanical scarification with physical scarification using boiling water. Once again, mechanical scarification emerged as pivotal. When sumac seeds were solely treated with boiling water and sown on pots, as reported by Olmez et al. (2007), the germination rate reached only the 14%.

Cold stratification is a method used to break the physiological dormancy, and it is considered effective especially for seeds of temperate climates plants, such as hops (Liberatore et al. 2018). Several authors have combined this treatment with chemical scarification for enhancing germination rates in sumac seeds in vivo sown (Olmez et al. 2007; Pipinis et al. 2017; Tilki and Bayraktar 2013), and the results obtained are controversial. Olmez et al. (2007) who applied cold stratification on in vivo cultured seeds, reported results similar to those obtained in this study (24% vs. 25%), while other authors (Pipinis et al. 2017; Tilki and Bayraktar 2013) obtained higher germination percentages (around 50% after 4 weeks of stratification and around 65% after 8 weeks).

What could have affected the sumac seed response in this study is the combination of cold stratification and chemical scarification, that could have compromised seed viability.

Imbibition represents another method used to overcome seed dormancy; in particular, the treatments carried out on previously mechanically scarified seeds, were imbibition in water or in a gibberellin solution for 24 h. To the best of our knowledge, the only other authors who have performed a similar treatment on sumac seeds were Pipinis et al. (2017); even if these authors combined the imbibition treatment with a chemical scarification and sown sumac seed on wet paper, they obtained results comparable with those reported in this study (10% vs. 14% with water and 28% vs. 20% with gibberellic acid solution).

Tissue culture techniques are widely used to produce high quality bioactive compounds with the added value that they guarantee a continuous supply of plant material, independently of the seasonality and in reduced time and space (Jakovljević et al. 2022; Koufan et al. 2020).

The increasing interest in extracts of natural origin and the awareness, supported by scientific research, of the richness in bioactive compounds of sumac leaves and fruits (Abu-Reidah et al. 2015) make it worth to study not only the in vitro germination process, but also the total polyphenol content and antioxidant activity of the vitro-derived sprouts of this promising species. In fact, sprouts obtained from in vitro germinated seeds have already been demonstrated to be a precious source of secondary metabolites, as observed in *Moringa oleifera* (Cirlini et al. 2022).

The vitro-derived sumac sprouts obtained in this study exhibited a richness in bioactive compounds and tannins, showcasing promising biotechnological applications. Prior research has investigated various types of sprouts commonly consumed as food, such as broccoli, red radish, radish, mizuna, kale, taatsa, pak choi, cabbage, turnip, rapeseed, chicory, alfalfa, and buckwheat, aiming to assess their

antioxidant content and activity (Park et al. 2019). To the best of the authors' knowledge, there is currently no available information regarding the Total Phenolic Content (TPC), as well as the antioxidant activities measured through DPPH, ABTS, and FRAP assays, specifically on vitro-derived sumac sprouts and their components. Consequently, a direct comparison between TPC and antioxidant activity could not be established for vitro-derived sumac sprouts. Instead, comparisons were made with data published on various sprouts or microgreens from different species. Authors evaluated the total phenolic content and antioxidant activity of sumac vitro-derived sprouts. Specifically, authors analyzed sprouts aged 2 and 4 weeks, as well as 6-week-old plantlets recovered from agarized culture medium. For seedlings, the analysis encompassed the entire sprout, while for plantlets, leaves and roots were assessed separately.

The 70% aqueous methanol extracts showed higher TPC in comparison to water extracts, that increases with the development stage (I stage = 48.36 ± 0.15 mg GAE/g DM, II stage = 71.14 ± 0.58 mg GAE/g DM). Our results exceed those of previous studies conducted on in vitro plant material analyzed at comparable stages of development. For example, Di Bella et al. (2020), analyzed freeze-dried samples of sprouts (germinated seeds without coats and roots), microgreens (2–3 weeks after germination, plantlets with the first true leaf) and baby leaves (5 weeks after germination, plantlets with almost three true leaves) of *Brassica oleracea* L. (cabbage) in which they have found 41.9 mg GAE/g DM, 36.9 mg GAE/g DM and 34.8 mg GAE/g DM, respectively. In sprouts of broccoli grown for 2 weeks and extracted in ethanol, Marchioni et al. (2021) have found a value of TPC of 32.67 mg GAE/g DM in five microgreens of the Brassicaceae family. Kim and Huh (2022) analyzed *Rhus verniciflua* leaves, and found 163.80 mg/GAE DM, an amount higher than that reported in the present study, probably due to different species, but most of all, to the different starting material analyzed and growing conditions considered.

The antiradical and reducing activities were measured with DPPH, ABTS and FRAP assays which showed a significant increase ($p < 0.05$) from I to II stage sprouts and from roots to leaves. In literature, there are no data available regarding the antioxidant assay of vitro-derived sumac sprouts, but other authors have analyzed several types of different microgreens, grown in controlled conditions. More in detail, Xiao et al. (2019) analyzed thirty varieties of commercial microgreens available in the Brassicaceae family showing an exceptional antioxidant activity measured by DPPH assay, mainly for Radish ruby and Broccoli microgreens that contained 80 mM TEAC FW and 38 mM TEAC FW, respectively. The ABTS assay performed by Ghooora et al. (2020) from culinary microgreens belonging to eight

botanical families found the radical scavenging activity highest in the fennel microgreens (2.28 mM TEAC FW). Dorman et al. (2003) studied the aqueous extracts of the commonly consumed herbs and reported an antioxidant activity with ABTS higher in rosemary, oregano and sage (14.1 mM TEAC; 14.9 mM TEAC; 14.5 mM TEAC) than thymus (6.8 14.5 mM TEAC).

Due to their high tannin content, sumac leaves were used as a tanning agent in the past. As reported by Kosar et al. (2007), the hydrolysable gallotannins, units of polyol-D-glucose esterified by gallic acid, are the most important tannins in plants of the *Rhus* family. Previous studies have investigated the antioxidant capacity of both leaves and fruits, highlighting the strong activity of these fractions of *Rhus coriaria* L. (Zalacain et al. 2000, 2002). It is known that extracts from sumac fruits are rich in tannins, which can improve the nutritional value and oxidative stability of animal products such as milk and meat, as reported by Batiha et al. (2022). In addition, in the plant, tannins explain their main function in protection against pathogenic microorganisms and insects (hydrolysable tannins) or herbivorous animals, and their synthesis is also related to extreme environmental conditions, such as stress due to lack of water or UV radiation (Furlan 2010). As there is no literature on the evaluation of hydrolysable gallotannins from in vitro-derived sumac sprouts, in the present work an evaluation of their amount in the tested starting material was performed, finding 2.44 and 18.36 mg GAE/g DM in sprouts at stage I and stage II, respectively; the hydrolysable gallotannins extracted from in vitro-derived roots were about half of those extracted from leaves (3.02 mg GAE/g DM versus 6.50 mg GAE/g DM). This study reports that roots obtained in vitro contain a lower amount of hydrolysable gallotannins (3.02 mg GAE/g DM) than leaves (6.50 mg GAE/g DM). The content of hydrolysable tannins in sumac root barks determined in Ben Mahmoud et al. (2015) is 85.6 mg TAE/g DM and the methanol extract of leaves from the Turkish province of Kahramanmaraş, contained 0.365 mg TAE/mg extract. However, this value and the value determined by us could be considered low compared to the tannin amount found in dried sumac fruits (*Rhus coriaria* L.) from adult plants, as reported in a previous study by Fereidoonfar et al. (2019).

Conclusion

Sumac (*Rhus coriaria* L.) is a plant species widely used in the Middle East, which is valued both as a spice and for its medicinal properties, and whose roots go back to ancient times. Recently, the growing demand for natural products has attracted the interest of various industries such as agri-food, pharmaceuticals and cosmetics. While numerous

studies have explored the beneficial properties of various parts of the sumac plant, there is a notable research gap in relation to its sprouts, which, like other species, provide a rich source of bioactive compounds; in fact, during germination, sprouts convert the nutrients from the seeds into remarkable compounds that have high nutritional value and powerful antioxidant, antiviral and antibacterial properties.

To utilise the potential of sumac sprouts, overcoming the double dormancy of sumac seeds is a major challenge. To speed up the germination process and enable year-round cultivation independent of the environment conditions, a protocol for inducing in vitro seed germination was established. Sumac seeds subjected to various treatments prior the in vitro sowing germinated at a higher percentage and faster rate when they were first mechanically and then chemically scarified and sown on media containing gibberellic acid. This protocol allows for a germination percentage comparable to that present in literature and a significantly lower average germination time.

The chemical characterization of sumac sprouts obtained in vitro yielded promising results: the total polyphenol content and antioxidant activity exceed those of edible sprouts of other plant species, such as microgreens. In addition, the gallotannins characteristic of sumac were also detected in the sumac sprouts obtained in vitro, albeit in slightly lower quantities than in the adult plant.

This study forms the basis for further research aimed at developing treatments to overcome sumac dormancy and accelerate germination. The chemical properties discovered in sumac sprouts warrant further research, making them not only a source of bioactive compounds, but also a potential novel food with significant nutritional and health benefits.

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Author contributions Conceptualization: V.G., M.C., B.C.; Data curation: C.G., L.L., B.C. M.C.; Formal analysis: C.G., L.L., B.C. M.C.; Investigation: C.G., L.L., L.D.V.; Methodology: V.G., M.C., B.C.; Project administration: V.G., M.C., B.C.; Resources: V.G., M.C., B.C.; Software: M.C., B.C.; Supervision: V.G., M.C., B.C.; Validation: V.G., M.C., B.C.; Visualization: V.G., M.C., B.C.; Roles/Writing - original draft: C.G., L.L., L.D.V., B.C., M.C. and Writing - review & editing: V.G., M.C., B.C.

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Data availability All data supporting the findings of this study are available within the paper.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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