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In Vitro Cultures of *Brassica oleracea* L. var. *costata* DC: Potential Plant Bioreactor for Antioxidant Phenolic Compounds

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In this work were studied the phenolic composition of in vitro material (shoots, calli, and roots) of *Brassica oleracea* var. *costata* and its antioxidant capacity. Samples were obtained in different culture medium, with distinct supplementations to verify their influence on those parameters. Phenolic determination was achieved by HPLC-DAD. Antioxidant activity was assessed against DPPH•. In calli and roots no phenolic compound was identified. In shoots was verified the presence of 36 compounds, which included hydroxycinnamic acids, flavonoids (kaempferol and quercetin derivatives), and hydroxycinnamic acyl glycosides (with a predominance of synapoyl gentiobiosides). MS liquid medium supplemented with 2 mg/L benzylaminopurine (BAP) and 0.1 mg/L naphthaleneacetic acid (NAA) revealed to be the best in vitro condition to produce shoot material with highest phenolic compound contents and stronger antioxidant potential, thus with a possible increase of health benefits.

KEYWORDS: ***Brassica oleracea* L. var. *costata* DC; shoots; calli; roots; phenolic compounds; antioxidant activity**

INTRODUCTION

In recent years attention has been focused on plants as a source of phytochemicals with chemopreventive and chemotherapeutic potential. These phytochemicals comprise different structures and involve several protective mechanisms, plant secondary metabolites being the most likely candidates for health-promoting effects. Among those presenting antioxidant capacity, phenolic compounds constitute one of the most important groups (*1*). Several studies report that these compounds show a preventive effect against chronic diseases, such as cancer, atherosclerosis, nephritis, diabetes mellitus, rheumatism, Alzheimer's, Parkinson's, ischemic and cardiovascular diseases, which are associated with an excess of free radicals (*2,3*).

The chemical composition of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) has already been studied, revealing this to be a good source of interesting compounds, such as organic acids, phenolic compounds, and amino acids. The different tronchuda cabbage vegetal materials exhibit distinct qualitative and quantitative compositions (*4*−*8*). In addition, all of these matrices have already displayed antioxidant capacity against different reactive species of both oxygen (*4, 5, 9*) and nitrogen (*10*). Thus, as it is widely consumed, this species constitutes a dietary source of phytochemicals useful to achieve optimal health.

In many cases, the chemical synthesis of these metabolites is not possible or economically feasible. The obtainment of these compounds in plant cultivation or from plants grown in nature is not always satisfactory, because they are exposed to different environmental, nutritional, and stress conditions, which can alter the quantitative and qualitative profile of compounds in plants (*11*). In vitro cultures are found to be an attractive option relative to the traditional methods of planting: growth conditions can be controlled, they allow a continuous and rapid propagation of plants, the production of specific metabolites can be stimulated, and a well-defined production system can result in higher yield and more consistent quality of the products (*12*). According to this, the large-scale plant cell and tissue cultures have been considered as a viable alternative source of phytochemicals. They can be regarded as a bioreactor, where reactions take place under optimum external environment to meet the needs of the biological system, so that a high yield of

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a bioprocess is achieved. Using plants as bioreactors allows valuable natural products to be obtained in a short period of time and space, with a maximum profitability. These products may be highly concentrated in interesting phytochemicals, which can be further used for human consumption or pharmaceutical industry (*13*, *14*). Different media and hormonal supplementations can influence the production of biomass and of specific metabolites. The most important groups of hormonal supplements are auxins (such as naphthaleneacetic and dichlorophenoxyacetic acids) and cytokinins (such as benzylaminopurine), but gibberellins (gibberellic acid) are also often used (*12*). Generally, auxins are associated with cell growth and root initiation, cytokinins are responsible for cell division and shoot growth, and gibberellins influence stem elongation, leaf differentiation, and photomorphogenesis (*12*, *15*). Phenolic composition can be modulated to achieve the best antioxidant activity (*16*).

In vitro cultures of *B. oleracea* var. *costata* would afford a good model system for studying the accumulation of antioxidant compounds. Several studies reported the in vitro development of vegetable materials from *Brassica* and some of them involved *B. oleracea* species (*17*−*21*). As far as we know, only one work described the presence of phenolic compounds in *B. oleracea* var. *costata* shoots (*22*). The chemical structures of the phenolics present are complex, which renders their synthesis almost impossible. In addition, studies concerning the effects of medium and hormonal supplementations on the production of phenolic compounds and antioxidant capacity of shoots and other in vitro materials are nonexistent.

The aim of this study was to evaluate the influence of different media and distinct supplementations on the phenolic composition and antioxidant potential of *B. oleracea* var. *costata* materials obtained from in vitro culture (roots, calli, and shoots). The phenolic profile was established by reversed-phase HPLC-DAD, and the antioxidant capacity was assessed against DPPH radical.

MATERIALS AND METHODS

Standards and Reagents. Chlorogenic, *p*-coumaric, ferulic, and sinapic acids, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-galactoside were purchased from Extrasynthése (Genay, France). DPPH was obtained from Sigma Chemical Co. (St. Louis, MO), methanol was purchased from Merck (Darmstadt, Germany), acetic acid was from Fisher Scientific (Leicestershire, U.K.), and hydrochloric acid was from Pancreac (Barcelona, Spain). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Solid-Phase Extraction (SPE) Columns. The C18 non-end-capped (NEC) columns (50 μm particle size, 60 Å porosity; 10 g of sorbent mass/70 mL of reservoir volume) were obtained from Chromabond (Macherey-Nagel, Germany).

Plant Material and Sterilization. *B. oleracea* L. var. *costata* DC seeds were dipped in a 90% ethanol solution for 10 min, followed by washing with sterilized water, before surface sterilization with a 5% sodium hypochlorite solution for 10 min. After a rinse with sterilized water, seeds were germinated on autoclaved MS (*23*) basal medium devoid of growth regulators, with 20 g/L sucrose and solidified with 8 g/L agar. Seed germination was performed in a growth chamber in complete darkness for 5 days and then under 16 h light and 23 °C/8 h dark and 16 °C cycles.

In Vitro Cultures. Internodal shoot segments (ca. 10 mm), obtained from aseptic 3-week seedlings, were used as primary explants in the development of shoot, calli, and root cultures. Root cultures were established on MS liquid medium with no hormonal supplementation or supplemented with 1 μM gibberellic acid (GA). Calli cultures were established on MS solid medium supplemented with 1 and 2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4D) or on B5 solid medium supplemented with 2 mg/L benzylaminopurine (BAP) combined with 0.1 mg/L naphthaleneacetic acid (NAA). The shoot cultures were established on MS and B5 media with distinct supplementations (**Table 1**). The culture conditions were those described above and were subcultured in the same medium with intervals of 3 weeks. At the end of the third subculture period, shoots, calli, and roots were withdrawn and lyophilized for 7 days.

Table 1. Characterization of *Brassica oleracea* var. *costata* Shoot Sample

sample	medium	type of medium	supplementation
A	MS	liquid	
B	MS	liquid	1 μM GA + 2 mg/L BAP + 0.1 mg/L NAA
C	MS	liquid	2 mg/L BAP + 0.1 mg/L NAA
D	MS	solid	2 mg/L BAP + 0.1 mg/L NAA + 2 mg/L $AgNO_3$
E	MS	solid	1 mg/L 2,4D
F	MS	solid	2 mg/L 2,4D
G	MS	solid	2 mg/L BAP + 0.1 mg/L NAA
H	B5	liquid	2 mg/L BAP + 0.1 mg/L NAA
I	B5	solid	2 mg/L BAP + 0.1 mg/L NAA

Phenolic Compound Extraction. Each sample (ca. 0.1 g) was thoroughly mixed with methanol until complete extraction of the phenolic compounds (negative reaction to 20% NaOH) and then filtered through a Büchner funnel. The methanolic extract was concentrated to dryness under reduced pressure (40 °C) and redissolved in water acidified to pH 2 with HCl. The solution obtained was applied in the C18 (NEC) column, previously conditioned with 30 mL of methanol and 70 mL of acidified water. Polar compounds were removed with the aqueous solvent, and the retained phenolic compounds were then eluted with 50 mL of methanol. The extract was concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (1 mL).

HPLC-DAD Analysis of Phenolic Compounds. The separation was carried out with a HPLC unit (Gilson) and a 250 × 4.6 mm i.d., 5 μm Spherisorb ODS2 column (Waters, Milford, MA). The solvent system was a mixture of 1% acetic acid in water (A) and methanol (B), at a flow rate of 1 mL/min. Elution started with 20% B and reached 50% B at 30 min, 80% B at 37 min, and 100% B at 40 min. Detection was achieved with a Gilson diode array detector. Spectroscopic data from all peaks were recorded at 330 nm. The data were processed on Unipoint system software (Gilson Medical Electronics, Villiers le Bel, France). Peak purity was checked by the software contrast facilities. The different phenolic compounds were identified by comparing their chromatographic behavior and UV−vis spectra in the 200−400 nm range with authentic standards and with published data (*22*).

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. The peaks in the chromatograms were integrated using a default baseline construction technique. Because standards of the compounds identified in the shoot methanolic extracts were not commercially available, the derivatives of caffeic, *p*-coumaric, ferulic, and sinapic acids were quantified as chlorogenic, *p*-coumaric, ferulic, and sinapic acids, respectively; kaempferol derivatives were quantified as kaempferol-3-*O*-rutinoside and quercetin derivatives as quercetin-3-*O*-galactoside. Ferulic, *p*-coumaric, and sinapic acids were quantified as themselves.

DPPH$^{\bullet}$ Scavenging Activity. The antiradical activity of the extracts was determined spectrophotometrically in a Multiskan Ascent plate reader (Thermo Electron Corp.), by monitoring the disappearance of DPPH$^{\bullet}$ at 515 nm, according to a described procedure (*5*, *9*). The reaction mixture in the sample wells consisted of 25 μL of methanolic extract and 200 μL of methanolic solution of DPPH$^{\bullet}$ 150 μM. The plate was incubated for 30 min at room temperature after the addition of DPPH$^{\bullet}$. Three experiments were performed in triplicate.

RESULTS AND DISCUSSION

Phenolics Composition. The analysis by HPLC-DAD of the methanolic extracts of *B. oleracea* var. *costata* shoots revealed the presence of several phenolic acids and flavonoid derivatives. Thirty-six compounds were identified (**Figure 1**), which included 6 chlorogenic acids, 3 free hydroxycinnamic acids, 8 hydroxycinnamic acid gentiobiosides, and 15 and 4 kaempferol

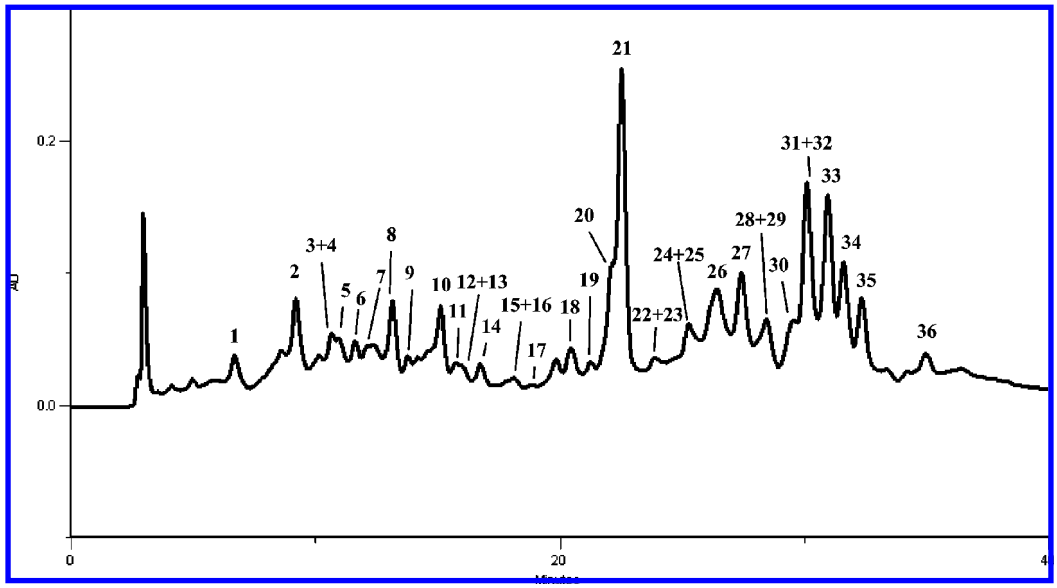

Figure 1. HPLC-DAD phenolics profile of *Brassica oleracea* var. *costata* shoots (sample C). Detection was at 330 nm. Peaks: (1) 3-caffeoylquinic acid; (2) 3-*p*-coumaroylquinic acid; (3) 3-feruloylquinic acid; (4) 4-caffeoylquinic acid; (5) kaempferol-3-*O*-(caffeoyl)-sophorotrioside-7-*O*-glucoside; (6) caffeoylferuloylquinic acid; (7) quercetin-3-*O*-(sinapoyl)sophorotrioside-7-*O*-glucoside; (8) kaempferol-3-*O*-(sinapoyl)sophorotrioside-7-*O*-sophoroside; (9) kaempferol-3-*O*-(sinapoyl)sophorotrioside-7-*O*-glucoside; (10) 4-*p*-coumaroylquinic acid; (11) kaempferol-3-*O*-(feruloyl)sophorotrioside-7-*O*-glucoside; (12) kaempferol-3-*O*-(sinapoyl)sophorotrioside-7-*O*-rhamnoside; (13) quercetin-3-*O*-(sinapoyl)sophorotrioside; (14) kaempferol-3-*O*-(methoxycaffeoyl)sophorotrioside; (15) kaempferol-3-*O*-(feruloyl)sophorotrioside-7-*O*-rhamnoside; (16) quercetin-3-*O*-sophoroside; (17) sinapoyl, caffeoyl, feruloyl-gentiobioside; (18) *p*-coumaric acid; (19) kaempferol-3-*O*-(caffeoyl)sophorotrioside; (20) ferulic acid; (21) sinapic acid; (22) kaempferol-3-*O*-(feruloyl)sophorotrioside; (23) kaempferol-3-*O*-sophoroside; (24) kaempferol-3-*O*-(methoxycaffeoyl/sinapoyl)sophorotrioside-7-*O*-sophoroside; (25) sinapoyl, methoxycaffeoyl-gentiobioside; (26) kaempferol-3-*O*-(methoxycaffeoyl/sinapoyl)sophorotrioside-7-*O*-glucoside; (27) kaempferol-3-*O*-(caffeoyl/sinapoyl)sophorotrioside-7-*O*-sophoroside; (28) kaempferol-3-*O*-(caffeoyl/sinapoyl)sophorotrioside-7-*O*-glucoside; (29) quercetin-3-*O*-(disinapoyl)sophorotrioside-7-*O*-glucoside; (30) disinapoyl, methoxycaffeoyl-gentiobioside; (31) disinapoyl, caffeoyl-gentiobioside; (32) kaempferol-3-*O*-(disinapoyl)sophorotrioside-7-*O*-glucoside; (33) 1-sinapoyl-2-feruloyl-gentiobioside; (34) 1,2,2′-trisinapoyl-gentiobioside; (35) 1,2′-disinapoyl-2-feruloyl-gentiobioside; (36) sinapoyl, diferuloyl-gentiobioside.

and quercetin derivatives, respectively. All of the detected compounds have already been reported in *B. oleracea* var. *costata* shoots (*22*), except *p*-coumaric, ferulic, and sinapic acids. However, qualitative and quantitative differences were noted between the phenolic profile of the several shoot samples, which were expected once they were obtained in different culture media, with distinct supplementations.

Data from the quantification of the identified compounds (**Table 2**) showed that MS liquid medium supplemented with 2 mg/L BAP and 0.1 mg/L NAA (sample C) allows the highest amount of phenolic compounds (ca. 19 g/kg) to be obtained. In a general way, the major compound in samples was 1-sinapoyl-2-feruloyl-gentiobioside (**33**), except for samples C, E, F, and G, in which the major compound was 1,2′-disinapoyl-2-feruloylgentiobioside (**35**).

When the media MS (samples C and G) and B5 (samples H and I), with the same hormonal supplementation (**Table 1**) were compared, higher production of phenolic compounds was observed in the MS medium (**Table 2**).

For liquid (samples C and H) and solid (samples G and I) media (**Table 1**) it was noted that liquid medium allows higher phenolic compound amounts (**Table 2**), which is not surprising; once in a liquid medium, there is higher nutrient availability, and their transport into the cells is easier (*24*). This occurs because in the liquid medium the absorption of nutrients can be made, theoretically, through all cellular surfaces, whereas in solid medium this absorption occurs only on the cells that directly contact the medium (*25*).

The addition of $AgNO_3$ (sample D) seems to lead to a decrease of phenolic production compared with the same medium without $AgNO_3$ (sample G) (**Tables 1** and **2**). This could be explained by the fact that $AgNO_3$ is an ethylene inhibitor, decreasing the levels of stress to which shoots were exposed and, consequently, the stimulation of phenolic compound accumulation (*26*).

The addition of GA (sample B) also seems to cause a decrease of the phenolic contents compared with the same medium without GA (sample C) (**Tables 1** and **2**). The hormone regulators can modulate key enzymes of phenolic biosynthesis pathways (*27*). In addition, the association of different hormone regulators can result in an antagonist or synergic response (*28*), which is a hypothesis to explain the observed decrease.

Two different concentrations of 2,4D were tested (samples E and F, **Table 1**), and it was verified that a higher phenolics production occurred in sample F, which corresponded to the addition of a higher concentration (2 mg/L) of this hormone regulator (**Table 2**). This finding is in accordance with a previous work in tobacco cells reporting the decrease of phenolic concentration with low level of 2,4D (*29*).

Independently of the shoot culture conditions, phenolic acid derivatives were the main compounds, varying between 77 and 93% of total phenolics (**Figure 2**). The synthesis of flavonoids was not preferred during shoot growth once the available nutrients are required for the primary metabolism, and mainly phenolic acids were synthesized (*7*). The sample supplemented with 2 mg/L BAP, 0.1 mg/L NAA, and 2 mg/L $AgNO_3$ (sample D) presents the higher flavonoid derivative relative amounts (23.7%), whereas sample H exhibits the lowest one (7.3%) (**Figure 2**).

By comparison of the media supplemented with different concentrations of 2,4D (samples E and F, **Table 1**) it was verified that the highest production of flavonoid derivatives occurred for higher supplementation of this growth regulator (sample F, **Figure 2**). The effect of 2,4D on some enzymes of

Table 2. Phenolic Composition of *Brassica oleracea* var. *costata* Shoots (Milligrams per Kilogram, Dry Basis)[a]

	sample[b]								
compound[c]	A	B	C	D	E	F	G	H	I
1			67.8 (0.4)	151.1 (1.1)	6.8 (1.6)	38.0 (0.5)	30.4 (0.7)		
2	2.4 (0.1)		95.0 (2.3)	62.5 (1.8)	19.5 (2.3)	43.9 (2.5)	28.2 (0.2)	2.8 (0.2)	2.6 (0.0)
3 + 4			115.8 (3.0)	80.0 (1.4)					
5	7.1 (0.7)		187.1 (0.8)						
6			126.5 (4.5)		35.6 (4.4)				
7			205.1 (6.3)	447.3 (18.4)			309.1 (35.7)		
8	98.7 (3.5)	47.5 (7.9)	304.6 (14.4)	342.2 (32.7)	80.3 (2.8)	537.7 (69.8)	418.8 (21.4)		
9	9.0 (0.5)	9.2 (0.5)	102.8 (0.6)	81.5 (3.5)			52.6 (0.2)		
10		30.7 (2.4)	106.6 (2.4)	24.8 (9.1)		28.1 (1.4)	2.3 (0.0)		
11			144.2 (0.1)						
12 + 13			157.5 (7.8)	11.8 (9.6)					
14	8.1 (0.5)	17.5 (0.2)	81.0 (3.3)						
15 + 16			42.3 (0.8)						
17			nq[d]						
18	3.9 (0.6)	1.2 (0.0)	30.4 (4.9)	2.3 (0.4)	2.5 (0.1)	4.4 (0.1)		2.1 (0.4)	2.7 (0.0)
19			5.9 (0.4)						
20	131.5 (7.6)	188.3 (2.1)	1617.2 (146.5)	462.1 (44.0)	390.5 (15.0)	539.3 (47.1)	217.3 (19.0)	61.3 (3.6)	186.9 (37.7)
21	254.4 (14.5)	257.1 (18.8)	2206.5 (209.2)	351.14 (42.8)	394.4 (28.1)	884.0 (23.1)	158.1 (1.4)	282.1 (25.2)	243.2 (10.9)
22 + 23	nq		71.8 (1.4)		6.0 (0.0)		32.9 (6.0)		
24 + 25			364.6 (16.5)	20.4 (14.5)	7.4 (1.0)	70.6 (0.7)	42.7 (4.2)		
26			694.4 (3.7)	290.4 (39.4)	44.8 (0.7)	230.6 (12.4)	144.2 (16.0)		
27			51.5 (2.8)		5.6 (0.0)	19.5 (0.5)	18.2 (0.2)		
28 + 29			330.3 (19.1)		44.2 (0.8)	198.0 (18.1)	108.2 (2.9)		
30			1051.5 (18.8)						
31 + 32	405.5 (5.8)	284.3 (17.4)	895.6 (9.9)	733.9 (25.4)	341.5 (8.3)	822.6 (60.2)	464.9 (55.2)	207.2 (4.4)	235.1 (5.1)
33	2493.3 (205.9)	2280.5 (110.1)	3213.5 (50.2)	1978.9 (151.6)	626.4 (29.2)	1226.5 (82.6)	843.4 (93.8)	1133.9 (123.2)	1026.6 (268.5)
34	186.0 (8.4)	243.5 (1.3)	1981.3 (31.1)	979.7 (68.6)	1052.1 (31.9)	3069.3 (48.7)	807.5 (71.1)	64.7 (1.4)	
35	584.8 (2.0)		3677.4 (45.8)	1619.6 (151.7)	1081.2 (156.8)	3340.9 (26.2)	2411.0 (110.6)	226.7 (13.8)	
36			1083.8 (30.8)	519.4 (25.9)	285.1 (85.6)	764.9 (14.9)	1812.0 (95.7)	850.2 (25.6)	
total	4184.9	3360.0	18969.5	8159.3	4423.7	11818.3	8905.0	2831.1	1697.0

[a] Results are expressed as means (standard deviation) of three determinations. [b] Sample characterization as in **Table 1**. [c] Compound numbers refer to peak numbers in **Figure 1**. [d] nq, not quantified.

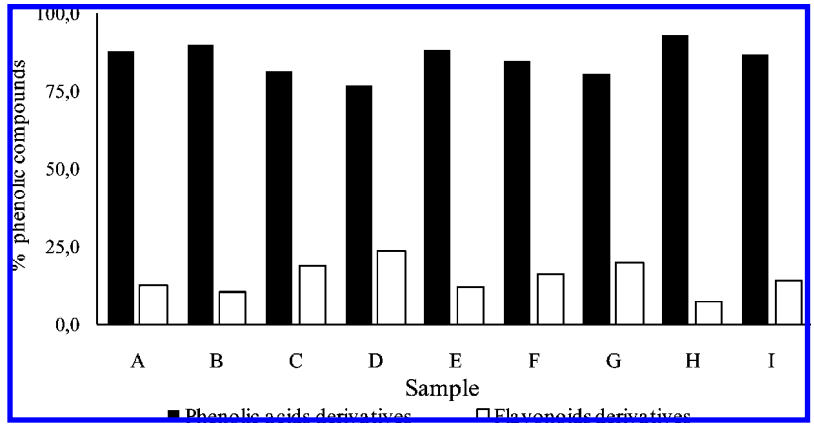

Figure 2. Relationship between phenolic acids and flavonoid derivatives in *Brassica oleracea* var. *costata* shoot samples. Characterization of samples was as in **Table 1**.

the flavonoid pathway, such as phenylalanine ammonia-lyase (PAL), was already reported. In studies with tobacco cells it was verified that PAL activity decreased for lower concentrations of 2,4D (*29*−*31*).

When comparing samples B and C (**Table 1**; **Figure 2**) we can see that the addition of GA to the medium supplemented with 2 mg/L BAP and 0.1 mg/L NAA caused a decrease of flavonoid derivatives. Previous works suggested that GA might inhibit enzymes involved in the biosynthesis of flavonoids, namely chalcone synthase, which may justify the decrease in flavonoid derivatives' production (*32*).

By comparison of the results obtained with those found for *B. oleracea* var. *costata* leaves obtained from in vivo cultures (*5*) it can be noted that the material produced in vitro presents a higher amount of phenolic compounds, independent of the medium and supplementation used.

In *B. oleracea* var. *costata* root and calli samples it was not possible to identify any phenolic compound. The lack of induction of phenolic metabolism in the roots may be explained by the fact that the available nutrients are being oriented for the primary metabolism (*33*). With respect to calli, this material may not express some genes responsible for the synthesis of certain secondary metabolites, such as phenolic compounds, because it is dependent upon plant differentiation: only in stages of greater differentiation are these promoters present, allowing the production of phenolics (*34*).

Antioxidant Activity. The DPPH$^{\bullet}$ assay provides basic information on the antiradical activity of extracts (*35*). The

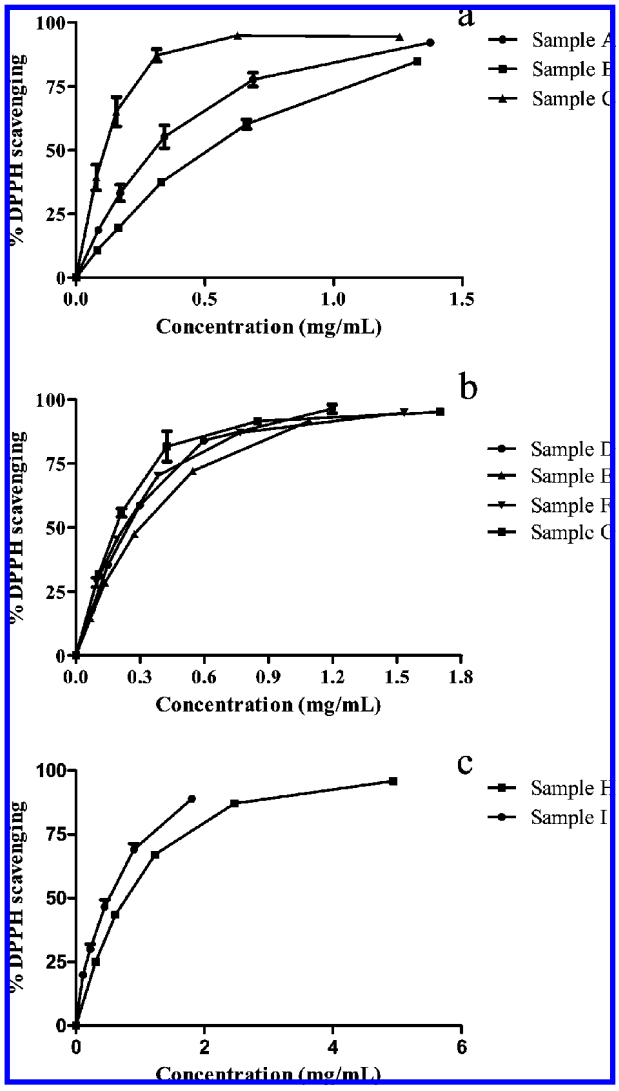

Figure 3. DPPH scavenging ability of methanolic extracts of *Brassica oleracea* var. *costata* shoots obtained on (**a**) liquid MS medium, (**b**) solid MS medium, and (**c**) B5 medium. Values show mean ± SE from three experiments performed in triplicate. Characterization of samples as in **Table 1**.

antioxidant activity was appraised in *B. oleracea* var. *costata* shoots because only this plant material presented phenolics. All of the analyzed shoot samples' methanolic extracts displayed a strong concentration-dependent antioxidant potential. The results were grouped by taking into account the medium characteristics (**Figure 3**).

Considering liquid MS medium (**Table 1**), supplementation with 2 mg/L BAP and 0.1 mg/L NAA (sample C) allowed us to obtain the in vitro material with the highest antioxidant activity (EC_{50} = 0.111 mg/mL). The shoots obtained on medium supplemented with GA (sample B) show less activity (EC_{50} = 0.516 mg/mL) than the medium without any supplementation (sample A; EC_{50} = 0.303 mg/mL) (**Figure 3**).

With regard to solid MS medium (**Table 1**), as for the liquid one, the shoots obtained on medium supplemented with 2 mg/L BAP and 0.1 mg/L NAA (sample G) exhibited the highest antioxidant activity (EC_{50} = 0.136 mg/mL), whereas supplementation with 1 mg/L 2,4D (sample E) led to the lowest one (EC_{50} = 0.301 mg/mL) (**Figure 3**).

By comparison of B5 media (**Table 1**), the sample obtained on the solid medium (sample I) exhibited higher antioxidant capacity (EC_{50} = 0.520 mg/mL) than the one developed on liquid medium (sample H, EC_{50} = 0.789 mg/mL) (**Figure 3**).

The observed antioxidant capacity can be related, at least in part, to shoot phenolic composition, once previous studies involving *B. oleracea* var. *costata* in vivo cultures and containing the same type of compounds (*5*, *6*, *9*) revealed these effects. In fact, in a general way, samples with higher phenolic content displayed the strongest antiradical ability, whereas those with lower levels of these compounds were less effective. In addition, *B. oleracea* var. *costata* in vitro material exhibited higher antioxidant potential than the one produced in vivo (*5*).

By analyzing all of the results it can be observed that MS liquid medium supplemented with 2 mg/L BAP and 0.1 mg/L NAA (sample C, **Table 1**) is the one which allows *B. oleracea* var. *costata* shoots with the highest antioxidant activity (**Figure 3**) and highest phenolic amount (**Table 2**) to be obtained. Thus, this medium can be considered to be the most appropriate for the production of *B. oleracea* var. *costata* shoots when it is intended to obtain material with these characteristics.

In conclusion, this work suggests that *B. oleracea* var. *costata* shoots may bring health benefits once they constitute a potential source of valuable secondary metabolites, such as antioxidant phenolic compounds. This species is considered to be a possible plant bioreactor to produce those metabolites, and shoots obtained in MS liquid medium supplemented with 2 mg/L BAP and 0.1 mg/L NAA seem to be the best option. This perspective deserves to be explored by food and pharmaceutical industries to obtain material that can be used either as nutraceuticals, food additives, or antioxidant supplements.

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