BRIEF COMMUNICATION

Carbon isotope composition as a tool to control the quality of herbs and medicinal plants

J.A. MARCHESE[*,+], L.C. MING[**], C. DUCATTI[***], F. BROETTO[***], E.T. DA SILVA[***], and M. LEONARDO[**]

Laboratory of Biochemistry and Plant Physiology, Agronomy College, Federal University for Technology of Paraná, Pato Branco-PR, CEP 85503-390, Brazil[*]
Agronomic Sciences College, São Paulo State University, Botucatu-SP, CEP 18603-970, Brazil[**]
Biosciences Institute, São Paulo State University, Botucatu-SP, CEP 18618-000, Brazil[***]

Abstract

Isotope screening is a simple test for determining the photosynthetic pathway used by plants. The scope of this work was to classify the photosynthetic type of some herbs and medicinal plants through studies of the carbon isotope composition ($\delta^{13}C$). Also, we propose the use of carbon isotope composition as a tool to control the quality of herbs and medicinal plants. For studies of $\delta^{13}C$, $\delta^{13}C‰ = [R\ (sample)/R\ (standard) - 1] \times 10^{-3}$, dry leaves powdered in cryogenic mill were analyzed in a mass spectrometer coupled with an elemental analyzer for determining the ratio $R = {}^{13}CO_2/{}^{12}CO_2$. In investigation of $\delta^{13}C$ of 55 species, 23 botanical families, and 44 species possessed a C_3 photosynthetic type. Six species found among the botanical families Euphorbiaceae and Poaceae were C_4 plants, and 5 species found among the botanical families Agavaceae, Euphorbiaceae, and Liliaceae possessed CAM-type photosynthesis. Carbon isotope composition of plants can be used as quality control of herbs and medicinal plants, allowing the identification of frauds or contaminations. Also, the information about the photosynthetic type found for these plants can help in introducing and cultivating exotic and wild herbs and medicinal plants.

Additional key words: C_3, C_4, and CAM plants; photosynthetic mechanisms.

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Modern eco-physiological research often uses isotope composition differences in plant biomass from that of the atmosphere. The isotope composition of plants with different photosynthetic pathways/types (*i.e.* C_3, C_4, and CAM) can be differentiated based on their isotope composition. Carbon isotope screening is a simple test for determining the photosynthetic type and is widely used as a criterion for C_3 and C_4 classification (Smith and Brown 1973, Farquhar *et al*. 1989, Pyankov *et al*. 2000). The information on photosynthetic types of plants is important when one wants to introduce and cultivate exotic and wild species, such as herbs and medicinal plants. In general, plants with the C_4 photosynthetic mechanism adapt better in hot climates, while the C_3 plants will be better in milder climates (Loomis and Connor 1992, Hall and Rao 1995, Körner and Bazzaz 1996, Lambers *et al*. 1998, Lawlor 2001). An example of medicinal plant widely introduced in many countries is *Artemisia annua* L. (used for treatment of malaria). *A. annua* is originally from China and has $\delta^{13}C‰$ values of –31.76, which is typical for a C_3 species. It is cultivated in Brazil, with the best yield of biomass and artemisinin on the Southern portion of that country, where the low temperature allows the decrease of CO_2 losses by photorespiration and good biomass production for C_3 plants (Marchese *et al*. 2005).

Also, we propose in this paper that isotope composition of plant biomass can be used to control the quality of herbs and medicinal plants, allowing the identification of frauds or contamination through the analysis of stable isotope composition. Over the last few years determinations of stable isotope ratios of the light elements, especially of carbon, hydrogen, and oxygen, have not only been applied to the elucidation of biochemical pathways and reaction mechanisms, but they have obtained increasing importance for authenticity control and origin assessment of food or food ingredients (Rossmann 2001).

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[+]Corresponding author; fax + 55 46 32202500, e-mail: abramo@olemiss.edu

The photosynthetic types or biochemical pathways of photosynthesis are highly conserved. Most plants are C_3 plants, in which the first product of photosynthesis is the three-carbon compound phosphoglyceric acid. A second biochemical pathway that allows for high concentration of CO_2 in leaves (decrease of photorespiration) has evolved in C_4 plants, which initially fix inorganic carbon in mesophyll cells, through the enzyme phospho*enol*-pyruvate carboxylase (PEPC), into the four-carbon compound oxaloacetic acid. In C_4 plants, oxaloacetate is converted into malate or aspartate, which then diffuses into bundle sheath cells that surround the vascular bundles where decarboxylation supplies large amounts of CO_2 to ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). In higher plants the C_4 pathway involves both biochemical and anatomical modifications, but it is not clear which of these modifications had evolved first. Some plants that possess characteristics of both C_3 and C_4 plants have been classified as C_3-C_4 intermediates and these may represent transitional stages in the evolution of C_4 photosynthesis from C_3 photosynthesis (Caemmerer 1992, Lawlor 2001, Hibberd and Quick 2002). Finally, CAM plants assimilate atmospheric CO_2 into C_4 acids, through the enzyme PEPC, predominantly at night and subsequently re-fix this CO_2 through RuBPCO into saccharides during the following day (Griffiths 1992, Cushman and Bohnert 1997, Lambers *et al*. 1998, Lawlor 2001).

The carbon dioxide in the earth's atmosphere is composed of different carbon isotopes. The majority is $^{12}CO_2$ (98.9 %); only approximately 1.1 % of the total amount of CO_2 in the atmosphere is $^{13}CO_2$; an even smaller fraction (10^{-10} %,) is the radioactive species $^{14}CO_2$. The chemical properties of $^{13}CO_2$ are identical to those of $^{12}CO_2$, but because of the slight difference in mass (2.3 %), plants use less $^{13}CO_2$ than $^{12}CO_2$. C_3 plants ($\delta^{13}C$ about –28 ‰) discriminate more $^{13}CO_2$ than the C_4 plants ($\delta^{13}C$ about –14 ‰). The largest isotope discrimination step is the carboxylation reaction catalyzed by RuBPCO, which has an intrinsic discrimination value ($\Delta^{13}C$) of –30 ‰. By contrast, PEPC has a much smaller isotope discrimination effect ($\Delta^{13}C$ = –2.0 to 5.7 ‰) (Sternberg *et al*. 1984, Farquhar *et al*. 1989, O'Leary *et al*. 1992, O'Leary 1993, Lambers *et al*. 1998, Condon *et al*. 2002). C_3 species represent approximately 85 % of all plant species, C_4 species account for about 5 %, and CAM species for 10 % (Lambers *et al*. 1998).

The scope of this work was to classify the photosynthetic type of some herbs and medicinal plants through studies of the carbon isotope composition ($\delta^{13}C$). Leaf samples were collected from the Medicinal Plants Collection of the Horticulture Section, Agronomic Sciences College, São Paulo State University, Brazil (22º52'S, 48º28'W, elevation 750 m). The plant species were identified by Dr. Lin Chau Ming and the first author.

The leaves of plants were analysed in the Isotopes Stables Center of São Paulo State University. Leaves were dried at 80 °C overnight and powdered in cryogenic mill (–196 °C). Triplicate samples were analysed in a mass spectrometer coupled in an analyser elemental for determination of the ratio $R = {}^{13}CO_2/{}^{12}CO_2$. The standard ratio is that of Pee Dee belemnite. Carbon isotope composition ($\delta^{13}C$) is a measure of the $^{13}C/^{12}C$ ratio in a sample of plant relative to the value of the same ratio in an accepted international standard, the limestone Pee Dee belemnite. Thus,

$$\delta^{13}C\,[‰] = (R_p/R_s - 1) \times 1\,000$$

where R_p is the $^{13}C/^{12}C$ ratio measure in plant material and R_s is the ratio of standard. $\delta^{13}C$ provides a means of relating samples of diverse origin for carbon isotope content. Samples of contemporary plant material have negative values of $\delta^{13}C$ because the $^{13}C/^{12}C$ ratio in the atmosphere is less than in Pee Dee belemnite and because there is a net discrimination against ^{13}C by plants during uptake and fixation of CO_2 into plant dry matter (O'Leary *et al*. 1992, O'Leary 1993, Condon *et al*. 2002, Dawson *et al*. 2002).

The carbon isotope composition ($\delta^{13}C$) of 55 vascular plant species from 50 genera and 27 families was investigated. 44 species from 43 genera and 23 botanical families possessed a C_3 photosynthetic type, presented a $\delta^{13}C$ average around –28 ‰. From C_3 plants, 25 species are exotic and 19 species are native from Brazil. With reference to growing habit, 22 species are herbs, 10 subshrubs, 5 shrubs, 5 trees, and 2 plant creepers. The total of 6 species from 5 genera found among the botanical families Euphorbiaceae (1 species) and Poaceae (5 species) were C_4, presenting a $\delta^{13}C$ average around –14 ‰. From C_4 plants, 4 species are exotic and 2 species are native. With reference to growing habit, 5 species are herbs and 1 is tree. Only 5 species from the botanical families Agavaceae (1), Euphorbiaceae (1), and Liliaceae (3) possessed the CAM photosynthesis and presented a $\delta^{13}C$ average around –14 ‰, like C_4 plants. In this case, we compared our $\delta^{13}C$ results with literature to support CAM species definition, because $\delta^{13}C$ values from C_4 and CAM plants are similar. From CAM plants, all species are exotic, 4 species possess the herbaceous growing habit, and 1 has the shrub growing habit.

Most of the analysed plants, 23 botanical families and 44 species, presented C_3 photosynthetic type (see Table 1), that possess on the average a $\delta^{13}C‰ = –28$ (Farquhar *et al*. 1989, O'Leary 1993). C_3 species represent approximately 85 % of all plant species (Lambers *et al*. 1998). $^{13}C/^{12}C$ in dry matter of C_3 plants is the result of discrimination against ^{13}C during several processes. These processes include discrimination during diffusion of CO_2 through the stomata; discrimination by RuBPCO during the carboxylation of CO_2 into the first photosynthate, and some downstream fractionations associated with metabolism and (possibly) respiration (O'Leary

Table 1. Carbon isotope composition and photosynthetic types of some herbs, spices, and medicinal plants from collection of Horticulture Section of the São Paulo State University, Botucatu-SP, Brazil, 2004. AR = arboreous; EX = exotic; HB = herbaceous; NT = native; PC = plant creeper; SB = shrub; SS = sub-shrub; $\delta^{13}C$ = carbon isotope composition.

	Botanical name	Local name	$[\delta^{13}C‰]_{PDB}$	Photosynth. type	Origin/ growing habit
Agavaceae	*Sansevieria zeylanica* Willd.	espada-de-são-jorge	–15.29 ± 0.04	CAM	EX/HB
Amaranthaceae	*Pfaffia glomerata* [Spreng] Pedersen	fáfia	–27.55 ± 0.92	C_3	NT/SB
	Alternanthera brasiliana [L.] O. Kuntze	terramicina	–27.88 ± 0.09	C_3	NT/HB
Anacardiaceae	*Myracrodruon urundeuva* Allemão	aroeira	–28.01 ± 0.03	C_3	NT/AR
Asteraceae	*Achillea millefolium* L.	mil-folhas	–28.97 ± 0.52	C_3	EX/HB
	Artemisia camphorata L.	artemisia	–28.76 ± 0.09	C_3	EX/HB
	Baccharis trimera Less.	carqueja	–31.72 ± 0.16	C_3	NT/SS
	Mikania glomerata Spreng.	guaco	–30.71 ± 0.03	C_3	NT/PC
	Tanacetum vulgare L.	catinga-de-mulata	–29.32 ± 0.07	C_3	EX/HB
Bignoniaceae	*Jacaranda decurrens* Cham.	carobinha	–28.41 ± 0.12	C_3	NT/SS
Boraginaceae	*Symphytum officinalis* L.	confrei	–28.64 ± 0.10	C_3	EX/HB
Cactaceae	*Peireskia aculeata* Mill.	ora-pro-nobis	–30.88 ± 0.05	C_3	EX/SB
Caesalpiniaceae	*Bauhinia forficata* Link.	pata-de-vaca	–27.84 ± 0.22	C_3	NT/SB
Cecropiaceae	*Cecropia glaziovii* Snethlage	embaúba	–29.75 ± 0.23	C_3	NT/AR
Celastraceae	*Maytenus aquifolium* Mart.	espinheira-santa	–27.84 ± 0.03	C_3	NT/AR
	Maytenus ilicifolia Reissek	espinheira-santa	–30.27 ± 0.16	C_3	NT/AR
Chenopodiaceae	*Chenopodium ambrosioides* L.	erva-de-santa-maria	–28.19 ± 0.14	C_3	NT/SS
Equisetaceae	*Equisetum hiemale* L.	cavalinha	–28.42 ± 0.08	C_3	NT/HB
Euphorbiaceae	*Euphorbia* sp.	janaguba	–17.45 ± 0.05	C_4	NT/AR
	Euphorbia tirucalli L.	aveloz; coroa-de-cristo	–14.97 ± 0.18	CAM	EX/SB
Geraniaceae	*Pelargonium graveolens* Art.	malva-cheirosa	–29.29 ± 0.05	C_3	EX/SS
Iridaceae	*Eleutherine bulbosa* [Mill.] Urb.	palmeirinha	–29.36 ± 0.20	C_3	NT/HB
Lamiaceae	*Melissa officinalis* L.	melissa verdadeira;	–28.01 ± 0.03	C_3	EX/HB
	Mentha sp.	hortelã	–29.13 ± 0.09	C_3	EX/HB
	Ocimum basilicum L.	manjericão, alfavaca	–31.73 ± 0.01	C_3	EX/SS
	Ocimum selloi Benth.	alfavaca-cheiro-de-anis	–29.05 ± 0.53	C_3	EX/HB
	Origanum vulgare L.	oregano	–29.73 ± 0.07	C_3	EX/HB
	Plectranthus barbatus Andr.	falso-boldo	–28.49 ± 0.23	C_3	EX/HB
	Plectranthus neochylus Schlechter	boldo-rasteiro	–29.42 ± 0.21	C_3	EX/HB
	Rosmarinus officinalis L.	alecrim	–27.83 ± 0.11	C_3	EX/SS
	Stachys lanata L.	orelha-de-coelho	–27.66 ± 0.77	C_3	EX/HB
	Tetradenia riparia [Hochst.] N.E.Br.	incense	–28.53 ± 0.01	C_3	EX/SS
Liliaceae	*Aloe arborescens* Mill.	babosa	–18.35 ± 0.04	CAM	EX/HB
	Aloe sp.	babosa	–12.44 ± 0.25	CAM	EX/HB
	Aloe vera [L.] Burm. F.	babosa	–15.63 ± 0.03	CAM	EX/HB
Lythraceae	*Cuphea carthagenensis* [Jacq.] Macbr.	sete-sangrias	–29.86 ± 0.04	C_3	NT/HB
Myrtaceae	*Eugenia uniflora* L.	pitanga	–29.16 ± 0.04	C_3	NT/AR
Piperaceae	*Piper aduncum* L.	falso-jaborandi	–29.49 ± 0.05	C_3	NT/SB
	Potomorphe umbellata [L.] Miq.	pariparoba	–28.19 ± 0.28	C_3	NT/HB
Poaceae	*Cymbopogon citratus* [DC.] Stapf.	capim-limão	–14.63 ± 0.46	C_4	EX/HB
	Cymbopogon flexuosus [DC.] Stapf.	capim-santo	–13.93 ± 0.10	C_4	EX/HB
	Cymbopogon winterianus Jowitt	citronela	–14.32 ± 0.20	C_4	EX/HB
	Elionurus latiflorus Nees	barba-de-bode	–13.99 ± 0.09	C_4	NT/HB
	Vetiveria zizanioides Stapf.	vetiver	–14.83 ± 0.11	C_4	EX/HB
Portulacaceae	*Talinum triangulare* [Jacq.] Willd.	maria-gorda	–25.26 ± 0.52	C_3	NT/HB
Rutaceae	*Ruta graveolens* L.	arruda	–28.79 ± 0.04	C_3	EX/HB
Umbelliferae	*Eryngium foetidum* L.	coentro-de-caboclo	–28.93 ± 0.37	C_3	NT/HB
	Foeniculum vulgare [Mill.] Gaertner	funcho	–29.65 ± 0.00	C_3	EX/HB
Verbenaceae	*Aloysia triphylla* [L' Hérit] Britt.	cidró	–27.53 ± 0.03	C_3	EX/SS
	Lippia alba [Mill.] N.E. Br.	erva-cidreira-brasileira	–29.40 ± 0.67	C_3	NT/SS
		cidreira-brasileira	–30.29 ± 0.16	C_3	NT/SS
	Lippia sidoides Cham.	alecrim-pimenta	–30.11 ± 0.15	C_3	NT/SB
Vitaceae	*Cissus verticillata* [L.] Nich. & C.E. Jarvis	insulina	–27.73 ± 0.06	C_3	NT/ PC
Zingiberaceae	*Curcuma longa* L.	açafrão	–27.77 ± 0.63	C_3	EX/HB
	Costus spicatus [Jacq.] Sw.	cana-do-brejo	–26.44 ± 0.54	C_3	NT/HB

et al. 1992, O'Leary 1993, Condon *et al*. 2002).

The species within the Euphorbiaceae and Poaceae families presented C_4 photosynthetic type, with the $\delta^{13}C‰ = -14$ (see Table 1). In C_4 plants most of the $^{13}CO_2$ that is discriminated against RuBPCO does not diffuse back to the atmosphere. This is prevented first by the diffusion barrier between the vascular bundle sheath and the mesophyll cells. Second, mesophyll cells contain large amounts of carbonic anhydrase and of PEPC, which discriminate less against the $^{13}CO_2$, and scavenge most of the CO_2 that might escape from the bundle sheath (O'Leary 1993, Farquhar *et al*. 1989, Lambers *et al*. 1998).

Species from the Agavaceae, Euphorbiaceae, and Liliaceae families have CAM photosynthesis (see Table 1). Like RuBPCO from C_3 and C_4 plants, the enzyme from CAM plants discriminates against $^{13}CO_2$. The fractionation is considerably less than that of C_3 plants and similar to that of C_4 species (Sternberg *et al*. 1983, Lambers *et al*. 1998). Because $\delta^{13}C$ from C_4 and CAM are similar, we compared our $\delta^{13}C$ results with literature to support the CAM species definition. Previous reports have confirmed that other species of this genus exert CAM photosynthesis (Martin *et al*. 1990, Luo *et al*. 1991, Griffiths 1992, Winter and Holtum 2002).

With reference to use of carbon isotope analysis as a tool for quality control of medicinal plants, we describe in this paper a typical example of fraud that occurs in Brazil, where some sellers place *Cymbopogon citratus* [DC.] Stapf. (lemon grass) powder inside the tea packets, and sell as *Melissa officinalis* L. (lemon balm), because lemon grass is less expensive and more available than lemon balm. *M. officinalis* presented a $\delta^{13}C‰ = -28.01$, a typical result for C_3 photosynthetic type, while *C. citratus* presented a $\delta^{13}C‰ = -14.63$, a typical result for C_4 photosynthetic type (see Table 1). Carbon isotope composition analysis of the raw material easily differentiates both species. A carbon isotope sample analysis cost is around US$ 25.00, which is not too expensive for the industry.

There is limitation on the use of carbon isotope biomass composition for fraud detection when the species in question have the same photosynthetic pathways/type or the same $\delta^{13}C$, like C_4 and CAM species. In such case, the analysis of other atom isotopes such as hydrogen, oxygen, sulphur, and nitrogen, together with the carbon isotopes, is necessary for a better identification.

Therefore, carbon isotope composition results of plants from this study can be used as quality control of herbs and medicinal plants, allowing the identification of frauds or contaminations. Also, the information about the photosynthetic type found for these plants can help one wants to introduce and cultivate exotic and wild herbs and medicinal plants.

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Kumar, S., Fladung, M. (ed.): **Molecular Genetics and Breeding of Forest Trees. –** Forest Products Press, New York – London – Oxford 2004. ISBN 1-56022-959-4. 436 pp., USD 44,96 (softbound).

Fifty-seven scientists from 12 countries of the world contributed to this book (Australia, Belgium, Canada, China, Finland, France, Germany, Italy, Japan, New Zealand, Spain, USA). It is divided to four parts: Forest Tree Functional Genomics, Molecular Biology of Wood Formation, Forest Tree Transgenesis, and Genome Mapping in Forest Trees.

Four chapters of Part I deal, among others, with expressed sequence tag databases, proteomics, and exploring the transcriptome of ectomykorrhizal symbiosis.

Part II consists of four chapters on genomics of wood formation, including celullose biosynthesis, lignin metabolism, and *in vitro* systems.

In five chapters of Part III, genetic modification in conifer forestry is dealt with. Insect resistance, modification of flowering, stability of transgene expression, and asexual production of marker-tree transgenosis are the main topics. Four chapters of Part IV analyse the use of high-density linkage maps, microsatelittes in forest tree species, and genome mapping in some tree species.

Main trees studied in this connection were pines, poplars, eucalipti, acacias, and species specific for some countries (*e.g.* China). In some cases model plants (*Arabidopsis*, yeasts) were used for comparison.

I recommend this book for students and scientists who are interested in forest genetics and tree breeding. I only regret that an information about legislation and regulation in the field of transgenic trees' use in forestry is missing.

J. KOBLIHA (*Praha*)