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Ferulic acid in C_4 grasses.

Marco Antônio Menezes Neto [*,1], **Miguel Pedro Guerra** [2, 3]

[1] Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil.

[2] Graduate Program in Plant Genetic Resources, Federal University of Santa Catarina, Florianópolis, SC, P.O. Box 476, 88040-900, Brazil

[3] Graduate Program in Agricultural and Natural Ecosystems, Federal University of Santa Catarina, Curitibanos, Campus Ulysses Gaboardi Road, Km 3, 89520-000, Curitibanos, SC, Brazil

*For correspondence. E-mail: neto@ufpa.br (+55 (91) 98819-1119)

Abstract

In order to identify the phenolic substance that is responsive in a new C_4-safranin histochemical test for determination of the photosynthetic pathway in grasses, we developed a fluorescence microscopy aided protocol for extraction and quantification of the target substance, with subsequent mass spectrometry analysis for molecular identification. The results indicate the target phenolic substance in C_4 grasses' bundle sheath cells is ferulic acid. In addition, we observed differences in distribution and compartmentalization of ferulic acid depending on the plants' photosynthetic pathway. In C_3 grasses, ferulic acid is restricted to mesophyll cells and concentrated on cell walls, while in C_4 grasses it is restricted to bundle sheath cells, and concentrated in the membrane adjacent to radial and tangential walls. Taken together these results indicate that ferulic acid may have an important function related to the

C_4 photosynthetic pathway in grasses. Such function may be unprecedented and different from any other previously ascribed to this substance.

Keywords: C4 grasses, fluorescence microscopy, ferulic acid.

Introduction

A new histochemical method to determine the photosynthetic pathway in grasses using safranin O as indicator (henceforth termed 'C_4-safranin histochemical test') was proposed by Neto, Mardegan and Barbosa [1]. In this method, safranin concentrates exclusively in bundle sheath cells of C_4 grasses, as opposed to C_3 grasses, coloring them into intense red, and indicating the presence of one or more phenolic compounds, since safranin is a non-specific phenolic marker (Lewis e Yamamoto [2]).

Neto and Guerra [3] used the C_4-safranin histochemical test to screen 58 grass species (C_3 and C_4 of different types: NAD dependent malic enzyme (ME-NAD), NADP dependent malic enzyme (ME-NADP) and phosphoenolpyruvate carboxykinase (PCK)), confirming the efficacy of the method in grasses, although apparently it does not apply in Cyperaceae. Furthermore, these authors observed that the phenolic substances are located within the protoplast of bundle sheath cells, not on the cell walls.

The main group of phenolic compounds are hydroxycinnamic acids, namely phenolic acids, as ferulic, ρ-coumaric, o-coumaric, caffeic, sinapic, syringic, ρ-hydroxybenzoic, vanillic and protocatechuic acid (Das & Singh [4-5]; Guo & Beta [6]; Roberts [7]; Van Hung [8]). Ferulic acid (4-hydroxy-3-methoxycinnamic) is the most abundant phenolic compound found in whole maize grain, representing about 3.1 - 4% of maize grain dry weight (Buanafina [9]; Mathew & Abraham [10]; Santiago & Malvar [11]; Saulnier, Vigouroux & Thibault [12]). It derives from phenylpropanoid metabolism (Buanafina [9]), being related to lignin biosynthesis from phenylalanine and tyrosine (Zhao & Moghadasian [13]), abundant in plant epidermis, xylem vascular elements, bundle sheath and sclerenchyma (Faulds and Williamson [14]; Lambert et al. [15]), and present in primary and secondary walls of grass cells (Harris and Hartley [16]).

The results found by Neto, Mardegan and Barbosa [1] and Neto and Guerra [3] suggest the presence of one or more phenolic substances exclusively stored in the protoplasm of C4

grasses' bundle sheath cells. This apparently compartmentalized location suggests a possible physiological function, which could differ from previously known functions of ferulic acid in grasses (Carpita [17]; Buanafina [9]; Buanafina *et al*. [18]; Buanafina *et al*. [19]). Given this, the objective of this study was the isolation and identification of the phenolic compounds present in the protoplasm of C4 grasses' bundle sheath cells.

Materials and Methods

Fluorescence microscopy

We scrutinized the identity of the phenolic compounds responsible for the positive response in the C_4-safranin histochemical test comparing two grass species: *Arundo donax* (C_3) and *Urochloa brizantha* (C_4). Both species were previously identified and investigated in the study by Neto and Guerra [3].

We hand-sectioned leaves transversally in the middle portion using steel blades. We mounted sections with water between microscopy glass slides and coverslips. After observing the sections' auto fluorescence, they were treated with a 2% sodium hypochlorite solution to clarify the tissue. After discoloration, sections were observed again in a fluorescence microscope. After that, we treated the sections with pure methanol to eliminate all hydroxycinnamic acids, and once again mounted and observed in the fluorescence microscope. We took photomicrographs during all steps of the process, using an Olympus IX80 inverted fluorescence microscope attached to an Olympus DP71 camera.

Mass spectrometry

The epifluorescence study of C_3 and C_4 grass leaves allowed us to develop an extraction protocol for the phenolic compounds present exclusively in the C_4 grasses' bundle sheath cells and in C_3 grasses' mesophyll cells, with subsequent mass spectrometry analysis, as follows: we cut leaves from both grasses in transversal sections (<1mm in width), and treated sections with 2% sodium hypochlorite solution until complete discoloration to eliminate influences from natural pigments, such as chlorophyll. Subsequently sections were washed in running water to remove hypochlorite residues. We added one gram of this material (transversal sections of leaves) to a 10 ml falcon tube filled with methanol. After a two-hour incubation, tubes were centrifuged for one minute at 100 g^{-1}, the supernatant was poured in a watch glass

and left to dry in a stove at 40 ºC. After drying, we scraped the material left in the bottom of the watch glass and put it into an eppendorf tube for subsequent mass spectrometry analysis of phenolic compounds, carried out in the Multi-user Spectrometry Laboratory of Molecular Biology Center of Mass Structure - LabMMass/CEBIME of the Federal University of Santa Catarina, Brazil using a Brukeratuoflex III mass spectrometer, using: Method reflective positive; range m/z (0-1000 Da). Ferulic acid (4-hydroxy-3-methoxycinnamic acid, Sigma-90034), was used as standard. Samples and the standard were diluted in 60 µl methanol and added to a Dihydroxybenzoic acid matrix (DHB; 20mg/ml; 70% methanol/30% water/0.2% trifluoroacetic acid) at the proportion of 1 µl sample to 9 µl matrix.

Plasmolysis induction

Transversal sections of leaves from both investigated grass species were treated with 2% sodium hypochlorite until complete clarification, washed with distilled water, and mounted between microscopy glass slides and cover slips with a 10% sucrose solution to dehydrate the tissue and induce cell plasmolysis. After 10-minutes incubation, we observed sections in the fluorescence microscope.

Results

Fluorescence microscopy

Transversal sections of fresh leaves of *Urochloa brizantha* (C_4) and *Arundo donax* (C_3) presented autofluorescence (Figure 1 a-b, respectively). Bundle sheath cells in *A. donax* did not show any fluorescence, indicating the absence of chlorophylls in these cells. Although sodium hypochlorite treatment completely degraded chlorophyll molecules, we still observed fluorescence exclusively in bundle sheath cells of *U. brizantha*, while in *A. donax* fluorescence was restricted to mesophyll cells (Figure 1 c-d, respectively). The phenolic substance within the bundle sheath cells of *U. brizantha* and mesophyll cells of *A. donax* was completely eliminated by the treatment with sodium hypochlorite and methanol (Figure 1 e-f, respectively).

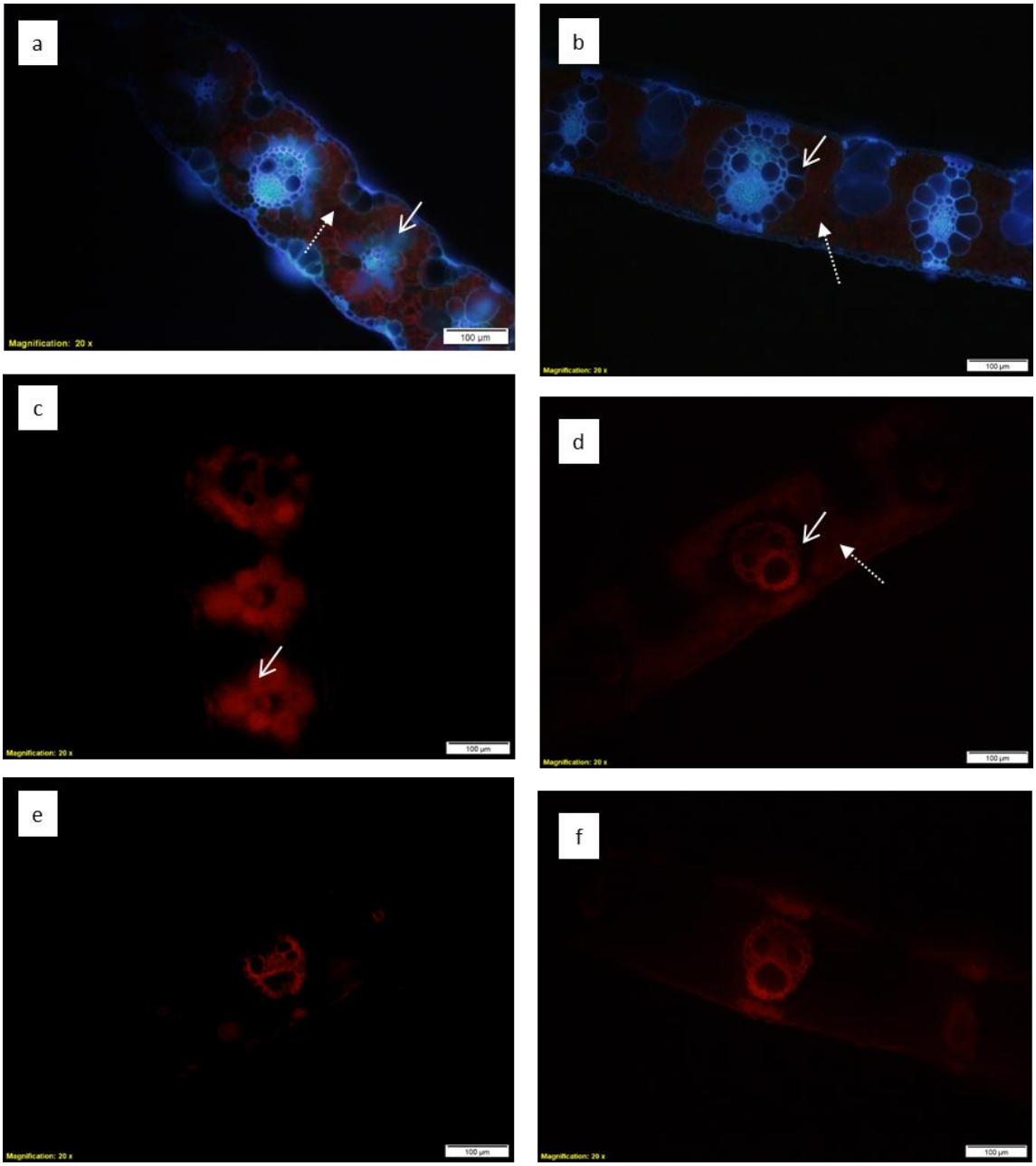

Figure 1. Transverse sections of leaves of *Urochloa brizantha* (a, c, e) and *Arundo donax* (b, d, f). Autofluorescence (UV) (a, b). Fluorescence after treatment with sodium hypochlorite (violet light) (c, d) and after treatment with hypochlorite and methanol (violet light) (e, f). Arrows indicate bundle sheath cells. Dotted arrows indicate mesophyll cells.

Mass spectrometry

Standard ferulic acid presented two forms (176, 275 Da and 194,348 Da) (Figure 2-a). Mass spectrometry analysis of the methanolic extracts from leaves of the studied C_3 and C_4 grasses, obtained using the protocol carried out after the initial observations under fluorescence

microscopy, showed that ferulic acid is the only phenolic substance present in both grasses (Figure 2 b-c, respectively). Fragmentation of ferulic acid molecules present in C_3 and C_4 grasses showed only the 176,275 Da form, while the 194,348 Da form was not observed (Figure 2 b-c).

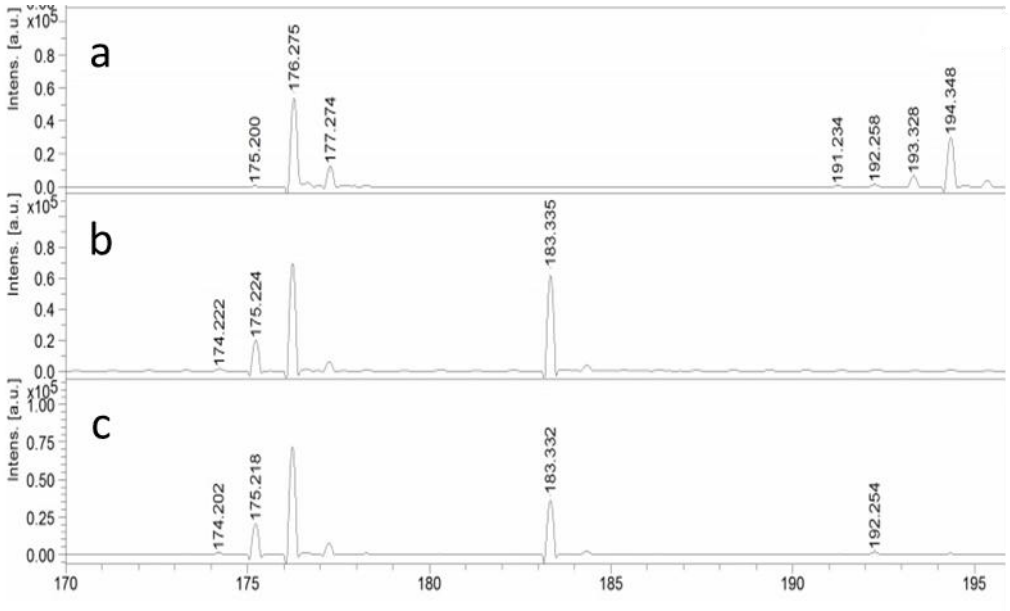

Figure 2. Mass spectrometry (MALDI-TOF) of ferulic acid standard (a) and extracts from leaves of *Arundo donax* (b) and *Urochloa brizantha* (c). The ferulic acid standard presented two peaks (176.275 and 194.348 Da). The grasses presented only the 176.275 Da peak of ferulic acid (b-c).

Comparing the results observed in fluorescence microscopy with results from mass spectrometry, we may infer that ferulic acid is the compound within the bundle sheath cells of C_4 grasses that causes positive results in the C_4-safranin histochemical test (Neto, Mardegan and Barbosa [1]). However, ferulic acid also occurs in leaves of C_3 grasses. The difference arises from a compartmentalized distribution of this phenolic compound. Ferulic acid was concentrated in the bundle sheath cells of the C_4 grass, while in the C_3 grass it was located only in mesophyll cells (Figure 1, c-d, respectively). In addition, we observed a compound with 183,335 Da in samples of grasses C3 and C4 (Figure 2 b-c).

After identifying the phenolic substance present in mesophyll cells of *A. donax* and bundle sheath cells of *U. brizantha* (ferulic acid), we observed that the application of methanol in *U. brizantha* leaf sections was sufficient to eliminate ferulic acid (Figure 3a), while in *A. donax* this required a treatment with sodium hypochlorite and methanol, regardless of the order (Figure 3b).

Dehydration of the C_4 grass bundle sheath cells caused the membrane to recoil and detach from the cell wall. This membrane dislocation led to a concentration of fluorescence on the cell membrane (Figure 3-c). Similar result was not observed in mesophyll cells of C_3 grass, since fluorescence was distributed throughout the cell surface (Figure 3-d).

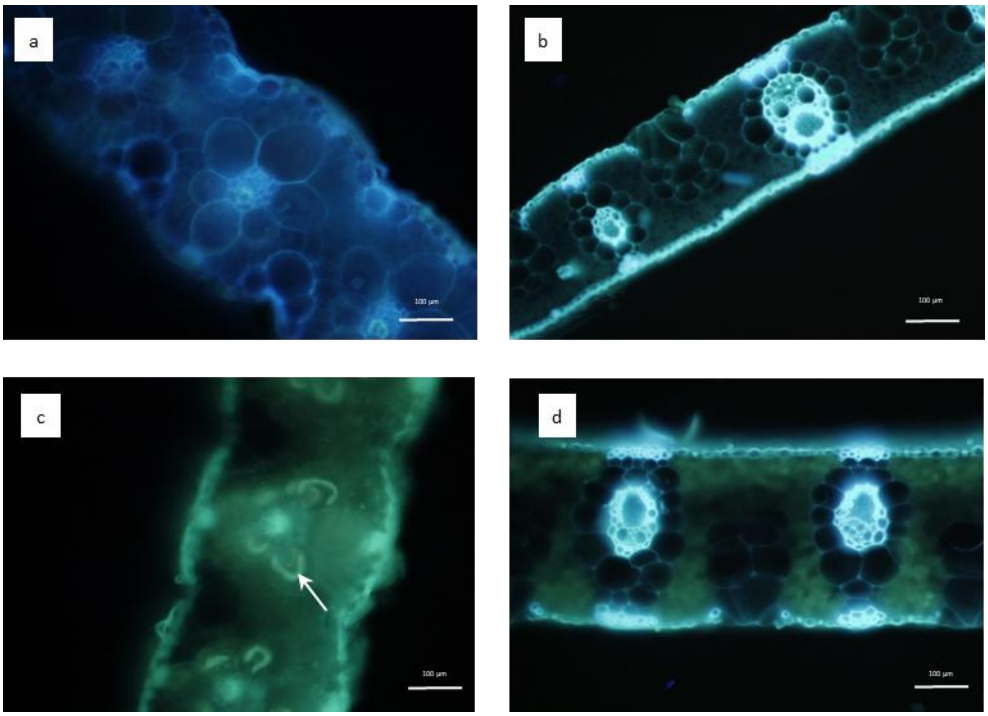

Figure 3. Transverse sections of *Urochloa brizantha* (a, c) and *Arundo donax* (b, d). Fluorescence after treatment with methanol (a). Fluorescence after clarification with hypochlorite and treatment with methanol (b). Fluorescence after clarification with sodium hypochlorite and plasmolysis with sucrose solution (c-d). Arrow indicates the cell membrane of the *Urochloa brizantha* bundle sheath cells with intense fluorescence of ferulic acid (blue-green), after detachment from the cell wall by the effect of the plasmolysis.

Discussion

The C_4-safranin histochemical test proposed by Neto, Mardegan and Barbosa [1] to determine the photosynthetic pathway in grasses derived from the observation of a responsive pattern in transversal sections of grass leaves, after discoloring with sodium hypochlorite and subsequent treatment with safranin O and astra blue. The authors also observed that only bundle sheath cells of C_4 grasses stained red, indicating that safranin reacts with some unknown phenolic substances present exclusively in C_4 grasses bundle sheath cells. Neto and Guerra [3] performed the histochemical test in 58 grass species with different photosynthetic pathways (C_3 and C_4 of all three types), comparing the results with isotope composition

analysis and relevant literature. The pattern observed by Neto, Mardegan and Barbosa [1] was confirmed by Neto and Guerra [3], proving the efficacy of the test and suggesting the possible involvement of the reactive phenolic substance(s) in the photosynthesis of C_4 grasses.

Phenolic compounds, as hydroxycinnamic acids, coumarins, stilbenes and styrylpyrones are strongly autofluorescent when irradiated with ultraviolet or violet light. For that reason, fluorescence microscopy is a powerful tool for studies on cellular location of these substances (Ibrahim and Barron [20]; Veit *et al*. [21]; Gorham [22]). Other techniques were used in the investigation of colorless and/or non-autofluorescent phenolic substances (Treutter [23]; Ibrahim [24]; Grandmaison and Ibrahim [25]; Vogt *et al*. [26]; Schnitzler *et al*. [27]; Reinold and Hahlbrock [28]).

Fluorescence microscopy was fundamental to identify the phenolic compound detected in the C_4 grass bundle sheath cells, allowing for the development of an efficient extraction protocol and subsequent identification of the target reactive substance.

Leaves of Poaceae species emit a higher blue-green fluorescence than dicot leaves when excited with UV-A. This fluorescence derives from a high concentration of ferulic acid in these plants. Ferulic acid seems to be main blue-green fluorescence emitter in leaves (Lichtenthaler and Schweiger [29]) (Figure 3 c-d). When excited by violet light, ferulic acid emits fluorescence in the red band (Figure 1 c-d)

Phenolic compounds perform a variety of functions in plant-environment interactions (Harborne [30]), including attraction of insects, plant-plant signaling, plant-symbiosis and plant-pathogen signaling, and protection against biotic and abiotic stresses (Hutzler [31]).

In *A. donax* (C_3 grass), ferulic acid seems to be confined in the cell walls of mesophyll cells and may only be extracted using methanol and sodium hypochlorite treatments, regardless of the treatment order (Figure 3 b). It seems obvious that safranin, used in the histochemical test proposed by Neto, Mardegan and Barbosa [1], readily reacts with the ferulic acid present in the membrane of bundle sheath cells of C_4 grasses. However, it does not react with the ferulic acid inside the walls of mesophyll cells of C_3 grasses.

The presence of ferulic acid only in 176,275 Da form in both C3 and C4 grass samples indicates that after treatment with hypochlorite and methanol during the extraction process, there was a loss of 18 Da, which was totally converted into the molecular ion form ($M^{+\bullet}$ less H_2O) (Figure 2 b-c), while the ferulic acid standard presented the two forms of the acid (176,275 and 194,348 Da) (Figure 2-a).

The 183,335 Da compound also observed in the grass samples was not identified, however, it is likely to be a molecular ion of a substance with an odd number of nitrogen atoms.

The phenolic esters cross-linkages of cell wall polysaccharides are formed through photochemical reactions or through coupling reactions catalyzed by peroxidases (Santiago & Malvar [11]). Ferulic acid is usually attached to the O-5 of some arabinopyranose residues (primary alcohol on the C5 carbon) of arabinoxylans, through an ester linkage (Buanafina [9]; Mathew & Abraham [10]; Santiago & Malvar [11]; Saulnier and Thibault [32]; Zhang, Smith and Li [33]), or be covalently linked to lignin (Buanafina [9]; Pedersen *et al*. [34]; Santiago and Malvar [11]).

In monocots, ferulic acid is found adhered to cell wall polymers, by ester bonds between its carboxyl group and the C5-hydroxyl of α-L-arabinosyl side chains of xylans (Hartley and Ford [35]; Ralph and Helm [36]; Wende and Fry [37]). Derived from the phenylpropanoid metabolism (Ou and Kwok [38]), ferulic acid is observed in primary and secondary cell walls of grasses (Harris and Hartley [16]), being more abundant in epidermis, xylem vessels, vascular bundle and sclerenchyma (Faulds and Williamson [14]; Lambert *et al*. [15]).

The occurrence of ferrulic acid within the cell walls of mesophyll cells of C3 grasses may hamper its reaction with safranin. It is likely that the connection of this acid to the components of the membranes of bundle sheath cells of C4 grasses allow the interaction with safranin, taunting the response observed by the histochemical test in the study of Neto, Mardegam and Barbosa [1].

Some studies indicate a high degree of compartmentalization for phenylpropanoids, flavonoids, and the enzymes involved in their biosynthesis (Knogge and Weissenböck [39]; Schmelzer, Jahen and Hahlbrock [40]; Haussühl, Rohde and Weissenböck [41]). Phenylpropanoid and flavonoid compounds are usually accumulated in central vacuoles of guard cells and epidermal cells, as well as in sub-epidermal leaf (Moskowitz and Hrazdina [42]; Weissenböck, Hedrich and Sachs [43]; Schnabl, Weissenböck, and Schar [44-45]) and branch cells (Ozimina [46]). Compartmentalization of ferulic acid in bundle sheath cells of C_4 grasses is a strong indication that it may perform other functions in addition to those previously proposed.

The results from Neto and Guerra [3], which corroborated the efficiency of the C_4-safranin histochemical test in 58 grass species, represent strong evidence that the results obtained for *U. brizantha* in our study may be extrapolated to other C_4 grasses, since all C_4 grasses analyzed

by Neto and Guerra [3] presented the phenolic substance in the bundle sheath cells. Negative results of the histochemical test on sedges (Neto and Guerra [3]) suggest that the presence of compartmentalized ferulic acid on the bundle sheath cells is a characteristic specific to grasses, indicating the possibility of synapomorphy of the PACMAD clade, formed only by Poaceae subfamily with C4 species.

Geldner [47], in a revision about endodermis, approached aspects relative to its structure and function, indicating that the main component substances of Caspary bands are lignin and suberin. Bundle sheath of C4 species share similarities with endodermic tissues in petioles, stems and roots (Nelson and Dengler [48]; Slewinski [49]). Naseer et al. [50] suggest ferulic acid may bind to a fatty acid and, by interacting with lignin during the synthesis of Caspary bands, may cause an alteration in their hydrophobicity, suggesting ferulic acid also performs an important function in root endodermis. Our results reinforce the hypothesis that Kranz-type C_4 mechanism is a manifestation of root endodermis in photosynthetic tissue (Slewinski [51]), and additionaly indicate a possible barrier function of ferulic acid, as it seems to be concentrated in the membranes adjacent to the tangential and radial walls of the bundle sheath cells in C4 grasses, configuring a "u" shape distribution (Figure 3c), similar to the Caspary streaks cells of root endodermis.

There is a diversity of papers that address aspects relative to ferulic acid on grasses, but these papers mention others plant parts, such as: stem, bran, endosperm, flour and seeds (Smith and Hartley [52]; Sun *et al*. [53]; Levigne *et al*. [54]; Schatz *et al*. [55]; Lopez-Martinez *et al*. [56]; Dobberstein and Bunzel [57]; Sun *et al*. [58]; Uddin *et al*. [59]; Dynkowska, Cyran and Ceglinska [60]; Malunga and Beta [61]). The majority of these papers highlight the importance of ferulic acid as structural components of the cell walls on a diversity of grasses tissues. None of these papers ever proposed a function of ferulic acid related to the photosynthetic process in C4 grasses.

The compartmentalization of ferulic acid in membranes of bundle sheath cell of C_4 grasses may be one of the causes for the extraordinary success of these plants, which expanded as no other plant group in the planet, dominating vast land areas. Considering this, further studies are necessary to confirm the role of ferulic acid in the physiology of C_4 grasses photosynthesis and, perhaps, in other botanical families with C_4 species.

Conclusions

Ferulic acid is the phenolic compound present and compartmentalized in the bundle sheath cells of C4 grasses, generating positive responses in the C_4-safranin histochemical test for determination of the photosynthetic pathway in grasses. The distribution of ferulic acid in the bundle sheath of C4 grasses suggests a novel physiological function for this compound in C4 photosynthesis.

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Author Contributions

M.A.M.N. and M.P.G. designed the experiments. M.A.M.N. collected the data. M.A.M.N. analyzed the data. M.A.M.N. wrote the manuscript. M.P.G. provided critical revisions to the manuscript.

References

[1] Neto, M.A.M., Mardegan, S.F. and Barbosa, S.M. Determinação da via fotossintética em gramíneas. In: Filho A P da S S, (ed). Poaceae Barnhart. Marques Editora: Belém, Pará, 103-122, (2017).

[2] Lewis, N.G. and Yamamoto, E. Lignin: ocorrence, biogenesis and biodegradation. Annual Review Plant Physiology.141: 455-496 (1999).

[3] Neto, M.A.M. and Guerra, M.P. A new method for determination of the photosynthetic pathway in grasses. Photosynthesis Research 140: 1-6 (2019). https://doi.org/10.1007/s11120-019-00646-5

[4] Das, A.K. and Singh, V. Antioxidative free and bound phenolic constituents in pericarp, germ and endosperm of Indian dent (Zea mays var. indentara) and flint (Zea mays var. indurate) maize. Journal of Functional Foods. 13: 363-374. 2015.

[5] Das, A.K. and Singh, V. Antioxidative free and bound phenolic constituents in botanical fractions of Indian specialty maiz (Zea mays L.) genotypes. Food Chemistry. 201: 298-306. (2016).

[6] Guo, W. and Beta, T. Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. Food Research International. 51: 518-525. (2013).

[7] Roberts, K. Handbook of plants science. Vol.1. Chichester: JohnWiley & Sons, Ltd. (2007).

[8] Van Hung, P. Phenolic compounds of cereals and their antioxidant capacity. Critical Reviews in Food Science and Nutrition. 56: 25-35. (2016).

[9] Buanafina, M.M. de O, Langdon, T., Hauck, B., et al. Manipulating the phenolic acid content and digestibility of Italian ryegrass (Lolium multiflorum) by vacuolar-targeted expression of a ferulic acid esterase. Applied Biochemistry and Biotechnology 130: 416-426 (2006).

[10] Mathew, S. and Abraham, T.E. ferulic acid: An antioxidant found naturally in plant cell walls and feruloyl esterases involved in its release and their applications. Critical Reviews in Biotechnology. 24: 59-83. (2004).

[11] Santiago, R. and Malvar, R.A. Role of ehydrodiferuletes im maize resistance to pests and diseases. International Journal of Molecular Sciences. 11: 691-703. (2010).

[12] Saulnier, L. Vigouroux, J. and Thilbault, J.F. Isolation and partial characterization of eruloylated oligosaccharides from maize bran. Crbohydrate Research. 272: 241-253. (1995).

[13] Zhao, Z. and Moghadasian, M.H. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. Food Chemistry. 109: 691-702. (2008).

[14] Faulds, C.B. and Williamson G. The role of hydroxycinnamates in the plant cell wall. J. Sci. Food and Agric. 79:393–395 (1999).

[15] Lambert, N., Kroon, P.A., Faulds, C.B., Plumb, G.W., McLauchlan, W.R., et al. Purification of cytosolic beta-glucosidase from pig liver and its reactivity towards flavonoid glycosides. Biochim. Biophys. Acta. 1435: 110–116 (1999).

[16] Harris, P.J. and Hartley, R.D. Detection of bound ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy. Nature. 259: 508–510 (1976).

[17] Carpita, N.C. Structure and biogenesis of the cell walls of grasses. Ann Rev Plant Physiol Plant Mol Biol. 47: 445-476 (1996).

[18] Buanafina, M.M. de O, Langdon, T., Hauck, B, et al. Expression of a fungal ferulic acid esterase increases cell wall digestibility of tall fescue (Festuca arundinaceae). Plant Biotechnology Journal. 6: 264-280 (2008)

[19] Buanafina, M.M. de O Feruloylation in grasses: current and future perspectives. Molecular Plant. 5: 861-872 (2009).

[20] Ibrahim, R.K., and Barron, D. Phenylpropanoids. In: Dey PM, Harbornej B (eds) Methods in Plant biochemistry, Vol 1. Plant phenolics. Academic Press, New York, 197-235 (1989).

[21] Veit, M., Geiger, H., Wray, V., Abou-Mandour, A.A., Rozdzinski, W., Witte, L., Strack, D. and Czygan, F.C. Equisetum pyrone, a styrylpyrone glucoside in gametophytes from Equisetum arvense. Phytochemistry. 32: 1029-1032 (1993).

[22] Gorham, J. The biochemistry of the stilbenoids. Chapman & Hall, London (1995).

[23] Treutter, D. Chemical reaction detection of catechins and proanthocyanins with 4-dimethylamino-cinnamaldehyde. Journal of Chromatography. 467: 185-193 (1989).

[24] Ibrahim, R.K. Immunolocalization of flavonoid conjugates and their enzymes. In: Stafford HA, Ibrahim RK, eds. Phenolic metabolism in plants. Plenum Press, New York, 25-61 (1992).

[25] Grandmaison, J. and Ibrahim, R.K. Evidence for nuclear binding of flavonol sulphate esters in Flaveria chloraefolia. Journal of Plant Physiology. 147: 653-660 (1996).

[26] Vogt, T., Pollak, P., Tarlyn, N. and Taylor, L.P. Pollination or wound-induced kaempferol accumulation in petunia stigmas enhances seed production. Plant cell. 6: 11-23(1994).

[27] Schnitzler, J.P., Junggblut, T.P., Heller, W., Hutzler, P., Heinzmann, U., Schmelzer, E., Ernst, D., Langebartels, C. and Sandermann, H. Tissue localization of UV-B screening pigments and chalcone synthase mRNA in Scots pine (Pinus sylvestris L.) needles. New phythologist. 132: 247-258 (1996).

[28] Reinold, S. and Hahlbrock, K. In situ localization of phenylpropanoid biosynthetic mRNAs and proteins in parsley (Petroselinum crispum). Botanica Acta. 110: 431-443 (1997).

[29] Lichtenthaler, H.K. and Schweiger, J. Cell wall bound ferulic acid, the major substance of the blue-green fluorescence emission of plants. Journal of Plant Physiology. 152: 272-282 (1997).

[30] Harborne, J. Introduction to ecological biochemistry. Academic Press, London (1993).

[31] Hutzler, P., Fischbach, R., Heller, W., Jungblut, T.P., Reuber, S., Schmitz, R., Veit, M., Weissenbock, G. and Schnitzler, J.P. Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. Journal of Experimental Botany. 4: 953–965 (1998).

[32] Saulnier, L. and Thilbault, J.F. Ferulic acid and diferulic acids as components of sugar beet pectins and maize bran heteroxylans. Journal of the Science of Food and Agriculture. 79: 396-402. (1999).

[33] Zhang, Z., Smith, C., & Li, W. Extraction and modification technology of arabinoxylans from cereal by-products: A critical review. Food Research International. 65: 423-436 (2014).

[34] Pedersen, M.B., Bunzel, M., Schafer, J., Knudsen, K.E.B., Sørensen, J.F., Yu, S. & Lærke, H.N. Frulic acid dehydrodimer and dehydrotrimer profiles of distiller's dried grains with solubles from different cereal species. Journal of Agricultural and Food Chemistry. 63: 2006-2012. (2015).

[35] Hartley, R.D. and Ford, C.W. Phenolic constituents of plant-cell walls and wall biodegradability. Acs Symposium Series 399: 137-145 (1989).

[36] Ralph, J. and Helm. R.F. Lignin/hydroxynnamic acid/polysaccharide complexes: synthetic models for region chemical characterization. In Forage Cell Wall: Structure and Digestibility, Jung JHG Buxton DR and Hartley RD, (eds) (Madison: ASA-CSSA-SSSA), pp 201-246 (1993).

[37] Wende, G. and Fry, S.C. 2-O-beta-D-xylopiranosyl-(5-O-feruloyl)-L-arabinose, a widespread component of grass cell walls. Phytochemistry. 44: 1019-1030 (1997).

[38] Ou, S.Y. and Kwok, K.C. Ferulic acid: pharmaceutical functions, preparation and applications in foods. Journal of the Science of Food and Agriculture. 84: 1261-1269 (2004).

[39] Knogge, W. and Weissenböck, G. Tissue-distribution of secondary phenolic biosynthesis in developing leaves of Avena sativa L. Planta 2: 196-205 (1986).

[40] Schmelzer, E., Jahnen, W. and Hahlbrock, K. In situ localization of light-induced chalcone synthase mRNA, chalcone synthase and flavonoid end products in epidermal

cells of parsley leaves. Proceedings of the National Academy of Sciences, USA 85: 2989-2993 (1988).

[41] Haussühl, K., Rohde, W. and Weissenböck, G. Expression of chalcone synthase genes in coleoptiles and primary leaves of Secale cereal L. after induction by UV radiation: evidence for a UV-protective role for the coleoptile. Botanica Acta. 109: 229-238 (1996).

[42] Moskowitz, A.H. and Hradzima, G. Vacuolar contents of fruit sub epidermal cells from Vitis sp. Plant Physiology. 68: 686-692 (1981).

[43] Weissenböck, G., Hedrich, R. and Sachs, H. Secondary phenolic products in isolated guard cell, epidermal cell and mesophyll cell protoplasts- distribution and determination. Protoplasma. 134: 141-148 (1986).

[44] Schnabl, H., Weissenböck, G. and Scharf, H. In vivo micro spectrophotometric characterization of flavonol glycosides in Vicia faba guard and epidermal cells. Journal of Experimental Botany. 37: 61-72 (1986).

[45] Schnabl, H., Weissenböck, G. and Scharf, H. Cellular distribution of UV-absorbing compounds in guard and subsidiary cells of Zea mays L. Journal of Plant Physiology. 135: 249-252 (1989).

[46] Ozimina, I.I. Flavonoids of Spartium junceum. 1. Flavones and flavonoids. Chemistry of Natural Compounds. 16 (3): 763-764 (1979).

[47] Geldner, N. The endodermes. Annual Review Plant Biology. 64: 531-558 (2013).

[48] Nelson, T. and Dengler, N. Leaf vascular pattern formation. The Plant Cell. 9: 1121-1135 (1997).

[49] Slewinski, T.L. Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. Journal of Experimental Botany. 63: 4647-4670 (2012).

[50] Naseer, S., Lee, Y., Lapierre, C, Franke, R., Nawrath, C. and Geldner, N. Casparian strip diffusion barrier in Arabidopsis is made of a lignin polymer without suberin. Proceedings of the National Academy of Sciences of the United States of America. 25:10101-10106 (2012).

[51] Slewinski, T.L. Using evolution as a guide to engineer Kranz-type C4 photosynthesis. Frontiers in Plant Science. 4:1-13 (2013).

[52] Smith, M.M. and Hartley, R.D. Occurrence and nature of ferulic acid substituition of cell-wall polysaccharides in graminaceous plants. Carbohydrate Research. 118: 65-80. (1983).

[53] Sun, R., Sun, X., Wang, S. Zhu, W. Wang, X. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. Industrial Crops and Products. 15: 179-188. (2002).

[54] Levigne, S.V., Ralet, M.C.J., Quéméner, B.C., Pollet, B.N.L., Lapierre, C. and Thibault, J.F.J. Isolation from sugar beet cell walls of arabinan oligosaccharides esterified by two ferulic acid monomers. Plant Physiology. 134: 1173-1180. (2004).

[55] Schatz, P.F., Ralph, J., Lu, F., Guzel, I.A. and Bunzel, M. Synthesis and identification of 2, 5-bis (4-hidroxy-3-methoxyphenyl)-tetrahydrofuran-3, 4-dicarboxylic acid, an unanticipated ferulate 8-8-coupling product acylating cereal plant cell walls. Organic & Biomeolecular Chemistry. 4: 2801-2806. (2006).

[56] Lopez-Martinez, L.X. Oliart-Ros, R.M., Valerio-Alfaro, G. Lee, C.H., Parkin, K.L. and Garcia, H.S. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexicam maize. LWT-Food Science and Technology. 42: 1187-1192. (2009).

[57] Dobberstein, D. and Bunzel, M. Separation and detection of cell wall-bound ferulic acid dehydrodimers and dehydrotrimers in cereals and outher plant materials by reversed phase high-performance liquid chromatography with ultraviolet detection. Journal of Agricultural and Food Chemistry 58: 8927-8935 (2010).

[58] Sun, X.F., Fowler, P., Rajaratam, M. and Zhang, G. Extraction and characterization of hemicelluloses from maize stem. Phytochemical Analysis. 21: 406-415. (2010).

[59] Uddin, M.N. Hanstein, S., Faust, F., Eitenmüller, P.T., Pitann, B. and Schubert, S. Diferulic acids in the cell wall may contribute to the suppression of shoot growth in the first phase of salt stress in maize. Phytochemistry. 102: 126-136. (2014).

[60] Dynkowska, W.M. Cyran, M.R. and Ceglinska, A. Soluble and cell wall-bound phenolic acids and ferulic acid Dehydrodimers in rye flour and five bread model systems: Insight into mechanisms of improved availability. Journal of the Science of Food and Agriculture. 965: 1103-1115. (2015).

[61] Malunga, L.N. and Beta, T. Isolation and identification of feruloylated arabinoxylan mono and oligosaccharides from undigested and digested maize and wheat. Heliyon. E00106. (2016).