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REPORT

Discovery of ferrochelatase 1 in *Nicotiana tabacum* L.: Its role in the response to abiotic stress and plant development

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ABSTRACT

Cuba is threatened by climate change. Some enzymes act as a shield against oxidative stress, always associated with salinity, water deficit, or excess and extreme temperatures; those enzymes have Heme as a cofactor. Heme is a tetrapyrrole, similar to chlorophyll, but with Fe instead of Mg as the central atom. The response associated with Heme was investigated in tobacco plants under saline, water, and temperature stress, together with the model plant *Arabidopsis thaliana*. The presence of the enzyme Ferrochelatase 1 (responsible for chelating iron to tetrapyrrole) was reported in tobacco tissues for the first time for science. Plants have two isoforms of Ferrochelatases (FC1 and FC2). Mutating the model plant for the Ferrochelatase 1 gene was lethal for the embryos. By comparing FC1 and FC2 mRNA levels in wild-type plant embryos, it was demonstrated that FC1 is the predominant isoform during embryo maturation. Therefore, the regulation of FC expression in time and space during embryogenesis is a prerequisite for the proper development of the embryo. It is concluded that the Heme produced by FC1 is essential for embryogenesis in addition to stress responses. Antisense lines for FC1 had an early flowering phenotype on short days; this made it possible for the first time to establish a relationship between the Heme produced by FC1 and the flowering process. Heme produced by the FC1 isoform is important in reproductive metabolism. This knowledge in the hands of breeders could be used to obtain productive cultivars with the capacity to acclimatize to abiotic stresses associated with climate change, which would potentially give us some advantage to mitigate its effects. This work has stimulated scientific exchange with Germany. The results are included as pedagogical techniques for the teaching of modern molecular biology methods, in the high-level training of young Cuban and Latin American scientists at the University of Havana, in the 5 editions of the joint course "Basic methods in plant molecular biology and plant physiology" with the Humboldt University of Berlin. This work received the Annual Award of the Cuban Academy of Sciences for the year 2020.

Keywords: tobacco, abiotic stress, ferrochelatase, embryogenesis, heme

RESUMEN

Descubrimiento de ferroquelatasa 1 en *Nicotiana tabacum* L.: papel en la respuesta al estrés abiótico y el desarrollo de las plantas. Cuba está amenazada por el Cambio Climático. En la fisiología de las plantas participan enzimas que actúan como escudo al estrés oxidativo, siempre asociado a la salinidad, el déficit o exceso hídrico y las temperaturas extremas; esas enzimas tienen Hemo como cofactor. Hemo es un tetrapirrol, semejante a clorofila, pero con Fe en lugar de Mg como átomo central. El objetivo fue investigar la respuesta asociada al hemo en plantas de tabaco bajo estrés salino, hídrico y de temperatura, junto a la planta modelo *Arabidopsis thaliana*. Se reportó, en tejidos de tabaco, por primera vez para la ciencia, la presencia, de la enzima Ferroquelatasa 1 (responsable de quelar el hierro al tetrapirrol). Las plantas tienen dos isoformas de Ferroquelatasas (FC1 y FC2). La mutación para el gen de la Ferroquelatasa 1, en la planta modelo, resultó letal para los embriones. Al comparar los niveles de ARNm de FC1 y FC2 en embriones de plantas tipo salvaje, se demostró que FC1 es la isoforma predominante durante la maduración del embrión. Por lo tanto, la regulación de la expresión de FC en tiempo y espacio durante la embriogénesis, es prerrequisito para el adecuado desarrollo del embrión. Se concluye que el hemo producido por FC1, es esencial para la embriogénesis además de las respuestas al estrés. Líneas antisentido para FC1 tuvieron fenotipo de floración temprana en día corto; esto hizo que por primera vez se estableciera una relación del hemo producido por FC1 con el proceso de la floración, deduciéndose así que el Hemo producido por FC1 es importante en el metabolismo reproductivo. Estos conocimientos en manos de mejoradores, pudieran servir para obtener cultivares productivos con capacidad de aclimatación a los estreses abióticos asociados al cambio climático, lo que potencialmente nos daría alguna ventaja para mitigar sus efectos. El trabajo ha estimulado el intercambio científico con Alemania. Los resultados son incluidos como técnicas pedagógicas para la enseñanza de métodos modernos de biología molecular, en la formación de alto nivel de científicos jóvenes cubanos y latinoamericanos en la UH, en las 5 ediciones del curso conjunto "Basic methods in plant molecular biology and plant physiology" con la Universidad Humboldt de Berlín. Este trabajo mereció el Premio Anual de la Academia de Ciencias de Cuba para el año 2020.

Palabras clave: tabaco, estrés abiótico, ferroquelatasa, embriogénesis, hemo

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*I*ntroduction

In Cuba, 20 % of arable lands are in danger due to salinization. Provinces where tobacco (*Nicotiana tabacum* L.) is cultivated have this problem [1]. The soils of southern plain of Pinar del Río have limiting factors: actual and potential salinity, low capacity for cationic exchange, etc., with soils of light texture in most of the areas [2]. Since the 1950's [3], it is known that in today southern Artemisa and Mayabeque, it started to manifest marine intrusion in arable lands. Along their life cycle, every plant is exposed to biotic and abiotic external stressing factors [4]. Knowing the response of the plants to abiotic stress constitutes a current line of research in the biology of the effects of stress on agriculture. Knowing the basis for tobacco resistance to saline, water and extreme-temperature stress is important in Cuba and a growing concern for agriculture and other countries.

This work was aimed to investigate the responses of tobacco plants (*N. tabacum*) to saline, water and temperature stress, with special attention to tobacco cultivation (including the model plant *Arabidopsis thaliana*), with respect to the influence of heme group on the adaptation to stress conditions. Heme is similar to chlorophyll, but without phytol chain and with Fe instead of Mg at tetrapyrrole center [5].

Heme is one of the biosynthesis products of tetrapyrroles. Ferrochelatase (FC) is the enzyme catalyzing the final reaction of the heme synthesis, by chelating ferrous iron in the ring of protoporhyrin IX (Proto) [6]. There has been described that several species of plants have two isoforms of enzyme FC (FC1 and FC2). Woodson *et al*. [7] suggested that both isoforms synthetize different heme pools. In this way, each heme pool would supply hemoproteins that act in the defensive responses to stress conditions. In *N. tabacum*, FC2 was described by Papenbrock *et al*. [8]; however, FC1 had not been described.

Heme plays a key role in the enzymes of the anti-oxidative metabolism, in the close association of oxidative stress to the physiology of the plants in the presence of abiotic stresses. Also considering tobacco as a relevant crop in the Cuban economy, in this work, we analyzed the characteristics of heme synthesis, its concentration in the tissues, the molecular biology of its metabolism and distribution in different organs, tissues and cellular organelles of tobacco plants, affected or not by three of the main types of stress associated to climate change.

*M*aterials and methods

Tobacco (*N. tabacum*) was the primary plant, and a model plant (*A. thaliana*) was included for elucidating the biological function of enzyme FC1 and its product, heme, under abiotic stress conditions. Some of the tobacco cultivars used were developed in Cuba and are used in the tobacco production and the program of genetic improvement.

The response of *N. tabacum* L. to salinity and extreme-temperature was studied under laboratory conditions, semicontrolled in soil and in hydroponics with Hoagland solution [9]. *A. thaliana* was used in complementary studies in knockout mutants of ferrochelatase 1, for elucidating the function of FC1 during development.

There were applied laboratory techniques such as spectrometry, enzymatic assays [10-12], fluorescence confocal microscopy, chromatography, etc., as well as molecular techniques for DNA [13] and RNA isolation, electrophoresis, cDNA sequencing and RT-PCR [14, 15], generation and complementation of transgenic plants [16-18], evolutionary molecular analysis using the MEGA software, version 5 [19], among others. Developing several of these techniques was possible due to the collaboration with the Humboldt University in Berlin.

*R*esults and discussion

Only one FC isoform has been reported for animals, fungi, bacteria and algae; however, in some species of plants, two FC isoforms (FC1 and FC2) have been described [20-25]. In *N. tabacum*, the gene coding for FC2 was previously described by Papenbrock [8], but not FC1. The cDNA of ferrochelatase 1 was first described in this work, and accepted for publication and international registry at the NCBI database (Accession number JF428143.1) [26]. In our study, there was found that the cDNA of FC1 sequence of *N. tabacum* (*NtFC1*) consisted of 1935 nucleotides, containing the 1464 base pairs of the open reading frame, as well as 168 and 303 nucleotides of the 5′ and 3′ non-coding regions, respectively.

Upon comparing the new sequence reported for NtFC1 protein with sequences obtained in other plants and also available at NCBI, it was demonstrated the homology of NtFC1 with those previous reports. There was built a phylogenetic tree, in which there was deduced the evolutionary relationship of FC1 and FC2 [27]. Both FC isoforms in plants are grouped separately from the FC sequences of other organisms. FC1 is more related to FC2 than with mitochondrial FC in non-photosynthetic organisms, supporting that both plant FC genes derive from a gene duplication process.

Once the sequence of amino acids of ferrochelatase was described, there were determined the expression profile, activity in different tissues of tobacco plant (*N. tabacum*) and heme.

The relative expression of FC1 and FC2 is different in leaves with respect to roots. The levels of FC2 transcripts was 2.5 times higher than FC1 levels in leaves, while in the roots the ratio of FC2 transcripts levels is five times lower than those of FC1 (Figure 1). These results were published in the scientific journal Plant and Cell Physiology [28], and in the PhD thesis of the main author [29].

Mutants of *N. tabacum* with decreased FC1 expression showed no modifications in the phenotype (Figure 2). Therefore, they were subjected to abiotic stress conditions (salinity and low temperature values) to detect possible phenotypic changes and any modification in the metabolism due to the decreased FC1 levels. In this sense, there was demonstrated the contribution of the heme produced by FC1 in the responses to stress. Salinity reduced the shoots length,

1. González Núñez LM, Tóth T, García D. Integrated management for the sustainable use of salt-affected soil in Cuba. Universidad y Ciencia. 2004;20(40):85-102.

2. García M, Díaz AL, Valdés MA. El mejoramiento de los suelos: una experiencia desde la agroecología en la Cooperativa de Producción Agropecuaria "Celso Maragoto Lara". Revista Científica. 2014;16(4):317-28.

3. Ortega F, Obregon A, Hernández A, Borreto M, editors. Los suelos salinos y salinizados de Cuba. Resúmenes de las Actas del Primer Seminario Científico de Pedología para la región de Centroamérica y el Caribe: Suelo y Agua; La Habana; 1985.

4. Roháček K, Soukupová J, Barták M. Chlorophyll fluorescence: A wonderful tool to study plant physiology and plant stress. In: Schoefs B, editor. Plant Cell Compartments. Kerala: Research Signpost; 2008; p. 41-104.

5. Tanaka R, Tanaka A. Tetrapyrrole biosynthesis in higher plants. Annu Rev Plant Biol. 2007;58:321-46.

6. Fan T, Grimm B, Layer G. Porphyrin and heme synthesis. Adv Bot Res. 2019;91: 89-131.

7. Woodson JD, Perez-Ruiz JM, Chory J. Heme synthesis by plastid Ferrochelatase I regulates nuclear gene expression in plants. Curr Biol. 2011;21:897-903.

8. Papenbrock J, Mishra S, Mock H-P, Kruse E, Schmidt EK, Petersmann A, et al. Impaired expression of the plastidic ferrochelatase by antisense RNA synthesis leads to a necrotic phenotype of transformed tobacco plants. Plant J. 2001;28:41-50.

9. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular. 1950;347:1-32.

10. Sharma P, Bhardwaj R, Arora N, Arora HK. Effect of 28-homobrassinolide on growth, Zn metal uptake and antioxidative enzyme activities in Brassica juncea L. seedlings. Braz J Plant Physiol. 2007;19:203-10.

11. Kato M, Shimizu S. Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic- dependent peroxidative degradation. Can J Bot. 1987;65:729-35.

12. Papenbrock J, Mock H-P, Kruse E, Grimm B. Expression studies in tetrapyrrole biosynthesis: inverse maxima of magnesium chelatase and ferrochelatase activity during cyclic photoperiods. Planta. 1999;208:264-73.

13. Edwards K, Johnstone C, Thompson C. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 1991;19(6):1349.

14. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protocols. 2008;3(6):1101-8.

15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402-8.

16. Horsch RB, Fry JE, Hoffman NL, Eichholtz SG, Rogers SG, Fraley RT. A simple and general method for transferring genes into plants. Biol Sci. 1985;221:1229-31.

but the decrease was the same for both wild-type and mutant plants (Line AS37). Some necrotic areas appeared in the leaves of the antisense lines, specially under saline-stress conditions.

The expression of ferrochelatase 1 (FC1) and ferrochelatase 2 (FC2) genes were not affected under saline conditions in any of the lines studied, including antisense lines for FC1. In fact, FC1 and FC2 are differently regulated under low-temperature conditions; the levels of FC1 transcripts increase while FC2 levels decrease (Figure 3). Changes in the content of heme could be explained by a modified expression of FC genes and an altered enzymatic activity. Although the contribution of FC1 in leaves to the heme pool is lower than that of FC2 (Figure 2), a higher expression of FC1 under temperature-stress conditions indicates a higher contribution, with respect to FC2, to the heme pool (Figure 3).

Ferrochelatase is an enzyme encoded in the cell nucleus, which is translocated to subcellular location where its function takes place [30]. Most of the reports indicate that the heme synthesis occurs in plastids. Despite, to date, few publications suggest that the last two steps of the heme synthesis occur, in parallel, in plastids and mitochondria [25, 31-33].

In silico prediction studies of protein NtFC1 suggested a location mainly in chloroplasts; nevertheless, the location in mitochondria is also possible, according to the prediction values of some of the software used. *In silico* studies were complemented with confocal microscopy to detect the protein FC1 fused together with green fluorescent protein (GFP) in plants expressing a transient construct bearing the sequences of the NtFC1 gene and the green fluorescent protein. Transient transformation by gene gun supported the detection of fluorescence in chloroplasts of tobacco leaves and petals. Location in chloroplasts was easily distinguishable due to the red self-fluorescence of the chlorophyll contained into chloroplasts (Figure 4). By this method, no NtFC1 protein was detected in mitochondria, possibly due to the higher expression in chloroplasts. Nevertheless, using mutant plants that overexpress FC1, there could be quantified the FC activity in isolated mitochondria, indicating that this protein is present in mitochondria as well as in chloroplasts [28].

FC2 enzyme is restrained to plastids [8] and FC activity is present in both organelles, even though to a lesser extent in mitochondria. Therefore, it was proposed that FC1 supplies heme for mitochondrial proteins, while the portion located in plastids provides the heme required for plastid and cytosol hemoproteins. Moreover, there was studied the role of the heme produced by FC1 at different phases of plant development. For that purpose, there were used antisense *N. tabacum* plants for FC1 and FC1 knock-out *A. thaliana* plants as model.

Tobacco FC1 antisense lines exhibited an early-flowering phenotype as compared to the wild-type lines. This implicates that the absence in leaves of the heme produced by FC1 triggers the mechanism that causes a (FT) signal to travel to the apical meristem and modifies the expression of genes related to the flowering process (Figure 5), as proven in the PhD thesis related to this work [29].

The *A. thaliana* FC1 knock-out lines not expressing the protein were defective starting on the embryogenesis maturation phase. Embryo development is arrested at the globular stage in the absence of

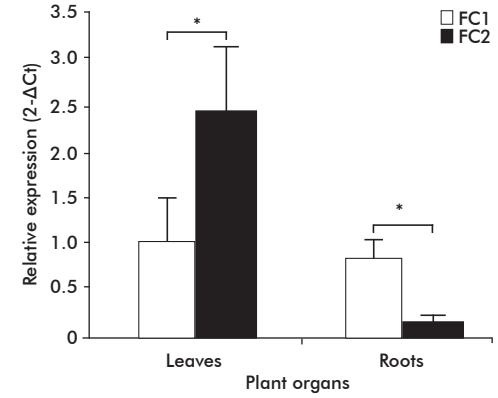

Figure 1. Quantitative analysis of FC1 and FC2 transcripts, in leaf No. 4 and roots. Plants of *N. tabacum* (SNN) were grown in Hoagland solution (half of strength), during ten days. The expression levels were quantified by real time PCR and calculated by means of 2-ΔCt method, using the UBI expression as standard. The expression data was shown as means of six biological replicates ± standard deviation. *: significant differences of paired t test, indicated by square brackets ($p < 0.05$).

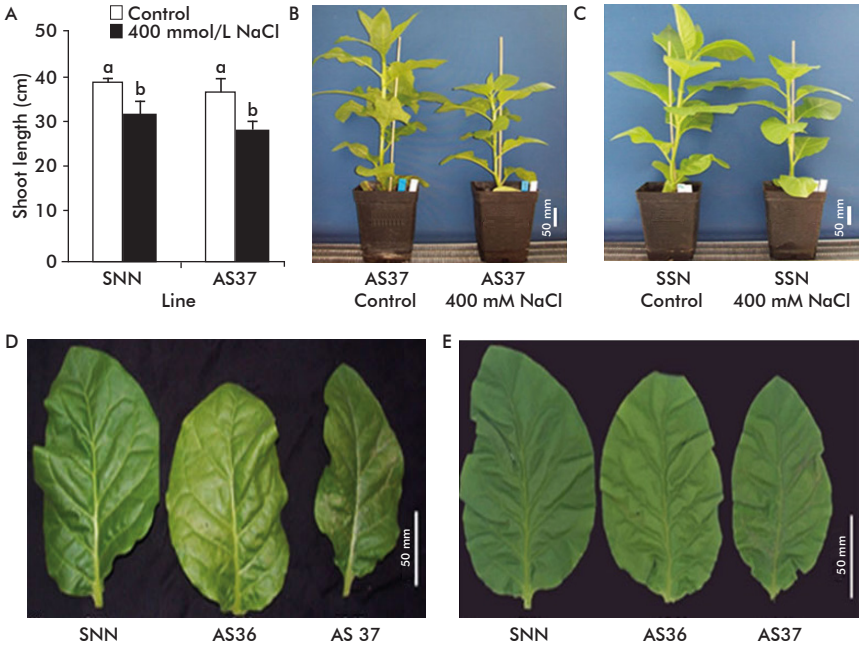

Figure 2. Effect of abiotic stress (salinity and cold) on growth and phenotype of wild-type N. tabacum (SNN) and FC1 antisense lines (AS36 and AS37), during seven days. A) Effect of salinity on the growth of shoot´s length. The bars represent the mean of six biological replicates ± SD. Different letters indicate significant differences among samples by the Tukey's test ($p < 0.05$). B) and C) Leaves of plants belonging to the wild line (SNN) and FC1 antisense line (AS37), respectively, under control (left) and salinity (right; 400mmol/L NaCl). D) and E) Leaves under saline stress conditions (400 mmol/L NaCl) and low-temperature conditions (10 °C), respectively.

17. Sudhir P, Murthy SDS. Effects of salt stress on basic processes of photosynthesis. Photosynthetica. 2004;42(4):481-6.

18. Clough SJ, Bent AF. Floral dip: a simplified method for *Agrobacterium*-mediated transformation in *Arabidopsis thaliana*. Plant J. 1998;16(6):735-43.

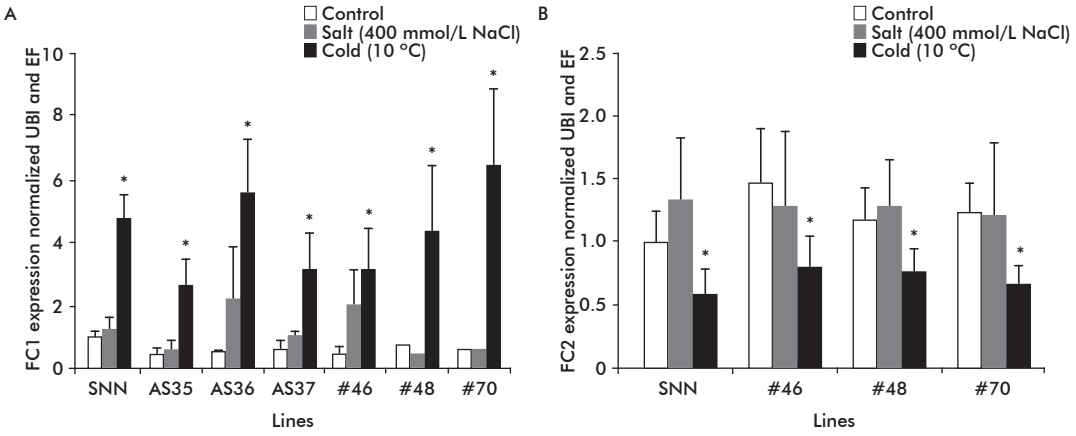

Figure 3. Analysis of gene transcripts (qRT-PCR) coding for enzymes of the tetrapyrrole pathway in leaves of *N. tabacum* (SNN and FC1 lines) 24 hours after stress. A) Ferrochelatase 1 (FC1) using primers qNt FC 1/2 fw and qNt FC 1/2 rev. B) Ferrochelatase 2 (FC2) using primers qNt FC 2/1 fw and qNt FC 2/1 rev. Expression data are shown as the mean of at least three biological replicates ± SD.

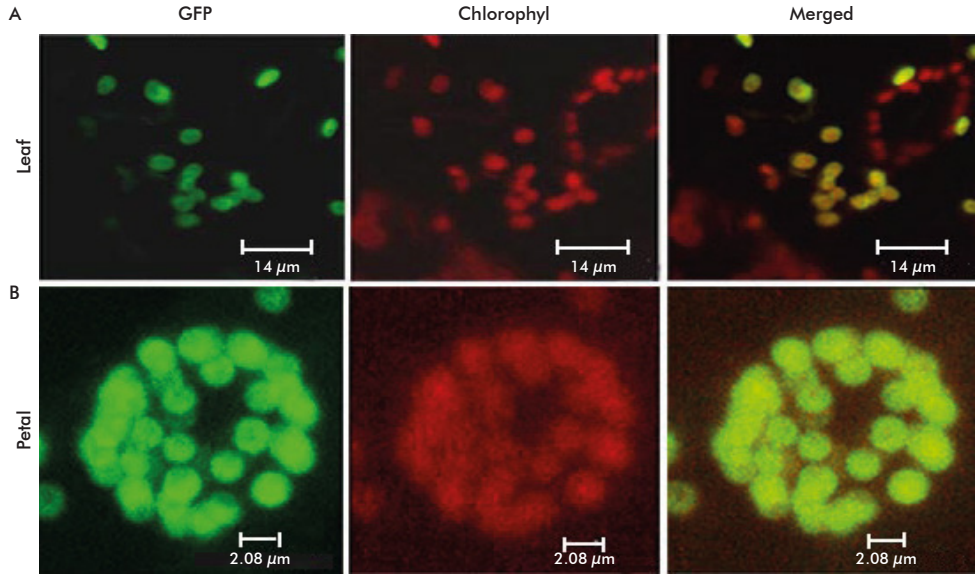

Figure 4. Location of the NtFC1-GFP fusion protein of FC1 to the green fluorescent protein (GFP) in *N. tabacum*. Images were taken using a confocal laser scanning microscope (CLSM). The green fluorescent protein (GFP) is represented in green color (left panel), while the self-fluorescence of the chlorophyll appears in red (central panel). The overlapping of the green and red fluorescence images is shown in the right panel. A) Leaf. B) Petal.

FC1. The heme produced by FC2 at this stage does not supplies the necessity for a proper development, being vital the presence of FC1, as demonstrated in this work [34]. During the embryogenesis maturation phase, the embryo becomes photosynthetically active, producing oxygen for seed respiration [35]. The heme produced by FC1 during embryogenesis can be incorporated to the hemoproteins necessary for oxygen homeostasis, and the respiratory and photosynthetic cytochromes required for the energy transduction.

As proposed by other authors [7] and based on the results obtained in this work, both FC isoforms contribute, in a different manner, to the heme pool in the cell. FC2 produces heme for sites of the photosynthetic machinery in the chloroplast (for instance, cytochromes). On the other hand, FC1 is the enzyme providing heme as cofactor at basal levels for the complete cell and for signaling to the nucleus, under abiotic stress conditions, at development phases, specially flowering, and during the embryogenesis maturation (Figure 5).

In summary, in this work, we have demonstrated the presence of isoform 1 of ferrochelatase in *N. tabacum* (*NtFC1*), detecting the transcript in leaves, roots and flowers. The heme produced by FC1 plays an important role in the flowering process of plants, in the response to the abiotic stress and in the development of plant embryos. Otherwise, its absence causes the lethality of the embryo. The heme is produced by FC1 both in mitochondria and plastids for proteins containing heme as cofactor, which are essential for maintaining cell

19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-9.

20. Banks JA, Nishiyama T, Hasebe M, Bowman JL, Gribskov M, dePamphilis C, *et al*. The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. Science. 2011;332(6032):960-3.

21. Soderlund C, Descour A, Kudrna D, Bomhoff M, Boyd L, Currie J, *et al*. Sequencing, mapping, and analysis of 27,455 maizes full-length cDNAs. PLOS Genetics. 2009;5(11):e1000740.

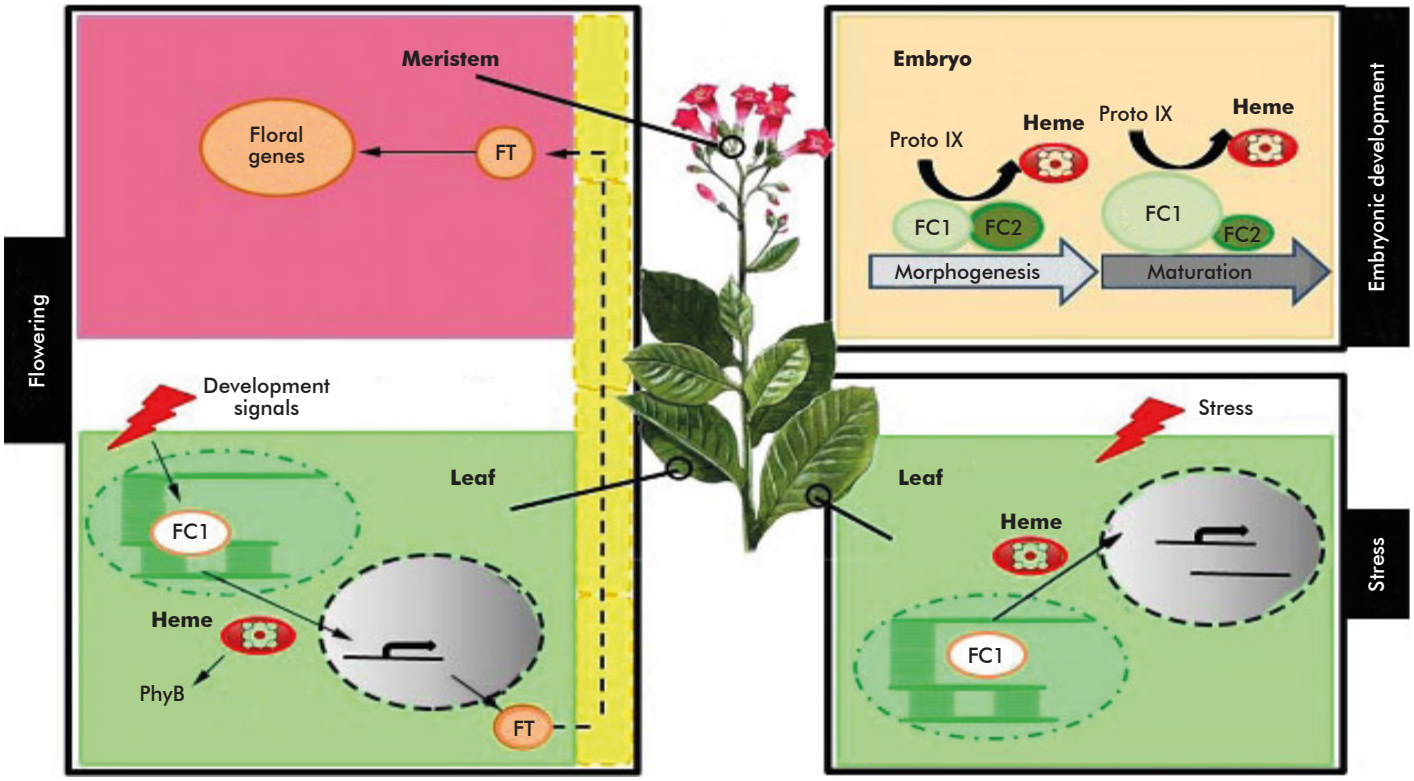

Figure 5. Model of functional distribution of Ferrochelatase 1 and 2 (FC1 and FC2, respectively) in plants. Both ferrochelatases are present in embryos and leaves. The left panel represents the changes during flowering. The signs are perceived in the leaves where heme is synthetized and the same modifies the expression of a movable element (FT) which travels from the phloem to the apical meristem changing there the gene expression of proteins responsible for flowering. The upper right panel represents what occurs during embryonic development in seeds. During the maturation phase, FC1 is the predominant ferrochelatase isoform that replaces heme for completing the embryo maturation. FC2 produces heme, mainly, in photosynthetically active leaves. Under stress conditions (lower right panel), FC1 synthetizes heme in plastids, which generates a feedback signaling that modulates the expression of genes coding for enzymes that modify plant metabolism, thereby contributing to their defense.

homeostasis. Likewise, it triggers the nucleus-signaling pathway, which modifies the expression of the different cell proteins. As a result of high social impact related from this work, five editions of the course "Basic methods in plant molecular biology and plant physiology" has been organized and delivered. These courses, taught as a partnership between the Plant Physiology Laboratory of the University of Havana and the Humboldt University of Berlin, have taken advantage of the theoretical and practical knowledge and the plant material derived from this research. Fifty six students from 15 Cuban institutions and two from Brazilian Universities (Federal University of Rio Grande do Norte and the Federal Rural University of the Semi-arid Region).

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

22. Schnable PS, Ware D, Furton RS, Stein JC, Wei F, Pasternak S, *et al*. The B73 maize genome: complexity, diversity, and dynamics. Science. 2009;326(5956):1112-5.

23. Kang K, Lee K, Park S, Lee S, Kim YS, Back K. Overexpression of rice ferrochelatase I and II leads to increased susceptibility to oxyfluorfen herbicide in transgenic rice. J Plant Biol. 2010;53:291-6.

24. Suzuki T, Masuda T, Singh DP, Tan F-C, Tsuchiya T, Shimada H, *et al*. Two types of ferrochelatase in photosynthetic and non-photosynthetic tissues of cucumber. Their difference in phylogeny, gene expression, and localization. J Biol Chem. 2002;227: 4731-7.

25. Chow KS, Singh DP, Walker AR, Smith AG. Two different genes encode ferrochelatase in *Arabidopsis*: mapping, expression and subcellular targeting of the precursor protein. Plant J. 1998;15:531-41.

26. Alawady A, Alawady A, Ortega P, Grimm B. Nicotiana tabacum ferrochelatase isoform I mRNA, complete cds; plastid [GenBank Accession Number JF428143.1]. 2016.

27. Ortega-Rodes P, Grimm B, Ortega E. Evolutionary, physiological and biotechnological aspects of ferrochelatase and heme in higher plants. Biotecnol Apl. 2014;31(3):176-186.

28. Hey D, Ortega-Rodés P, Fan T, Schnurrer F, Brings L, Hedtke B, et al. Transgenic tobacco lines expressing sense or antisense FERROCHELATASE 1 RNA show modified Ferrochelatase activity in roots and provide experimental evidence for dual localization of Ferrochelatase 1. Plant Cell Physiol. 2016;57(12): 2576-85.

29. Ortega-Rodés P. Ferrochelatase 1 and heme in *Nicotiana tabacum*: their relationship with stress physiology [PhD thesis]. La Habana: Universidad de la Habana; 2014.

30. Papenbrock J, Grimm B. Regulatory network of tetrapyrrole biosynthesis of intracellular signaling involved in metabolic and developmental control of plastids. Planta. 2001; 213:667-81.

31. Porra RJ, Lascelles J. Studies of ferrochelatase. The enzymatic formation of haem in proplastids, chloroplasts and plant mitochondria. Biochem J. 1968;108:343.

32. Smith AG, Santana MA, Wallace-Cook ADM, Roper JM, Labbe-Bois R. Isolation of a cDNA encoding chloroplast ferrochelatase from *Arabidopsis thaliana* by functional complementation of a yeast mutant. J Biol Chem. 1994;269:13405-13.

33. Chow K, Singh DP, Roper JM, Smith AG. A single precursor protein for Ferrochelatase-I from *Arabidopsis* is imported *in vitro* into both chloroplasts and mitochondria. J Biol Chem. 1997;272(44): 27565-71.

34. Fan T, Roling L, Meiers A, Brings L, Ortega-Rodés P, Hedtke B, *et al*. Complementation studies of the *Arabidopsis* fc1 mutant substantiate essential functions of ferrochelatase 1 during embryogenesis and salt stress. Plant Cell Environ. 2019;42(2):618-632.

35. Borisjuk L, Rolletschek H. The oxygen status of the developing seed. New Phytol. 2009;182:17-30.

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