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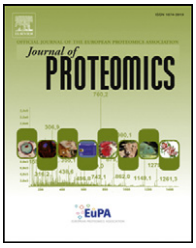

Effect of greenhouse conditions on the leaf apoplastic proteome of *Coffea arabica* plants☆

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ABSTRACT

This work describes the coffee leaf apoplastic proteome and its modulation by the greenhouse conditions. The apoplastic fluid (APF) was obtained by leaf vacuum infiltration, and the recovered proteins were separated by 2-DE and subsequently identified by matrix assisted laser desorption/ionization time of flight-mass spectrometry, followed by homology search in EST coffee databases. Prediction tools revealed that the majority of the 195 identified proteins are involved in cell wall metabolism and in stress/defense responses. Although most of the proteins follow the classical secretory mechanism, a low percentage of them seem to result from unconventional secretion (leaderless secreted proteins).

Principal components analysis revealed that the APF samples formed two distinct groups, with the temperature amplitude mostly contributing for this separation (higher or lower than 10 °C, respectively). Sixty one polypeptide spots allowed defining these two groups and 28 proteins were identified, belonging to carbohydrate metabolism, cell wall modification and proteolysis. Interestingly stress/defense proteins appeared as more abundant in Group I which is associated with a higher temperature amplitude. It seems that the proteins in the coffee leaf APF might be implicated in structural modifications in the extracellular space that are crucial for plant development/adaptation to the conditions of the prevailing environment.

Biological significance

This is the first detailed proteomic study of the coffee leaf apoplastic fluid (APF) and of its modulation by the greenhouse conditions. The comprehensive overview of the most abundant proteins present in the extra-cellular compartment is particularly important for the understanding of coffee responses to abiotic/biotic stress.

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1. Introduction

Coffee is the most important commodity in the international agricultural trade, representing a significant source of income to several Latin American, African and Asian countries [1]. Many of these countries are heavily dependent on coffee, which can account for over 75% of their total export earnings. *Coffea arabica* represents 61% of the world coffee production, which is greatly affected by coffee-leaf-rust (CLR), a disease of worldwide distribution and important economic negative impact [2]. Breeding for resistance is environmentally and economically the most appropriate and sustainable strategy used against CLR. Since 1955, "Centro de Investigação das Ferrugens do Cafeeiro" (CIFC/IICT, Portugal) has been cooperating with coffee growing countries to solve this major crop disease at an international level [3–6]. Resistance against CLR leads to restricted fungal growth in early stages of the infection process, involving important cell surface interactions [5,7]. This fact has prompted us to study the proteome of the coffee leaf extracellular space (ECS).

The ECS (or apoplast), as the plant cell compartment external to the plasma membrane, includes the cell walls, the intercellular spaces and the apoplastic fluid (APF). It is a dynamic compartment, important in the regulation of the structure of the cell, the responses to biotic and abiotic stress and the processes of recognition and signaling [8]. APF is composed of secreted proteins, enzymes, metabolites and ions that have a fundamental importance in the intra/inter-cellular communications, providing a means of delivering materials to the cells, and playing a critical role in the plant interactions with the environment [9]. It has been shown that while some of the secreted proteins are APF constitutive, others are induced by signals of either biotic or abiotic nature [8].

We have been studying the involvement of proteins in the coffee–CLR interactions and noticed that greenhouse grown plants were somehow subjected to environmental influence that affects leaf APF characteristics [10,11]. Our goal was then, to establish the annotated *C. arabica* leaf apoplastic proteome and evaluate to which extent greenhouse conditions exert any influence.

We stress the fact that, so far only a low number of woody species have been investigated at the proteomic level, particularly those without a sequenced genome, like coffee [12,13]. Here we describe for the first time the *C. arabica* leaf apoplastic proteome, and analyze its alterations along the year.

2. Material and methods

2.1. Plant material

Five year old *C. arabica* S4 Agaro, genotype S_H4S_H5, that resulted from clonally propagated stem cuttings, were grown in 50 L pots in a mixture of soil:peat:sand (1:1:1) under greenhouse conditions (90% air humidity; temperature as recorded in Table 1). Nine distinct samples of young (10 to 12 days-old) fully expanded leaves were collected along the year, from May to December (Table 1), each sample represented a pool of six to eight pairs of leaves (12 ± 2.5 g fresh weight) from three to four different plants.

2.2. Plant protein extraction

Apoplastic fluid (APF) of the leaves was obtained by vacuum infiltration as previously described [11]. Briefly, square sections of about two cm^2 of leaves were vacuum infiltrated, for five periods of 30 s, in 100 mM Tris–HCl buffer (pH 7.6) solution, containing 50 mM L-ascorbic acid, 500 mM KCl and 25 mM 2-mercaptoethanol (at 4 °C). The blotted sections were centrifuged at 1000 *g*, during 15 min at 4 °C, and the collected APF frozen. This fraction was subsequently desalted and concentrated on centrifugal filter Vivaspin2 (Sartorius), followed by purification and buffer changing [IEF buffer: 8 M urea, 4% CHAPS (w/v), 65 mM DTT] in PD SpinTrap G-25 columns (GE Healthcare). Using a modified Bradford assay [14] the protein was quantified, yielding on average 78 ± 35 μg protein per gram of leaf fresh weight. Protein was stored at −80 °C prior to electrophoresis.

2.3. 2D electrophoresis

As previously described [15,16] IEF was performed in IPG strips with slight alterations. One hundred micrograms of protein was loaded to 13 cm IPG strips (linear pH gradient of 4–7; GE Healthcare). The Ettan IPGphor (GE Healthcare) was used

Table 1 – Coffee leaf samples collected for the experiments and greenhouse conditions (day length and temperature).

Leaf [a] APF samples	Collection date [a] (2011)	Day length [b]			Temperature (°C)			
		Sunrise	Sunset	Total duration (h)	Minimum	Maximum	Average	Amplitude
APF1	10 May	06:28	20:37	14:08	15.5	29.5	22.5	14.0
APF2	1 June	06:13	20:55	14:42	18.5	29.0	23.8	10.5
APF3	14 June	06:11	21:02	14:51	16.5	27.0	21.8	10.5
APF4	14 July	06:23	21:01	14:37	17.0	26.7	21.9	9.7
APF5	5 August	06:41	20:43	14:01	19.0	26.5	22.8	7.5
APF6	22 September	07:24	19:33	12:09	16.5	30.0	23.0	13.5
APF7	12 October	07:43	19:02	11:19	14.0	28.0	21.0	14.0
APF8	11 November	07:14	17:26	10:12	17.0	24.0	20.5	7.0
APF9	6 December	07:40	17:14	09:34	9.3	16.6	13.0	7.3

[a] Leaves (young fully expanded, 10–12 days old) were collected from 5 year old clonally propagated coffee plants. Leaf collection was performed monthly (twice in June, the very active growth period).
[b] http://www.sunrise-and-sunset.com/en/portugal/lisbon.