Journal of Plant Physiology 212 (2017) 1–12

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Daylily protein constituents of the pollen and stigma a proteomics approach☆

Roland R. Tremblay[a,*], Sylvie Bourassa[b], Benjamin Nehmé[b], Ezequiel L. Calvo[c]

[a] CHUL Research Center in Reproduction, Centre de Recherche du CHU de Québec,2705 Boulevard Laurier, Suite T3-67, Quebec City, QC, G1 V 4G2, Canada
[b] Proteomics Platform Quebec Genomics Center, CRCHUL, Centre de Recherche du CHU de Quebec, Canada
[c] Scientific Consultant in Genomics, 701 Leonard, Quebec City, QC, G1X 4C9, Canada

ARTICLE INFO

Article history:
Received 17 September 2016
Received in revised form 18 January 2017
Accepted 18 January 2017
Available online 20 January 2017

Keywords:
Daylily
Hemerocallis
Pollen
Stigma
Proteome

ABSTRACT

This study was aimed at the identification and quantification of the protein components of the pollen grains in parallel with the distal stigmatic tissue of tetraploid cultivars. Proteomes were analyzed using iTRAQ 4plex labeling, peptides separation by online RP-nano-LC and analysis by ESI–MS/MS. Protein identification and quantification were made using the Asparagales database as a reference. A total of 524,037 MS/MS spectra were produced from pollen and stigma samples. From these, a total of 8368 peptides wereidentified corresponding to 994 unique peptides and 432 protein groups. Among them, 128 differentially expressed proteins were retained for further analysis. In absence of the daylily genome availability, we exploited numerous databases and bioinformatics resources to exploring the putative biological functions of these proteins. The profile of differentially expressed proteins suggests an important representation of functions associated to the signalling and response against endogenous and environmental stresses, including several enzymes implicated in the biosynthesis of antibiotics. The abundance in stigma of several structural proteins of the ribosomal sub-units as well as of the core histones suggest that the translation processes and the regulation of gene expression in stigma is a more active mechanism than in pollen. In addition, pollen prioritizes the synthesis of fructose and glucose as opposed to sucrose in stigma as a source of energy. Finally, the modulated proteins in *Hemerocallis* point to several pathways that give potential clues concerning the molecular mechanisms underlying the functions of the pollen and the stigmatic fluid in daylily reproduction.

1. Introduction

Hemerocallis is a member of the vast clade of monocots and belongs to the Xanthorrhoeaceae family of the order of Asparagales. *Hemerocallis* is an economically interesting plant for which limited genomics and proteomics data are available. It has been suggested, however, by Rodriguez-Enriquez and Grant-Downton (2013) Tremblay (2014,2016) that *Hemerocallis* should be considered as a plant model to a similar extent as *Arabidopsis thaliana* (AT).

The Floral Genome Project (http://fgp.bio.psu.edu/. September 12, 2016) is on its way because of a large Consortium between several states; it is hosted at Penn State University. This project refers mainly to angiosperms where most flower diversity is observed. Hundreds of genes per species will eventually be available for researchers in order to complement the impressive genomic data gained with AT. In addition, the Tomato Genome Consortium (TGC) will provide new insights into fleshy fruits (Tomato Genome Consortium, 2012).

In 2015, a study funded in part by The American *Hemerocallis* Society (AHS) was published by Nageswara-Rao et al. (2014) and the goal of the study was « to gain information about which genes are important to make various tissues of a daylily ». A tulip DNA base was used as a comparator to their daylily transcripts. The use of proteomics has also led to a highly significant contribution to the topic of pollen and stigma interactions with emphasis on the plant ovule secretome (Yang et al., 2015). The omics technology has thus allowed valuable advances for these interactions in Liliopsida and Asparagales plants (Zhang et al., 2014; Rejón et al., 2013).

☆ This research was supported by the not-for-profit grant (CRYOP (L.R.Q, C-38, 1995) to Roland R. Tremblay, PhD.

* Corresponding author at: Centre de Recherche du CHU de Québec, 2705 Boulevard Laurier, suite T3–67, Quebec City, QC, G1V 4G2, Canada.
E-mail addresses: roland.r.tremblay@crchudequebec.ulaval.ca (R.R. Tremblay), bourassa@crchudequebec.ulaval.ca (S. Bourassa), Nehme@crchudequebec.ulaval.ca (B. Nehmé), ecalvo.omics@gmail.com (E.L. Calvo).

http://dx.doi.org/10.1016/j.jplph.2017.01.006
0176-1617/

At the origin of the daylily pollen tube growth, a molecular alliance must occur between two specialized tissues, the pollen and the stigma that produces a rather complex fluid. This conjunction is associated with defined functions and realized as a time clock. Indeed, mature pollen grains leaves their quiescent state to the contact of the stigmatic milieu to become extremely active to accomplish daylily fertilization.

Several studies were conducted to identify pollen proteomes in different species (for a review see Li et al., 2016a). Conversely, few studies about stigma proteome are available, in species like *Nicotiana tabacum* and *Petunia* (Verhoeven et al., 2005), *Brassica napus* (Sheoran et al., 2009; Samuel et al., 2011), tobacco and maize (Sang et al., 2012), *Glycine max* (Li et al., 2012), *Lilium longiflorum* and *Olea europaea* (Rejón et al., 2013), *Solanum pennellii* (Chalivendra et al., 2013), as well as in *Triticeae* (Nazemof et al., 2014). Almost all above-mentioned studies were made using semi-quantitative techniques. Quantitative proteome analysis by iTRAQ is actually one of the most sensitive and reasonably accurate methods for differential detection between samples. To date, few studies about pollen or stigma proteome have been performed by iTRAQ method including studies of pollen grains and pollen tubes from *Lilium davidii* (Han et al., 2010), pollen from domesticated tomato versus two wild relatives (Lopez-Casado et al., 2012), two different cell lines of rice pollen (Yan et al., 2014), sterile versus wild-type cotton anthers (Liu et al., 2015a,b), rice pistil responses during early post-pollination (Li et al., 2016b), and sterile vs fertile cybrid pummelo anthers (Zheng et al., 2014).

Knowing that a proteomics approach had been successfully applied to daylily leaves, petals, sepals and roots (Madhugiri et al., 2015), our objective was to identify and quantify the differential protein profiles between pollen grains and distal stigmatic tissue in tetraploid cultivars. Our aim was to explore the putative biological functions and pathways of those structures involved in sexual reproduction. Our goal was achieved by using iTRAQ and new «omics» technologies to identify/quantify constitutive and secreted proteins. Here, we describe for the first time an iTRAQ-based quantitative proteome analysis of pollen grains and stigma of *Hemerocallis*. These data will help us gain insight into the mechanisms associated with daylily pollen-pistil interactions and will be applied to future studies concerning the differences between cultivars with high and low fertility.

2. Materials and methods

2.1. Plant cultivation

The sampling of the materials under study was performed in July 2015 from two cultivars, *H.* Princesse Juliana (Turcotte, 2007) and *H.* Felicidad (Turcotte, 2007) that were developed in our garden (Les Jardins Merlebleu, Quebec) because of our hybridization program. These two registered cultivars in N.Y. were deemed to be excellent pollen and pod parents. Each cultivar was divided in 8–10 double fans during year 2010.

2.2. Developmental stage of stigma and pollen

The stigmas and pollen were collected between 10h00 and 11h00 at anthesis. A few stigmas were used for hybrydization by Turcotte with *H.* Viva Fiona (Turcotte, 2011) to guarantee the pollen receptivity. Similarly, the pollen grains of both cultivars under study were tested for their maturity on *H.* Maélie (Turcotte, 2008). Pod developments were as usual either with *H.* Viva Fiona or *H.* Maélie six weeks later.

2.3. Material used for extracts

Pollen grains and distal stigmas were collected in one ml Nalgene™ centrifugation tubes and alcohol treated scissors. The 12–15 stigmas samples corresponding to 48 mg and the 20 anthers corresponding to 60 mg of tissue were collected on the individual plants aged three years in 2015 and arising from the original clumps of *H.* Princesse Juliana (Turcotte, 2007) and *H.* Felicidad (Turcotte, 2007). So, multiple flowers but strict genetic homogeneity.

The collected material was kept on ice for 15 min at time of collection, then kept refrigerated at −20 °C and delivered to the laboratory on ice cubes for further exposure to −80 °C until processing.

2.4. Sample preparation

Frozen stigma and pollen grains were disrupted using a mortar and pestle and grounded to a fine powder. (Fig. S1). Then lysis buffer (50 mM ammonium bicarbonate, 50 mM DTT, 0.5% sodium deoxycholate) containing protease inhibitors cocktail (Roche) was added, and the sample preparation was homogenized on ice by sonication with a Sonic Dismembrator (Fisher) with 1 s. pulse (20 times). Samples were centrifuged 10 min at 16,000*g*. Supernatants were mixed with 5 vols of acetone (stored at −20 °C) and incubated overnight at −20 °C. Precipitated proteins were centrifuged 15 min at 16,000*g*. Protein pellets were air dried, and then resuspended in 0.5 M triethylammonium bicarbonate (TEAB) and 0.5% sodium deoxycholate (DOC). Finally, protein concentrations were determined by the colorimetric Bradford assay.

2.5. iTRAQ sample labeling

Protein extracts (25 μg each) from 2 samples of stigma and 2 samples of pollen were digested with trypsin and labelled with iTRAQ according to the iTRAQ kit manufacturer's instruction (Sciex). The labelled peptide extracts from the 4 samples were pooled and fractionated into 14 fractions by high pH (pH 10) reversed phase chromatography.

2.6. Mass spectrometry

Approximately 900 ng of peptide samples from each of the 14 high-pH reversed phase fractions were injected and separated by online reversed-phase (RP) nanoscale capillary liquid chromatography (nanoLC) and analyzed by electrospray mass spectrometry (ESI MS/MS) as described in Sheta et al. (2016).

2.7. Database searching

Spectra were searched against an *Asparagale* (tax id 73496) UniProt protein database (42,187 proteins) using Proteome discover (version 2.1 Thermo Scientific, San Jose, CA). The Asparagales database contains more than 1.5 million of nucleotidic sequences and 63,148 protein sequences. Trypsin enzyme parameter was selected with two possible missed cleavages. MMTS alkylation of cysteins was set as static modification as well as iTRAQ modification at the N-terminus of the peptide and on lysine or tyrosine but methionine oxidation and deamidation of Asn and Gln were set as variable modifications Mass search tolerance were 10 ppm and 25 mDa for MS and MS/MS respectively. For protein validation, a maximum False Discovery Rate of 1% at peptide and protein level was used based on a target/decoy search. All observations associated with the known contaminants like trypsin, BSA and keratin were manually removed before the analysis. Only those proteins with variability between replicates lower than 20% were conserved.