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Proteomic analysis of the mature Brassica stigma reveals proteins with diverse roles in vegetative and reproductive development

Nazila Nazemof[a,b], Philippe Couroux[a], Tim Xing[b], Laurian S. Robert[a,*]

[a] Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada
[b] Carleton University, Department of Biology, 1125 Colonel By Drive, Ottawa, ON, Canada

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ABSTRACT

The stigma, the specialized apex of the Brassicaceae gynoecium, plays a role in pollen capture, discrimination, hydration, germination, and guidance. Despite this crucial role in reproduction, the global proteome underlying Brassicaceae stigma development and function remains largely unknown. As a contribution towards the characterization of the Brassicaceae dry stigma global proteome, more than 2500 *Brassica napus* mature stigma proteins were identified using three different gel-based proteomics approaches. Most stigma proteins participated in Metabolic Processes, Responses to Stimulus or Stress, Cellular or Developmental Processes, and Transport. The stigma was found to express a wide variety of proteins with demonstrated roles in cellular and organ development including proteins known to be involved in cellular expansion and morphogenesis, embryo development, as well as gynoecium and stigma development. Comparisons to a corresponding proteome from a very morphologically different Poaceae dry stigma showed a very similar distribution of proteins among different functional categories, but also revealed evident distinctions in protein composition especially in glucosinolate and carotenoid metabolism, photosynthesis, and self-incompatibility. To our knowledge, this study reports the largest Brassicaceae stigma protein dataset described to date.

1. Introduction

In angiosperms, reproductive development relies on successful pollen-stigma interactions. The stigma, the specialized receptive portion of the female organ, plays key roles in pollen capture, discrimination, hydration, germination, and guidance [1]. It can vary both structurally and functionally among species and has been broadly classified into two main types: wet and dry stigmas [2]. In species with wet stigmas, such as in the Solanaceae and Liliaceae, a liquid exudate containing mainly lipids and/or carbohydrates and proteins is secreted onto the epidermal surface. In contrast, in species with dry stigmas, such as in the Brassicaceae and Poaceae, their epidermal cells or papillae are covered by a waxy cuticle coated by a proteinaceous pellicle and pollen-stigma interactions are more actively regulated. Members of these two families illustrate the extent of morphological variations observed among dry stigmas when comparing the plumose form found within the Poaceae which is adapted to capture wind dispersed pollen and the unicellular papillate dome-shaped form of the Brassicaceae where the heavier pollen grain can be carried by insects. Furthermore, members of the Poaceae species can exhibit gametophytic self-incompatibility (SI), whereas Brassicaceae SI is sporophytic [2].

The detailed molecular processes underlying the development and function of the dry stigma are still being elucidated with the study of SI in the Papaveraceae and Brassicaceae having made considerable progress [3]. Few transcriptional profiling studies specific to the Brassica stigma have been reported. Recently, a combination of laser microdissection and RNA-seq of *Brassica rapa* stigma papillar cells revealed that a large proportion of genes were involved in metabolic processes, transcription regulation, oxidation-reduction processes, translation, phosphorylation, and transport [4]. In addition, a recent Brassica microarray analysis identified 287 differentially expressed stigma genes associated with early responses following compatible or self-incompatible pollinations [5].

Abbreviations: SI, self-incompatibility; 2D PAGE LC–MS/MS, two dimensional polyacrylamide gel electrophoresis liquid chromatography mass spectrometry/mass spectrometry; IEF, isoelectric focusing; RP HPLC, reversed-phase high pressure liquid chromatography; OGE, off-gel electrophoresis; DIGE, differential in gel electrophoresis.

* Corresponding author.
E-mail addresses: NazilaNazemof@cmail.carleton.ca (N. Nazemof), Philippe.Couroux@agr.gc.ca (P. Couroux), Tim.Xing@carleton.ca (T. Xing), Laurian.Robert@agr.gc.ca (L.S. Robert).

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Although advances have been made in the characterization of the Brassicaceae stigma transcriptome, there remains a large gap in our knowledge of its proteome. Given the poor correlation demonstrated between floral mRNA and protein abundance [6,7], and the fact that transcriptomic studies provide limited information regarding post-translational modifications, protein localization and protein–protein interactions, the study of the proteome can reveal insights that cannot be predicted from knowledge of the transcriptome alone. As effectors of development and function, proteins thus provide an essential representation of cellular state. A number of studies have focused on the proteins involved in Brassicaceae SI [8], but there are no reports attempting to describe the global proteome of the Brassicaceae stigma.

In this study, *Brassica napus* serves as a representative of the Brassicaceae and the proteome of its dry mature stigma was analyzed using gel-based proteomics approaches. The Brassica mature stigma was found to express a broad range of proteins involved in various aspects of plant development. The only other global stigma proteome available resulted from a very similar analysis of the triticale (x *Triticosecale* Wittmack) mature stigma [7] which permitted a direct comparison between the Poaceae and Brassicaceae dry stigma proteomes. This comparison revealed that the Poaceae and Brassicaceae stigmas display similar trends in the distribution of their proteins within different functional categories despite being very dissimilar morphologically, but also demonstrated clear distinctions in protein composition.

2. Material and methods

2.1. Plant material

The doubled haploid *B. napus* line, DH12075 (Rakow, G. and Séguin-Swartz, G., Agriculture and AgriFood Canada) was grown in growth cabinets under a 16 h/8 h day-night cycle at 22 °C. Mature stigmas were manually dissected with a scalpel just before anthesis and individually inspected using a Leica ZOOM 2000 dissecting microscope (Leica Microsystems Inc., Concord, Canada) for the presence of contaminating style tissue or pollen before being flash frozen in liquid nitrogen.

2.2. Protein analysis

Additional details regarding these experimental procedures and analyses have previously been published in an equivalent analysis of the triticale mature stigma proteome by Nazemof et al. [7]. Briefly, for 2D PAGE LC–MS/MS, frozen *B. napus* stigmas were ground to a fine powder on dry ice and the frozen powder was immediately suspended in IEF loading buffer. Approximately 100 μg of stigma proteins were separated on an IEF pH3-10 gradient followed by SDS PAGE on a 12% acrylamide gel. A representative 2D gel is shown in Fig. 1. 190 individual spots were manually excised (total of 150 samples) and sent to the Québec Genomic Centre (http://proteomique.crchul.ulaval.ca/en/index.html) for LC–MS/MS analysis. Peptides were separated by online RP HPLC using a Jupiter 300 C18 column (Phenomenex, Torrance, USA). Analyses were performed with a LTQ Linear Ion Trap Mass Spectrometer equipped with a nanoelectrospray ion source (ThermoFisher Scientific, San Jose, USA) and mass spectra were acquired using a data dependent acquisition mode using the Xcalibur software version 2.0. Each full scan mass spectrum (440–2000 m/z) was followed by collision-induced dissociation of the seven most intense ions. Dynamic exclusion was set at 30 s and the relative collisional fragmentation energy at 35%. Since many spots contained more than one protein, no quantitative analysis of relative spot intensity was performed.

For the shotgun 1D SDS PAGE LC–MS/MS analysis, frozen *B. napus* stigma were ground as described above and immediately suspended in 1X SDS PAGE loading buffer proteins. Approximately (45 μg) were separated on a 7–15% acrylamide gradient gel, and the entire lane was excised into 15 bands and analyzed at the McGill University Genome Québec Innovation Centre (http://gqinnovationcenter.com/index.aspx). Peptides were separated on a Zorbax 300SB-C18 column (Agilent Technologies Inc., Mississauga, Canada) and analyzed with a Q-Tof micro™ mass spectrometer (Waters Corporation, Milford, USA). Doubly and triply charged ions from MS scans (350–1600 m/z) with an intensity exceeding 25 counts underwent MS/MS fractionation.

The OGE LC–MS/MS analysis was performed by Bioproximity Proteomics Services (http://www.bioproximity.com) on 50 μg of stigma proteins extracted with IEF loading buffer as described above. Briefly, peptides were separated into 24 fractions using an Agilent OFFGEL 24 cm fractionator (Agilent Technologies Inc., Mississauga, Canada) and a pH 3–10 gradient. Each peptide fraction was desalted, separated using a Zorbax 300SB-C18 column (Agilent Technologies Inc.) and analyzed with a LTQ Velos Dual-Pressure Linear Ion Trap mass spectrometer (ThermoFisher Scientific). Tandem mass spectra were acquired from the top 15 ions in the full scan (400–1400 m/z) with dynamic exclusion set at 30 s.

2.3. Data analysis

Peptide mass spectra data from the three different MS experiments were analyzed separately as a pooled sample using both Mascot version 2.5.1 (http://www.matrixscience.com) to search the UniProt Viridiplantae database. Searches were performed with a maximum of 2 missed trypsin cleavages, fixed carbamidomethyl alkylation of cysteine and variable methionine oxidation, as well as a 0.5 Da and 2.0 Da mass tolerance for fragment and parent ions respectively. Scaffold version 4.0.4 (Proteome software Inc., Portland, OR, USA) was used to validate MS/MS based peptide and protein identifications at a greater than 95% probability, and proteins with a single peptide match had to meet several criteria previously described [7]. Signal peptides were predicted using SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/). *B. napus* and triticale protein annotations were performed with BLAST2GO (www.blast2go.com) using the top 20 hits at 1e-30 and the NCBI non-redundant (nr) protein sequence database. Biological Process annotations were regrouped into categories based on the Ancestor Chart option of QuickGO (www.ebi.ac.uk/QuickGO/WebServices). Proteins involved in *B. napus* or triticale glucosinolate metabolism, carotenoid metabolism or photosynthesis were identified by searching their Biological Process GO annotations.

3. Results and discussion

3.1. Comparison of the different gel-based proteomics approaches

Gel-based and gel-free proteomic techniques have distinct advantages and disadvantages, and the selection of an approach often reflects the intended purpose of the study. While contributing to the global characterization of the stigma proteome, we also wanted to generate a 2D PAGE reference map of the mature Brassica stigma in which the major proteins had been identified to serve in comparative studies (e.g. DIGE). Other electrophoretic techniques that might correlate to the 2D data and also possibly be used for comparative purposes were evaluated. Consequently, 2700 unique *B. napus* mature stigma proteins (see Supplementary material Table S1 in the online version at DOI: 10.1016/j.plantsci.2016.05.020) were identified using 1D SDS PAGE LC–MS/MS, 2D PAGE LC–MS/MS and OGE LC–MS/MS with the different approaches