Article

Proteome and interactome linked to metabolism, genetic information processing and abiotic stress, in the gametophyte of two woodferns

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Abstract: Ferns and fern-allies, now known as Monylophytes, have received scant molecular attention, in relation to angiosperm group. The advent of high-throughput technologies allows to advance towards a greater knowledge of their elusive genome. In this work, samples of apogamous and sexual heart-shaped gametophytes from two ferns: the apomictic species *Dryopteris affinis* ssp. *affinis*and its sexual relative *Dryopteris oreades* were extracted and identified. In total, a set of 218 proteins shared by these gametophytes were analysed by using the STRING database, and the proteome associated to metabolism, genetic information processing and responses to abitotic stress is discussed. Specifically, there are reported proteins involved in metabolism of carbohydrates and lipids, biosynthesis of amino acids, metabolism of nucleotides, energy, and secondary compounds, oxido-reduction, transcription, translation, folding, sorting, and degradation, and response to abiotic stress. Some homologs of proteins found are MACCI-BOU (MAB1), MOSAIC DEATH 1 (MOD1), MAINTENANCE OF PHOTOSYSTEM II UNDER HIGH LIGHT 2 (MPH2), TRANSPARENT TESTA 5 (TT5), ALBINO OR GLASSY YELLOW 1 (AGY1), LEUCYL AMINOPEPTIDASE 1 and 3 (LAP1 and LAP3), or LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (LOS1). The interactome of the set of proteins was also studied, being the most common interactions database and textmining. All these data about the interactions that exist between the studied proteins of the ferns *D. affinis* and *D. oreades*, together with the description of their biological function, might contribute to better understand the functioning and development of ferns as well as to fulfil gaps of knowledge in plant evolution.

Keywords: *Dryopteris affinis* ssp. *affinis*; *Dryopteris oreades*; fern; gametophyte; non-seed plants; proteome; STRING database.

1. Introduction

Ferns and fern-allies, now known as Monylophytes, represent a genetic legacy of great value, being descendants of the first plants that developed vascular tissue, about 470 million years ago. They have received scant attention, concerning the angiosperm group, which relegated them to the background, after a splendid past. The appeal of its fronds or its use to alleviate ailments in traditional medicine is all they have traditionally inspired. Only a handful of species have been used to delve into basic developmental processes, such as photomorphogenesis (Wada, 2007), spore germination (Salmi et al., 2005; Salmi et

 doi:10.20944/preprints202304.0240.v1

al., 2007; Suo et al., 2015), cell polarity (Salmi et al., 2010), cell wall composition (Eeckhout et al., 2014), or reproduction, focussing on the gametophyte generation, which is an autonomous-living organism, easily for *in vitro* culture and sample collection (Fernández and Revilla 2003; Rivera et al., 2018). Although being an individual possessing a very simple structure of a one-cell layer, the gametophyte undergoes some degree of complexity: apical-basal polarity, dorsoventral symmetry, rhizoids, meristems in the apical or lateral part, reproduction organs (antheridia and archegonia, respectively), and trichomes distributed for the entire surface.

From a metabolic point of view, ferns contain secondary metabolites: flavonoids, alkaloids, phenols, steroids, etc., and exhibit various bioactivities such as antibacterial, antidiabetic, anticancer, antioxidant, etc. (Chen et al., 2015). The therapeutic use of pteridophytes is showing a great evolution, from its use in the traditional medicine of different peoples to the current stage, in which the principles of these plants are used in the form of nanoparticles (Femi-Adepoju et al., 2019). Finally, we recently assist the use of ferns to solve interesting problems in the plant world caused by biotic and abiotic stress. Drought is one of the most severe abiotic stress factors affecting plant growth and productivity, and ferns could contribute to managing it (Wang et al., 2010). Other important adaptations of ferns to extreme environments such as salinity, heavy metal, epiphytes, or invasiveness tolerance, are summarized by Rathinasabapathi (2006). Likewise, Dhir (2018) highlights the high efficiency of many species of aquatic and terrestrial ferns, to extract various organic and inorganic pollutants from the environment.

Increasingly, researchers become more interested in these plants, and sometimes this is possible by the advent of high-throughput technologies, such as omics, allowing to advance towards a greater knowledge of their elusive genome. The variation in gene expression, induced by whatever environmental or inner conditions, can be examined in non-model organisms because these techniques have become more feasible as automation and efficiency have reduced costs. Until present, some transcriptome and proteome datasets have been published for ferns, which include the species *Pteridium aquilinum* (Der et al., 2011), *Ceratopteris richardii* (Salmi et al., 2010; Cordle et al., 2012), *Blechnum spicant* (Valledor et al., 2014), *Lygodium japonicum* (Aya et al., 2015), *Dryopteris affinis* ssp. *affinis* (Grossmann et al., 2017; Wyder et al., 2020; Fernández et al., 2021), and *Dryopteris oreades* (Fernández et al. 2021; Ojosnegros et al. 2022). In the last case, both transcriptomic and proteomic analyses were performed by using next-generation sequencing (NGS) and shotgun proteomics by tandem mass spectrometry.

This work expands our knowledge of the proteomic data available in non-seed plants, less explored so far than in seed plants. It is the continuation of a previous work (Ojosnegros et al., 2022) in which samples of apogamous and sexual heart-shaped gametophytes from two ferns: the apomictic species *D. affinis* and its sexual relative *D. oreades*, were extracted and identified by using a species specific transcriptome database established in a previous project (Grossmann et al., 2017). The functional annotation was inferred by blasting identified full length protein sequences. The categorization of the proteins having in common both types of gametophyte is reported. Specifically, this work reveals new proteomic information involved in the metabolism of carbohydrates and lipids, the biosynthesis of amino acids, the metabolism of nucleotides and energy, as well as of secondary compounds, such as flavonoids, terpenoids, lignans, etc., important in plant defence against stress, in addition to the oxide-reduction processes; it also reveals proteins related to transcription, translation, folding, sorting or transport, and degradation.

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2. Results

A set of 218 proteins shared by the gametophytes of the apomictic fern *D. affinis* and its sexual relative *D. oreades* were analyzed by using the STRING version 11.5, and the CYTOSCAPE version 3.9.1 programs. To gain insights into their biological function, Gene Ontology (GO) and KEGG classification provided by the STRING platform were performed (Figs. 1 and 2). From the GO classification, most of the proteins found are involved in biological functions linked to the primary metabolism, and then, more specifically to other cellular processes, such as response to stimulus, protein metabolism, translation, etc.

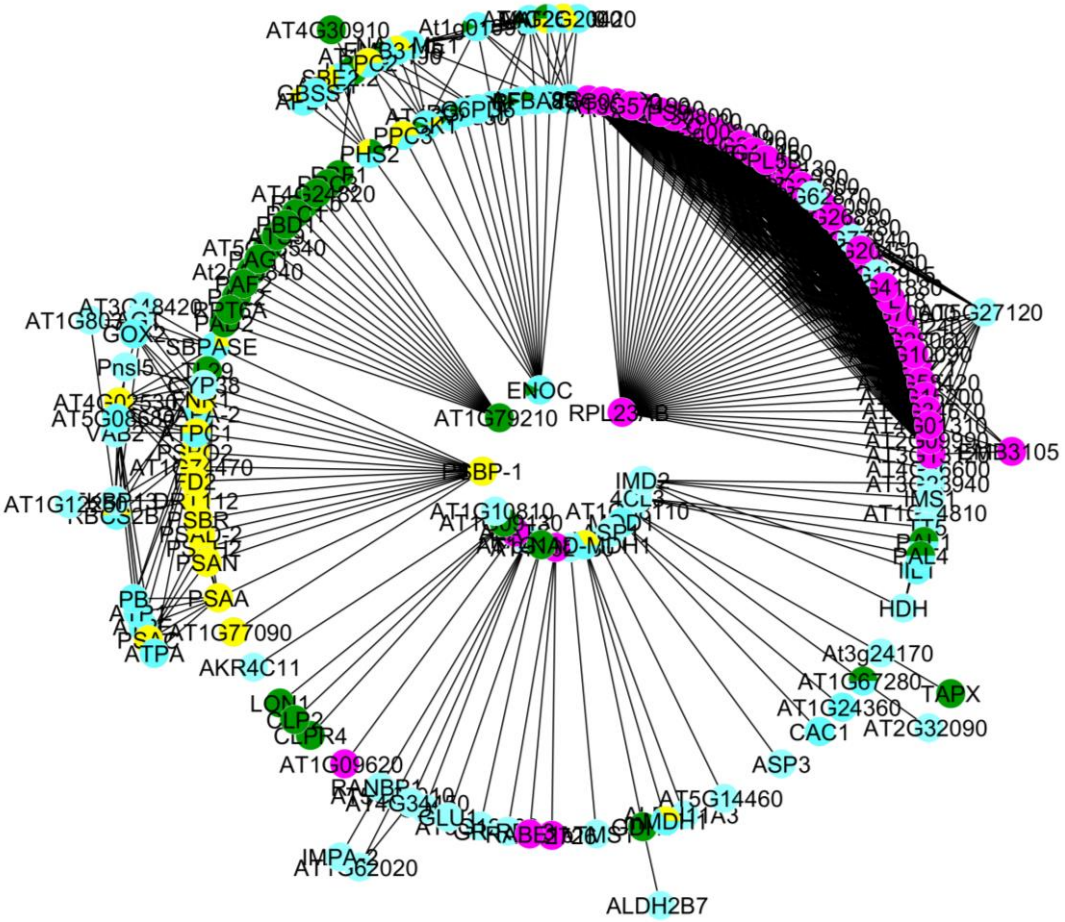

Figure 1. GO enrichment terms of the shared proteomes obtained from gametophytes of *Dryopteris affinis* and *D. oreades* according to the category biological function, analyzed with the STRING and CYTOSCAPE programs. Yellow color refers to the metabolism of energy, pink to transcription and translation, green to protein degradation, and blue to the metabolism of carbohydrates.

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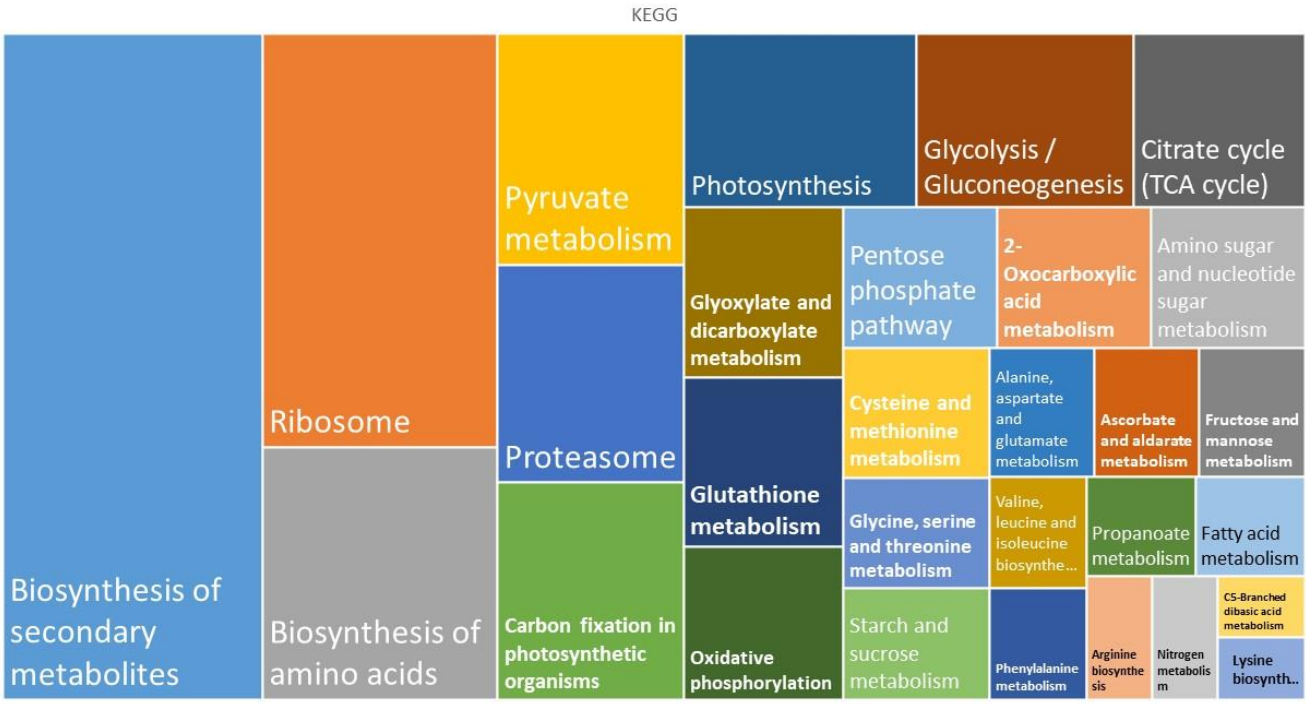

Figure 2. KEGG enrichment terms of the shared proteomes obtained from the gametophytes of *Dryopteris affinis*and *D. oreades,* analyzed with the STRING platform.

In turn, the KEGG classification reveals the presence of proteins mostly associated with the biosynthesis of secondary metabolites, the ribosome, and the biosynthesis of amino acids. These processes include the building of cellular organelles such as the ribosomes (Fig. 3), or the proteasomes (Fig. 4). Regarding the ribosomes, there are several proteins such as nucleic acid-binding proteins, ribosomal proteins, translation elongation factors, etc. On the other hand, the proteasomes intervene in the degradation of proteins, being anotated proteins linked to the proteolytic core: the 20S particle, and also regulatory factors.

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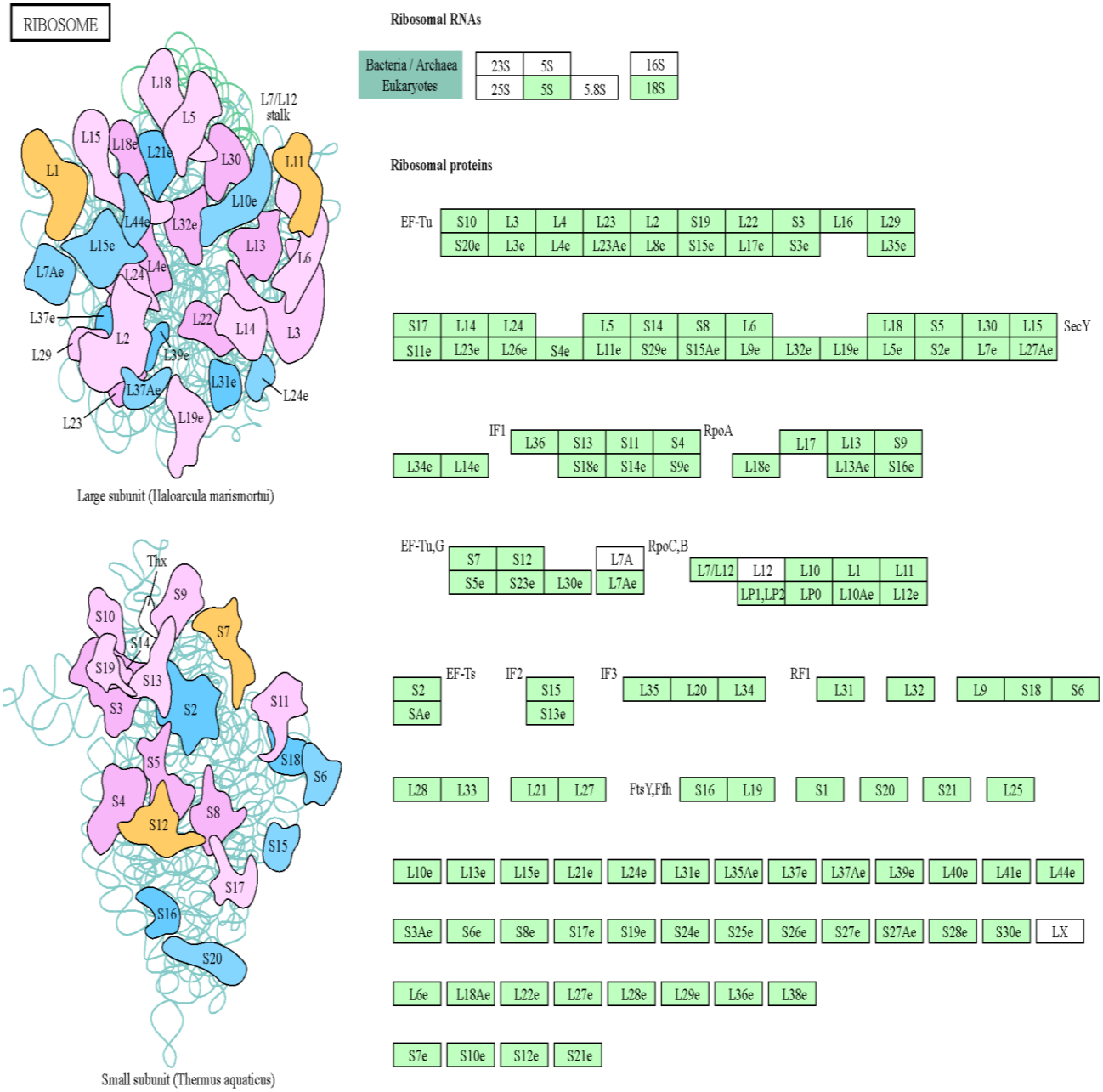

Figure 3. Proteins involved in ribogenesis found in the gametophyte of the ferns *Dryopteris affinis* and *D. oreades*. Imaged provided by STRING platform according to KEGG dataset. "Light-green" highlighted boxes are the identified proteins.

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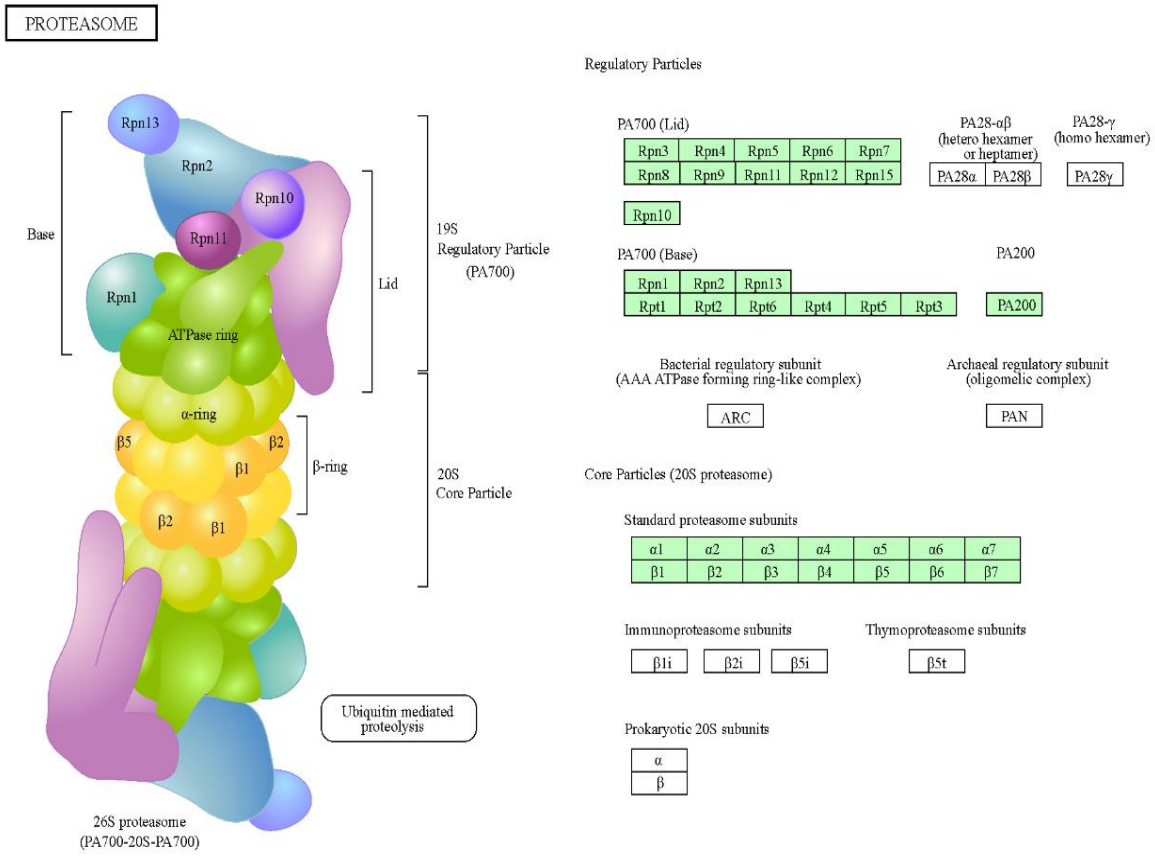

Figure 4. Proteins involved in proteasome found in the gametophyte of the ferns *Dryopteris affinis* and *D. oreades*. Imaged provided by STRING platform according to KEGG dataset. "Light-green" highlighted boxes are the identified proteins.

The protein domains more abundant in the studied groups are the pyruvate dehydrogenase E1 component, and the histidine and lysine active sites of the phosphoenolpyruvate carboxylase, related to carbohydrates metabolism. In the biosynthesis of amino acids, the domains more abundant are the aspartate aminotransferase and the pyridoxal phosphate-dependent transferase. In the metabolism of energy, the HAS barrel domain, and the F1 complex of the alpha and beta subunits of the ATP synthase. In the metabolism of secondary compounds, the aromatic amino acid lyase, the phenylalanine ammonia-lyase, and the N-terminal of the histidase. Finally, the domains more frequent in transcription and translation are the GTP-binding domain and domain 2 of the elongation factor Tu, and the conserved sites of the ribosomal proteins S10 and S4.

Looking at the interactome of this set of proteins, it represents a total network composed of 218 nodes and 1,792 interactions (p-value < 1 x e-16). In the group metabolism of carbohydrates, the proteins showing more interactions are CYTOSOLIC ENOLASE (ENOC) and PHOSPHOGLYCERATE KINASE 1 (PGK1), with 12 interactions each one; in the biosynthesis of amino acids is AT1G14810 with 6 interactions; in the metabolism of energy is ATPC1 with 20 interactions; in the metabolism of secondary compounds is 4-COUMARATE: COA LIGASE 3 (4CL3) with 3 interactions; in transcription and translation are SUPPRESSOR OF ACAULIS 56 (SAC56), RIBOSOMAL PROTEIN US11X (US11X), and RIBOSOMAL PROTEIN US17Y (US17Y) with 44 interactions each one; and finally, in transport is RAB GTPASE HOMOLOG 1A (RAB1A) with 4 interactions.

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The strength of the interactions can be weak or strong, (Table S1) according to a scale from 0 to 1, where a weak interaction will have a score close to 0 and a strong interaction a score close to 1. Taking into account only in each group of proteins studied, the number of interactions with a total score equal to or greater than 0.99, the results show that the proteins of transcription and translation are the ones that have more strongest interactions: 554, next proteins involved on energy with 29 interactions, carbohydrates with 16 interactions, and finally biosynthesis of amino acids, and transport with 5 and 3, respectively.

In addition to the number of interactions and the strength of interactions, we considered also in this study the type of interactions that exist between the proteins, provided by STRING (Fig. 5), which derived from textmining, experiments, co-expression, and databases. Specifically, we analyzed the groups of metabolism of carbohydrates (Fig. 5a), metabolism of energy (Fig. 5b), ribogenesis (Fig. 5c), and protein degradation (Fig. 5d).

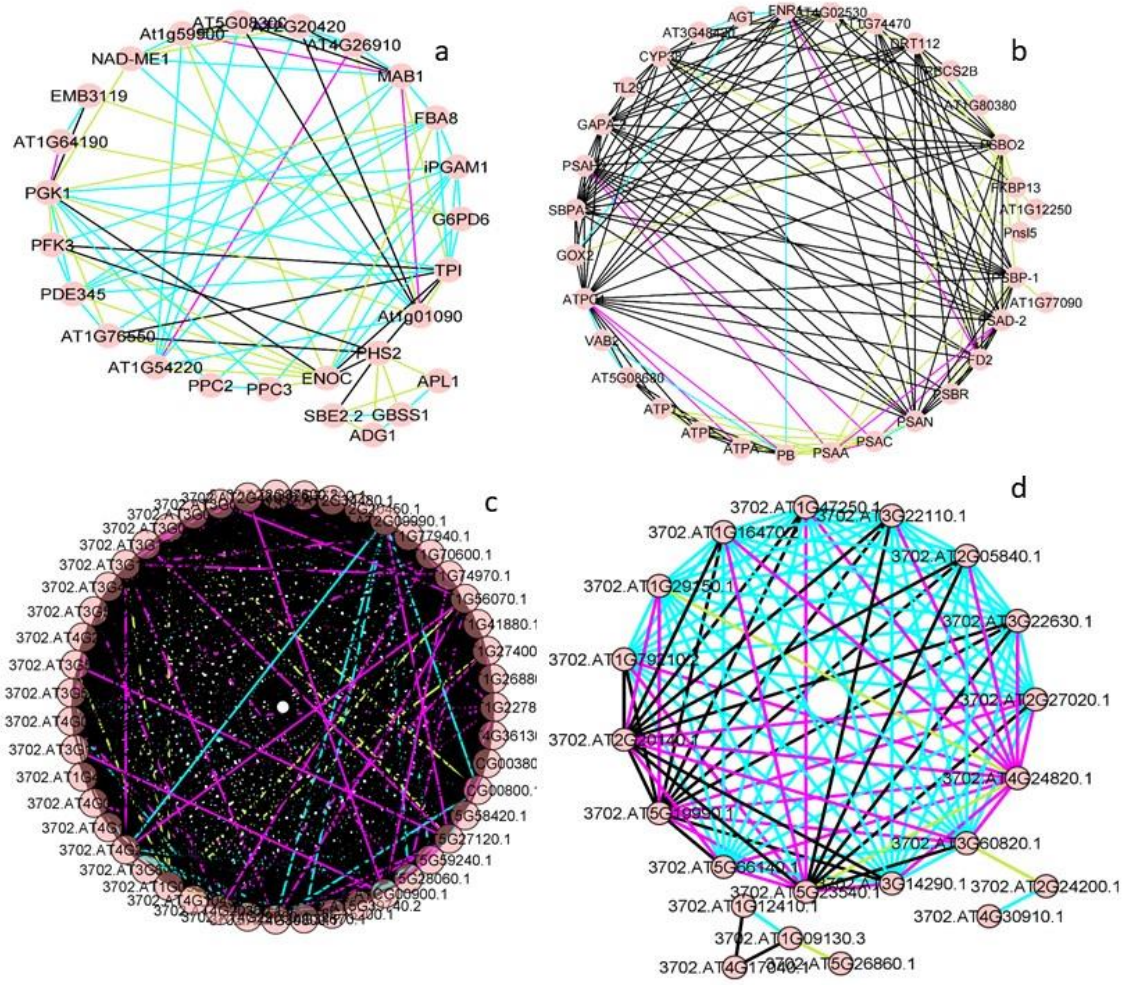

Figure 5. Circular representations obtained through STRING and CYTOSCAPE programs of proteins shared by the gametophytes *Dryopteris affinis* and *D. oreades* and classified in the following groups: **(a)** metabolism of carbohydrates, **(b)** metabolism of energy, **(c)** ribogenesis, and **(d)** protein degradation. The pinklines refer to experiments interactions, the green lines to textmining interactions, the black lines to co-expression interactions, and the blue lines to databases interactions.

Paying attention only the two main types of interactions of each group of proteins, their relations were analyzed (Fig. 6). Hence, database and text mining in metabolism of

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carbohydrates (Fig. 6a), the biosynthesis of amino acids (Fig. 6b), and also in the metabolism of secondary compounds, and transport; textmining and co-expression in the metabolism of energy (Fig. 6c), and experiments interactions and co-expression in transcription and translation (Fig. 6d). In the metabolism of carbohydrates and transcription and translation, the two interactions are alike, meanwhile, in the biosynthesis of amino acids and the metabolism of energy, one interaction is stronger than the other, specifically the textmining interaction in the first group and the co-expression interaction in the second. On the other hand, comparing the same type of interaction among the different groups of proteins, it is observed that the neighborhood interaction is higher in the biosynthesis of amino acids and transcription and translation; gene fusion in the metabolism of carbohydrates and the biosynthesis of amino acids; co-occurrence in the biosynthesis of amino acids and the metabolism of secondary compounds; co-expression in the metabolism of energy and transcription and translation; experiments in transcription and translation, and transport; database in the metabolism of carbohydrates and transport; and finally, textmining in the metabolism of secondary compounds and transport.

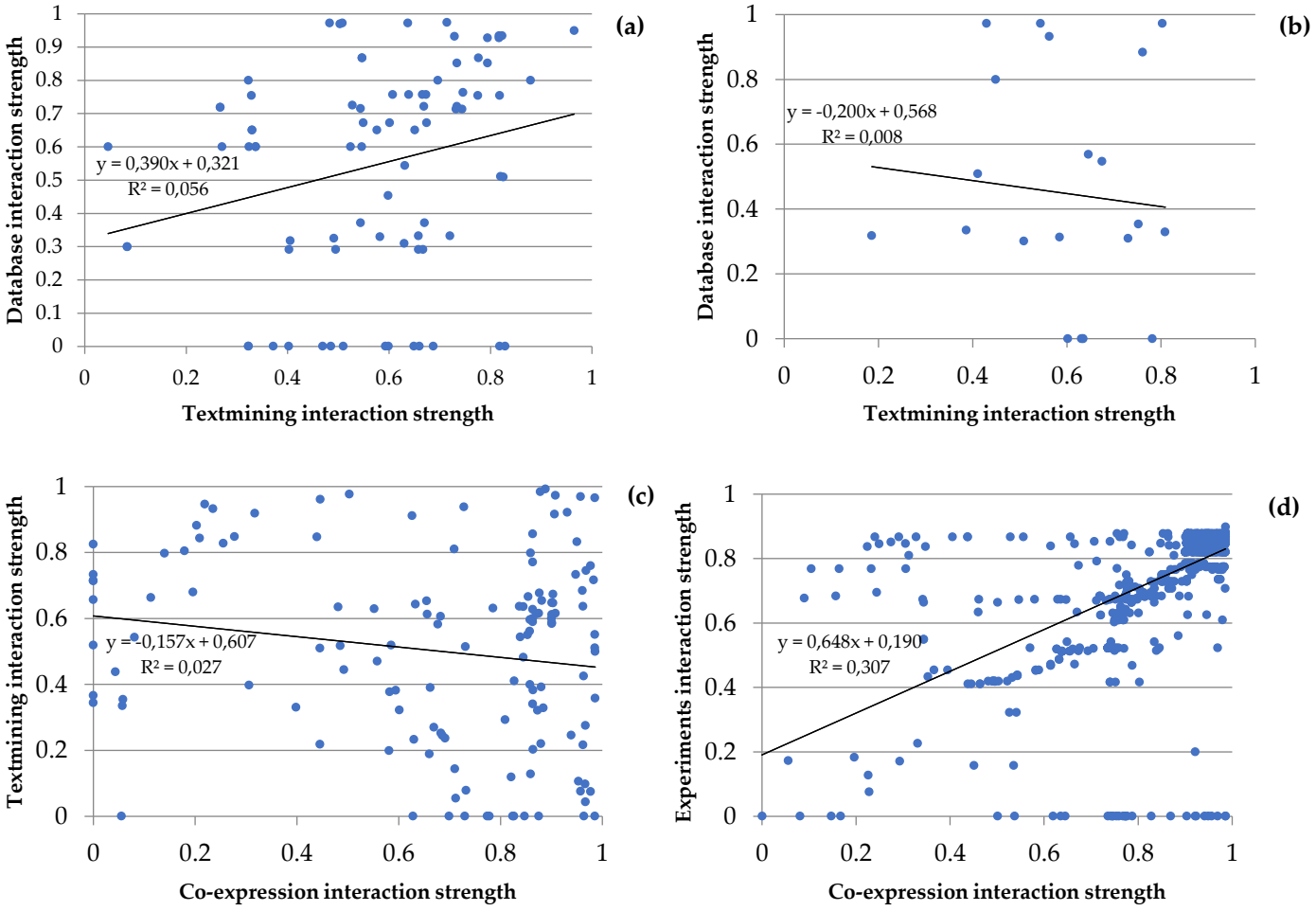

Figure 6. Plots of the two main types of interactions in the groups of proteins shared by the gametophytes of *Dryopteris affinis* and *D. oreades*: **(a)** metabolism of carbohydrates, **(b)** biosynthesis of amino acids, **(c)** metabolism of energy, and **(d)** transcription and translation. Each spot represents the intersection ofthe interactions between two proteins.

3. Discussion

In the context of a proteome commitment carried out in the gametophyte generation of the species of ferns: the apomictic *D. affinis* and its sexual relative *D. oreades*, the current work provides additional information about the proteome and gives continuity to previous studies that dealt with these ferns (Grossmann et al., 2017; Wyder et al., 2020; Fernández et al., 2021; Ojosnegros et al., 2022). Specifically, the proteins discussed here are

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grouped into two major categories to make easier the reading: metabolism and genetic information processing (Table 1). Their biological functions and protein-protein interactions are reported next.

Metabolism comprises two main branches: primary and secondary. Primary metabolism connects to the main metabolites directly involved in plant growth (carbohydrates, lipids, amino acids, nucleotides), as well as those reactions operating in fueling them such as photosynthesis, glycolysis, tricarboxylic acid cycle, etc. In plants, there is also a secondary metabolism, which connects with other transcendental metabolic routes, governing in most cases the defence and stress responses, needed to survive, specifically when the organism is anchored to the underground.

Proteins linked to the "**metabolism of carbohydrates**" turn around glycolysis, pyruvate metabolism, citrate/tricarboxylic acid cycle, pentose phosphate pathway, starch, and biosynthesis of nucleotide sugars. ***Glycolysis*** converts glucose in pyruvate, and in the gametophytes of the study, there are reported enzymatic proteins such as ATP-DEPENDENT 6-PHOSPHOFRUCTOKINASE 3(PFK3), involved in the first reaction; two enzymes participating in glycolysis and gluconeogenesis: FRUCTOSE-BISPHOSPHATE ALDOLASE 3 (FBA3), also named PIGMENT DEFECTIVE 345 (PDE345), and FRUCTOSE-BISPHOSPHATE ALDOLASE 8 (FBA8); and other catalyzing the decarboxylation of pyruvate to acetyl-CoA such as the mitochondrial component of pyruvate dehydrogenase MACCI-BOU (MAB1). Linked to ***pyruvate metabolism*** there are reported two phosphoenolpyruvate carboxylases (PPC2 and PPC3), which supply oxaloacetate for the tricarboxylic acid cycle, and the protein NAD-DEPENDENT MALIC ENZYME 1 (NAD-ME1), which is involved in the regulation of the metabolism of sugars and amino acids during the night (Tronconi et al., 2008). We mention here the protein 2,3-BIPHOSPHOGLYCERATE-INDEPENDENT PHOSPHOGLYCERATE MUTASE 1 (iPGAM1), also important in the functioning of stomatal guard cells and fertility in *A. thaliana* (Zhao et al., 2011).

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Table 1. Selected proteins equally regulated in gametophytes of *Dryopteris affinis*and *D. oreades*.

Category	Accession Number	UniProtKB/ Swiss-Prot	Gene Name	Protein Name	MW (kDa)	Amino Acids
Carbohydrates	58787-330_2_ORF2	Q94AA4	*PFK3*	Phosphofructokinase 3	53	489
Carbohydrates	135690-210_1_ORF2	Q9ZU52	*PDE345*	Pigment defective 345	42	391
Carbohydrates	tr|A9NMQ0|A9NMQ0_PICSI	Q9LF98	*FBA8*	Fructose-bisphosphate aldolase 8	38	358
Carbohydrates	38153-411_5_ORF2	Q38799	*MAB1*	Macci-bou	39	363
Carbohydrates	83096-276_3_ORF2	Q5GM68	*PPC2*	Phosphoenolpyruvate carboxylase 2	109	963
Carbohydrates	54280-344_1_ORF1	Q84VW9	*PPC3*	Phosphoenolpyruvate carboxylase 3	110	968
Carbohydrates	113756-233_2_ORF1	Q9SIU0	*NAD-ME1*	NAD-dependent malic enzyme 1	69	623
Carbohydrates	102811-246_6_ORF2	O04499	*iPGAM1*	2,3-biphosphoglycerate-independent phospho-glycerate mutase 1	60	557
Carbohydrates	70011-302_2_ORF1	O82662	*AT2G20420*	-	45	421
Carbohydrates	8279-816_3_ORF2	P68209	*AT5G08300*	-	36	347
Carbohydrates	222487-119_2_ORF2	P93819	*c-NAD-MDH1*	Cytosolic-NAD-dependent malate dehydro-genase 1	35	332
Carbohydrates	156827-185_4_ORF1	Q9SH69	*PGD1*	6-phosphogluconate dehydrogenase 1	53	487
Carbohydrates	12493-682_6_ORF2	Q9FJI5	*G6PD6*	Glucose-6-phosphate dehydrogenase 6	59	515
Carbohydrates	20760-547_4_ORF1	Q9LD57	*PGK1*	Phosphoglycerate kinase 1	50	481
Carbohydrates	69882-302_6_ORF2	Q9LZS3	*SBE2.2*	Starch branching enzyme 2.2	92	805
Carbohydrates	tr|Q5PYJ7|Q5PYJ7_9MONI	Q9MAQ0	*GBSS1*	Granule bound starch synthase 1	66	610
Carbohydrates	tr|A9SGH8|A9SGH8_PHYPA	P55228	*ADG1*	ADP glucose pyrophosphorylase 1	56	520
Carbohydrates	181563-155_3_ORF2	P55229	*APL1*	ADP glucose pyrophosphorylase large subunit 1	57	522
Carbohydrates	tr|D7MQA6|D7MQA6_ARALL	Q9LUE6	*RGP4*	Reversibly glycosylated polypeptide 4	41	364
Carbohydrates	162660-176_6_ORF1	P83291	*AT5G20080*	-	35	328
Lipids	20213-554_2_ORF1	Q9SLA8	*MOD1*	Mosaic death 1	41	390
Lipids	387953-27_4_ORF1	Q9SGY2	*ACLA-1*	ATP-citrate lyase A-1	49	443
Category	**Accession Number**	**UniProtKB/ Swiss-Prot**	**Gene Name**	**Protein Name**	**MW (kDa)**	**Amino Acids**

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Lipids	211149-128_1_ORF1	Q9LXS6	*CSY2*	Citrate synthase 2	56	514
Amino acids	47558-369_4_ORF2	P46643	*ASP1*	Aspartate aminotransferase 1	47	430
Amino acids	72506-296_4_ORF1	Q94AR8	*IIL1*	Isopropyl malate isomerase large subunit 1	55	509
Amino acids	125905-219_3_ORF2	Q9ZNZ7	*GLU1*	Glutamate synthase 1	179	1,648
Amino acids	393073-25_4_ORF2	Q94JQ3	*SHM3*	Serine hydroxymethyltransferase 3	57	529
Amino acids	tr|D8RLH8|D8RLH8_SELML	Q9C5U8	*HDH*	Histidinol dehydrogenase	50	466
Amino acids	294436-71_4_ORF2	Q9LUT2	*MTO3*	Methionine over-accumulator 3	42	393
Nucleotides	2121-1366_3_ORF2	Q9SF85	*ADK1*	Adenosine kinase 1	37	344
Nucleotides	59309-329_5_ORF1	Q96529	*ADSS*	Adenylosuccinate synthase	52	490
Nucleotides	152024-193_3_ORF2	Q9S726	*EMB3119*	Embryo defective 3119	29	276
Energy	164104-175_1_ORF1	Q9FKW6	*FNR1*	Ferredoxin-NADP(+)-oxidoreductase 1	40	360
Energy	sp|Q7SIB8|PLAS_DRYCA	P42699	*DRT112*	DNA-damage-repair/toleration protein 112	16	167
Energy	154679-189_1_ORF2	Q9S841	*PSBO2*	Photosystem II subunit O-2	35	331
Energy	218625-122_1_ORF2	O22773	*MPH2*	Maintenance of photosystem II under high light 2	23	216
Energy	6036-926_2_ORF1	Q9ASS6	*Pnsl5*	Photosynthetic NDH subcomplex l 5	28	259
Energy	250817-99_2_ORF2	Q94K71	*AT3G48420*	-	34	319
Energy	tr|A9RDI1|A9RDI1_PHYPA	Q944I4	*GLYK*	Glycerate kinase	51	456
Energy	297118-70_2_ORF2	Q56YA5	*AGT*	Alanine:glyoxylate aminotransferase	44	401
Energy	33137-439_6_ORF2	O48917	*SQD1*	Sulfoquinovosyldiacylglycerol 1	53	477
Energy	227095-115_1_ORF2	Q84W65	*CPSUFE*	Chloroplast sulfur E	40	371
Energy	311596-62_2_ORF2	Q9ZST4	*GLB1*	GLNB1 homolog	21	196
Energy	318906-58_1_ORF1	Q39161	*NIR1*	Nitrite reductase 1	65	586
Secondary compounds	156331-186_3_ORF2	P41088	*TT5*	Transparent testa 5	26	246
Secondary compounds	230420-113_2_ORF2	P34802	*GGPS1*	Geranylgeranyl pyrophosphate synthase 1	40	371
Secondary compounds	85783-271_1_ORF2	Q9T030	*PCBER1*	Phenylcoumaran benzylic ether reductase 1	34	308

Category	**Accession Number**	**UniProtKB/ Swiss-Prot**	**Gene Name**	**Protein Name**	**MW (kDa)**	**Amino Acids**
Secondary compounds	153413-190_1_ORF2	P42734	*CAD9*	Cinnamyl alcohol dehydrogenase 9	38	360

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Secondary compounds	156554-185_2_ORF1	Q9S777	*4CL3*	4-coumarate:coA ligase 3	61	561
Secondary compounds	223603-118_1_ORF1	P05466	*AT2G45300*	-	55	520
Oxido -reduction	133847-212_2_ORF2	Q9SID3	*GLX2-5*	Glyoxalase 2-5	35	324
Oxido -reduction	tr|E1ZRS4|E1ZRS4_CHLVA	Q9ZP06	*mMDH1*	Mitochondrial malate dehydrogenase 1	35	341
Oxido -reduction	34437-432_2_ORF1	Q9M2W2	*GSTL2*	Glutathione transferase lambda 2	33	292
Oxido -reduction	115571-230_4_ORF1	Q9LZ06	*GSTL3*	Glutathione transferase L3	27	235
Transcription	tr|A2X6N1|A2X6N1_ORYSI	Q96300	*GRF7*	General regulatory factor 7	29	265
Transcription	287872-75_1_ORF1	Q9C5W6	*GRF12*	General regulatory factor 12	30	268
Translation	209284-130_2_ORF2	Q9FNR1	*RBGA7*	MA-binding glycine-rich protein A7	29	309
Translation	293356-72_1_ORF1	Q9LR72	*AT1G03510*	-	47	429
Translation	26795-487_6_ORF2	Q0WW84	*RBP47B*	MA-binding protein 47B	48	435
Translation	20230-554_5_ORF2	Q9LES2	*UBA2A*	UBP1-associated protein 2A	51	478
Translation	174433-162_1_ORF1	Q9ASR1	*LOS1*	Low expression of osmotically responsive genes 1	93	843
Folding	26640-489_1_ORF2	Q9M1C2	*GROES*	-	15	138
Folding	189606-147_1_ORF2	Q9SR70	*AT3G10060*	-	24	230
Folding	149253-199_6_ORF2	O22870	*AT2G43560*	-	23	223
Folding	2524-1285_6_ORF2	Q9SKQ0	*AT2G21130*	-	18	174
Transport	19573-562_5_ORF2	Q9SYI0	*AGY1*	Albino or glassy yellow 1	117	1,042
Transport	248569-101_3_ORF1	P92985	*RANBP1*	RAN binding protein 1	24	219
Transport	146969-201_2_ORF1	F4JL11	*IMPA-2*	Importin alpha isoform 2	58	535
Transport	151836-193_1_ORF2	P40941	*AAC2*	ADP/ATP carrier 2	41	385

Category	**Accession Number**	**UniProtKB/ Swiss-Prot**	**Gene Name**	**Protein Name**	**MW (kDa)**	**Amino Acids**
Transport	161087-178_2_ORF2	Q8H0U5	*Tic62*	Translocon at the inner envelope membrane of chloroplasts 62	68	641
Transport	82340-277_1_ORF2	Q39196	*PIP1;4*	Plasma membrane intrinsic protein 1;4	30	287
Transport	154825-188_3_ORF2	Q9SMX3	*VDAC3*	Voltage dependent anion channel 3	29	274

 doi:10.20944/preprints202304.0240.v1

Transport	272341-85_2_ORF2	Q94A40	*alpha1-COP*	Alpha1 coat protein	136	1,216
Transport	29489-466_3_ORF1	Q0WW26	*gamma2-COP*	Gamma2 coat protein	98	886
Transport	38639-409_2_ORF3	Q93Y22	*AT5G05010*	-	57	527
Transport	43675-385_1_ORF2	Q67YI9	*EPS2*	Epsin2	95	895
Transport	68824-304_5_ORF2	Q9LQ55	*DL3*	Dynamin-like 3	100	920
Transport	-	F4J3Q8	*GET3B*	Guided entry of tail-anchored proteins 3B	47	433
Transport	3434-1154_1_ORF2	Q96254	*GDI1*	Guanosine nucleotide diphosphate dissociation inhibitor 1	49	445
Degradation	141778-205_4_ORF2	Q8L770	*AT1G09130*	-	40	370
Degradation	172993-163_5_ORF1	Q9XJ36	*CLP2*	CLP protease proteolytic subunit 2	31	279
Degradation	72587-296_2_ORF2	Q8LB10	*CLPR4*	CLP protease R subunit 4	33	305
Degradation	17420-593_1_ORF2	P93655	*LON1*	LON protease 1	109	985
Degradation	tr|A9SF86|A9SF86_PHYPA	Q9LJL3	*PREP1*	Presequence protease 1	121	1,080
Degradation	186732-150_2_ORF2	Q9FL12	*DEG9*	Degradation of periplasmic proteins 9	65	592
Degradation	170504-166_2_ORF2	P30184	*LAP1*	Leucyl aminopeptidase 1	54	520
Degradation	170504-166_2_ORF2	Q944P7	*LAP3*	Leucyl aminopeptidase 3	61	581

 doi:10.20944/preprints202304.0240.v1

Likewise, we identified some proteins associated with the ***citrate/tricarboxylic acid cycle***: AT2G20420 and AT5G08300, involved in the only phosphorylation step at the substrate level of this cycle. Other protein is the cytosolic MALATE DEHYDROGENASE 1 (c-NAD-MDH1), which catalyzes a reversible NAD-dependent dehydrogenase reaction involved in central metabolism and redox homeostasis between organelle compartments (Tomaz et al., 2010). In parallel with glycolysis, it is the ***pentose phosphate pathway***, generating NADPH and pentoses. This metabolic pathway is represented here by the proteins 6-PHOSPHOGLUCONATE DEHYDROGENASE 1 (PGD1), GLUCOSE-6-PHOSPHATE DEHYDROGENASE 6 (G6PD6), and PGK1. A mutation in the gene of the first protein might decrease cellulose synthesis and alters the structure and composition of the primary cell wall (Howles et al., 2006). G6PD6 is important for the synthesis of fatty acids and nucleic acids involved in membrane synthesis and cell division (Wakao et al., 2005). PGK1 contributes too to triggering the phosphorylation of the proteins FTSZ2-1 and FTSZ2-2, required for chloroplast division (Gargano et al., 2012). In addition***, Starch*** is the main reserve form of carbohydrate and energy in plants, being accumulated in chloroplasts during the day, and transported and degraded to provide energy and nutritional substances for growth and metabolism. The gametophytes of our ferns count for proteins involved in its synthesis, includingSTARCH BRANCHING ENZYME 2.2 (SBE2.2), andGRANULE BOUND STARCH SYNTHASE 1 (GBSS1), required this last together with STARCH DIRECTED PROTEIN (PTST) for amylose synthesis (Seung et al., 2015).

Apart from to the proteins mentioned above, others were found associated with the ***biosynthesis of nucleotide sugars*** such as two pyrophosphorylases (ADG1 and APL1); the protein REVERSIBLY GLYCOSYLATED POLYPEPTIDE 4 (RGP4), involved in the synthesis of non-cellulosic polysaccharides of the cell wall (Rautengarten et al., 2011); and AT5G20080.

Regarding the "**metabolism of lipids**", three proteins are reported in this study. The first protein is MOSAIC DEATH 1 (MOD1), which catalyzes the last reduction step of the *de novo* fatty acid synthesis cycle and the fatty acid elongation cycle. An alteration in the gene causes a decrease in the activity of the protein, reducing the number of fatty acids, that triggers mosaic premature cell death, and finally, it changes the plant morphology, such as chlorotic and curly leaves, distorted siliques, and dwarfism (Mou et al., 2000). The second protein is ATP-CITRATE LYASE A-1 (ACLA-1), necessary for the normal growth and development of plants because synthesizes acetyl-coA, a key compound functioning in many metabolic pathways (fatty acids and glucosinolates in chloroplasts; flavonoids, sterols, and phospholipids in the cytoplasm; and ATP and amino acids carbon skeletons in mitochondria). Moreover, it is the substrate for histone acetylation and transcription factors in the nucleus and regulates their function to maintain or alter chromosome structure and transcription (Fatland et al., 2002; Fatland et al., 2005). The third protein is CITRATE SYNTHASE 2 (CSY2), which synthesizes citrate in peroxisomes for the respiration of fatty acids in seedlings, and being also needed for seed germination (Pracharoenwattana et al., 2005).

Involved in the ***biosynthesis of aminoacids***, are reported the proteins aminotransferase ASP1; the ISOPROPYL MALATE ISOMERASE LARGE SUBUNIT 1 (IIL1), which act in the glucosinolate biosynthesis, molecules used by the plant to defend itself against insect attacks (Knill et al., 2009); the GLUTAMATE SYNTHASE 1 (GLU1), needed also for the re-assimilation of ammonium ions generated during photorespiration (Ishizaki et al., 2009); and SERINE HYDROXYMETHYLTRANSFERASE 3 (SHM3), HISTIDINOL DEHYDROGENASE (HDH), and METHIONINE OVER-ACCUMULATOR 3 (MTO3), which catalyze the formation of glycine, L-histidine, and methionine, respectively (Zhang et al., 2010; Petersen et al., 2010; Shen et al., 2002).

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It is reported here some proteins associated with the"**metabolism of nucleotides**", specifically to the AMP synthesis, such as ADENOSINE KINASE 1 (ADK1) and ADENYLOSUCCINATE SYNTHASE (ADSS). It deserves to be also mentioned the protein DEFECTIVE 3119 (EMB3119), essential in the synthesis of numerous compounds such as purines, pyrimidines, aromatic amino acids, NAD, and NADP (Howles et al., 2006).

As it is well known, metabolism demands "**energy**" in the form of ATP obtained from nutrients, and it comprises a series of interconnected pathways that can function in the presence or absence of oxygen. In the gametophyte of our ferns, several mitochondrial, chloroplastic, and vacuolar ATP synthases engaged in the oxidative phosphorylation process are found. Additionally, chemical energy can be obtained through ***photosynthesis***. The list of annotated proteins includes the protein FERREDOXIN-NADP(+)-OXIDOREDUCTASE 1 (FNR1), which regulates the flow of electrons to meet the demands of the plant for ATP and reduction power (Lintala et al., 2007), and others involved in repairing DNA damage such as DNA-DAMAGE-REPAIR/TOLERATION PROTEIN 112 (DRT112) (Kieselbach et al., 2000), PHOTOSYSTEM II SUBUNIT O-2 (PSBO2), which regulates the replacement of the protein D1 impaired by light (Lundin et al., 2007), and the protein maintenance OF PHOTOSYSTEM II UNDER HIGH LIGHT 2 (MPH2). This protein is necessary to carry out photosynthesis correctly and efficiently in two conditions: controlled photoinhibitory light and fluctuating light, since in nature plants experience rapid and extreme changes in sunlight, giving them an adaptive advantage (Liu et al., 2017). Involved in photosynthesis, there is the protein PHOTOSYNTHETIC NDH SUBCOMPLEX L 5 (Pnsl5), which modulates the conformation of the protein BRASSINAZOLE-RESISTANT 1 (BZR1) (Zhang et al., 2013). This protein binds to the promoter of the FLOWERING LOCUS D (FLD), suppresses its expression, and then FLOWERING LOCUS C (FLC) can repress flowering (Zhang et al., 2013). Finally, AT3G48420 degrades xylulose-1,5-bisphosphate, a potent inhibitor of the protein rubisco produced by itself (Bracher et al., 2015). On the other hand, ***photorespiration*** represents a waste of the energy produced by photosynthesis. The enzyme GLYCERATE KINASE (GLYK) catalyzes the final reaction of photorespiration allowing terrestrial plants to grow in an atmosphere with oxygen, reflecting the evolutionary origin of photosynthesis in an anaerobic environment towards an aerobic currently (Boldt et al., 2005). Another important protein in photorespiration is ALANINE GLYOXYLATE AMINOTRANSFERASE (AGT), which participate also in root development, both primary and lateral, in seedlings once they have germinated (Wang et al., 2019).

Likewise, there are some proteins about ***sulfur and nitrogen metabolism***. The first one is represented by two proteins: SULFOQUINOVOSYLDIACYLGLYCEROL 1 (SQD1), which converts UDP-glucose and sulfite to the precursor of the main group of sulfolipids: UDP-sulfoquinovose, preventing it from accumulating in the cell as it is very toxic (Sanda et al., 2001); and CHLOROPLAST SULFUR E (CPSUFE), a sulfur acceptor that activates cysteine desulfurases in plastids and mitochondria, vital during embryogenesis (Ye et al., 2006). As to nitrogen metabolism, there are the proteins GLNB1 HOMOLOG (GLB1), which is a nitrogen regulatory protein and intervenes in glycosaminoglycan degradation (Ferrario-Méry et al., 2008); and NITRITE REDUCTASE 1 (NIR1), which catalyzes the reduction of nitrite to ammonium (Takahashi et al., 2001). It has been seen that if the amount of this protein in the cell increases, the tolerance and assimilation of nitrogen dioxide by the plant improve, and as nitrogen dioxide is an air pollutant caused largely by motorized vehicles, the plant could act as a sink for this substance, i.e., it could be used as a biotechnological application of bioremediation (Takahashi et al., 2001).

Concerning the "**metabolism of secondary compounds",** proteins related to flavonoid biosynthesis are glossed in this work, such as TRANSPARENT TESTA 5 (TT5), responsible for the isomerization of chalcones into naringenin (Shirley et al., 1992). We found too enzymes involved in the biosynthesis of terpenoids: GERANYLGERANYL PY-

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ROPHOSPHATE SYNTHASE 1 (GGPS1); the biosynthesis of lignans: PHENYLCOUMARAN BENZYLIC ETHER REDUCTASE 1 (PCBER1); and the biosynthesis of phenylpropanoids: CINNAMYL ALCOHOL DEHYDROGENASE 9 (CAD9), very important in cell wall formation (Eudes et al., 2006). Also, the protein 4CL3, which produces coA thioesters of hydroxy- and methoxy-substituted cinnamic acids, is used to synthesize anthocyanins, flavonoids, isoflavonoids, coumarins, lignin, suberin, and phenols (Ehlting et al., 1999); and AT2G45300, which intervenes in the synthesis of chorismate, the precursor of the amino acids phenylalanine, tryptophan, and tyrosine (Klee et al., 1987). Linked to "**oxidoreduction**" processes, they are the proteins: GLYOXALASE 2-5 (GLX2-5), involved in cell detoxification in mitochondria (Marashinghe et al., 2005); the mitochondrial MALATE DEHYDROGENASE 1 (mMDH1), participating in redox homeostasis between organelle compartments, may limit photorespiration during the dark phase, and it is required for partitioning of carbon dioxide and energy in leaves (Lindén et al., 2016); and the transferasesGSTL2 and GSTL3, which catalyze the glutathione-dependent reduction of S-glutathionyl quercetin to quercetin (Dixon et al., 2010).

3.2. Genetic Information Processing

The processing of genetic information comprises transcription, translation, folding, sorting or transport, and degradation. In our gametophytes, two proteins are involved in "**transcription**", specifically the 14-3-3-like proteins: GF14 nu (GRF7) and GF14 iota (GRF12), which are associated with a DNA-binding complex that binds to the G-box, a DNA regulatory element (Rosenquist et al., 2001). Besides, and telated to "**translation**": RNA-BINDING GLYCINE-RICH PROTEIN A7(RBGA7), which has a role in RNA processing during stress, specifically in editing cytosine to uracil in mitochondrial RNA, controlling 6 % of mitochondrial editing sites (Shi et al., 2015); and others such as AT1G03510; RNA-BINDING PROTEIN 47B (RBP47B); and UBP1-ASSOCIATED PROTEIN 2A (UBA2A), which regulates mRNA and stabilize RNA in the nucleus (Lambermon et al., 2002). Apart from several ribosomal subunits, there are others involved in elongation, like the protein LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (LOS1), which is also involved in the response to cold (Guo et al. 2002).

Once the proteins have been formed, there is a control of quality to check that they have been synthesized completely and have been folded correctly. Among the proteins playing a major role in the acceleration of **folding** or the degradation of misfolded proteins are GROES, AT3G10060, AT2G43560, AT2G21130, etc. The gametophyte of ferns harbor proteins linked to the **sorting or transport** of molecules within the cell and between inside and outside of cells. In line with it, there are ALBINO OR GLASSY YELLOW 1 (AGY1), which has a role in coupling ATP hydrolysis with protein transfer across the thylakoid membrane, participating in photosynthetic acclimation and chloroplast formation (Skalitzky et al., 2011), RAN BINDING PROTEIN 1(RANBP1), moving proteins into the nucleus (Haizel et al., 1997), IMPORTIN ALPHA ISOFORM 2 (IMPA-2), acting on nuclear localization (Bhattacharjee et al., 2008), and the proteins ADP/ATP CARRIER 2 (AAC2), mediating the import of ADP into the mitochondrial matrix (Haferkamp et al., 2002), and TRANSLOCON AT THE INNER ENVELOPE MEMBRANE OF CHLOROPLASTS 62 (Tic62), into the chloroplasts (Küchler et al., 2002). In addition, we found others associated with the transport of water and small hydrophilic molecules through the cell membrane: PLASMA MEMBRANE INTRINSIC PROTEIN 1;4 (PIP1;4) (Lee et al. 2012), and VOLTAGE DEPENDENT ANION CHANNEL 3 (VDAC3) (Berrier et al., 2015). There are also proteins bound to dilysin motifs and associated with clathrin-uncoated vesicles that are transported from the endoplasmic reticulum to the Golgi apparatus and vice versa: ALPHA1 COAT PROTEIN (alpha1-COP), GAMMA2 COAT PROTEIN (gamma2-COP), and AT5G05010. Instead, the proteins EPSIN2 (EPS2) and DYNAMIN-LIKE 3 (DL3) are related to clathrin-coated vesicles, and the last one participates also in root hair positioning during planar polarity formation in root hair-forming cells (Stanislas et al., 2015). Reviewing our proteomic profile we found proteins such as GUANOSINE NUCLEOTIDE

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DIPHOSPHATE DISSOCIATION INHIBITOR 1 (GDI1), which regulates the GDP/GTP exchange reaction of most RAB proteins by inhibiting GDP dissociation and subsequent GTP binding (Zarsky et al., 1997).

On the other hand, many proteins found are related to protein catabolism or **degradation**, being subunits of proteasomes, i.e., complexes characterized by their ability to degrade proteins or ubiquitination structures. Others such as AT1G09130; CLP PROTEASE PROTEOLYTIC SUBUNIT 2 (CLP2); CLP PROTEASE R SUBUNIT 4 (CLPR4); LON PROTEASE 1 (LON1); PRESEQUENCE PROTEASE 1 (PREP1), which degrades in mitochondria the pre-sequences of proteins that have entered after being cut, since the other option, export them to the cytoplasm, costs a lot of energy and has low efficiency (Stahl et al., 2002); and also DEGRADATION OF PERIPLASMIC PROTEINS 9 (DEG9), which degrades the *A. thaliana* response regulator 4 (ARR4), a regulator which participates in light and cytokinin signaling pathways by modulating the activity of phytochrome B (Ouyang et al., 2017). Plants have to deal with heat stress, and for this, the gametophytes of the study count with the aminopeptidases LEUCYL AMINOPEPTIDASE 1 and 3 (LAP1 and LAP3), probably involved in the processing and turnover of intracellular proteins, and function as molecular chaperones protecting them from heat-induced damage (Scranton et al., 2012).

3.3. Protein-Protein Interactions

Although previously the interactions that exist between the proteins of some studied selected groups, have already been commented on, it is necessary to define all the types of interactions that exist clearly for a better understanding. The interactions between proteins can be of various types: (a) Experiments interaction: referred to proteins that have been shown to have chemical, physical, or genetic interactions in laboratory experiments; (b) Databases interaction: related to proteins found in the same databases; (c) Textmining interaction: occurs between proteins that are mentioned in the same PubMed abstracts or the same articles of an internal selection of the software STRING; (d) Co-expression interaction: indicates that the gene expression of those proteins is related; (e) Neighborhood interaction: between proteins whose genes are close in the genome; (f) Gene fusion interaction: indicates that in at least one organism the orthologous genes of the genes for those proteins have been fused into a single gene; and the last, (g) Co-occurrence interaction: related to proteins that have a similar phylogenetic distribution (Crosara et al., 2018).

Analyzing with the STRING platform thoroughly one by one the interactions of the groups of proteins studied, it is observed that in the metabolism of carbohydrates, co-expression, textmining and experiments interactions are stronger between the proteins AT2G20420 and AT5G08300, and database interaction between AT2G20420 and E2-OGDH1. AT2G20420 and AT5G08300 are both mitochondrial succinate-coA ligase subunits, and E2-OGDH1 catalyzes the conversion of 2-oxoglutarate to succinyl-CoA and CO2, i.e., the three proteins intervene in the tricarboxylic acid cycle (Condori-Apfata et al., 2021). Among the proteins of biosynthesis of amino acids, co-expression interaction is stronger between the proteins AT1G14810 and DIHYDROXYACID DEHYDRATASE (DHAD); and database interaction between DHAD and 2-ISOPROPYLMALATE SYNTHASE 1 (IMS1), between IIL1 and IMS1, and between IIL1 and ISOPROPYLMALATE DEHYDROGENASE 2 (IMD2). In fact, these proteins are involved in the synthesis of numerous compounds necessary for the plant: AT1G14810 for lysine, threonine, and methionine (Zhang et al., 2018); DHAD for isoleucine and valine (Yan et al., 2018); IMS1 and IMD2 for leucine (de Kraker et al., 2007; He et al., 2011); and IIL1 for glucosinolate (Knill et al., 2009). In the metabolism of energy, co-expression interaction is stronger between the proteins ATPC1 and GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE A SUBUNIT 2 (GAPA-2), and between DRT112 and FED A; experiments interaction between PSAA and PSAC, because in the photosynthesis the C-terminal of PSAC interacts

 doi:10.20944/preprints202304.0240.v1

with PSAA and other proteins such as PSAB and PSAD for its assembly into the photosystem I complex (Varotto et al., 2000); database interaction between AGT and GLYCOLATE OXIDASE 2 (GOX2), due to both proteins are necessary for photorespiration (Wang et al., 2019); and finally: textmining, between PSBO2 and PHOTOSYSTEM II SUBUNIT P-1 (PSBP-1), as the two proteins are chloroplastic oxygen-evolving enhancers that form part of the photosystem II (Lundin et al., 2007).

Continuing with the analysis of the interactions between the proteins of the groups studied, in the fourth group: the metabolism of secondary compounds, textmining interaction is the strongest, it occurs between PHE AMMONIA LYASE 1 (PAL1) and PHENYLALANINE AMMONIA-LYASE 4 (PAL4). Both proteins participate in the synthesis from phenylalanine of numerous compounds based on the phenylpropane skeleton, necessary for the plant's metabolism (Cochrane et al., 2004). Regarding: transcription and translation, co-expression interaction is stronger between ribosomal proteins such asEL34Z and UL22Z, RPL23AB, UL11Z, EL14Z, and RPL18; among a long list of proteins forming ribosomes, essential in translation. Finally, in transport, co-expression interaction is stronger between the proteins alpha1-COP and gamma2-COP; experiments interaction between alpha1-COP and AT5G05010; and textmining between AGY1 and GET3B.

As indicated in the results, in the group metabolism of carbohydrates, the proteins with more interactions are ENOC and PGK1, because they are both involved in the process of glycolysis (Li et al., 2019). In the biosynthesis of amino acids is AT1G14810, as it intervenes in several pathways of biosynthesis: lysine, isoleucine, methionine, and threonine (Zhang et al.,2018). In the following group: the metabolism of energy, is ATPC1, surely due to it being an ATP synthase chloroplastic (Takagi et al., 2017). The protein 4CL3 is the one with more interactions in the group metabolism of secondary compounds. It is key in the synthesis of numerous secondary metabolites, such as anthocyanins, flavonoids, isoflavonoids, coumarins, lignin, suberin, and phenols (Ehlting et al., 1999). In transcription and translation are the ribosomal proteins SAC56, US11X, and US17Y, necessary for the formation of ribosomes, and thus, for the translation (Carroll et al., 2008). Finally, in transport is RAB1A, as it participates in intracellular vesicle trafficking and protein transport (Ito et al., 2011).

All these data of the different interactions that exist between the studied proteins of the ferns *D. affinis* and *D. oreades*, together with the description of the possible biological function associated, contribute to expanding the scarce information that exists to date on the development and functioning of these two species of ferns.

4. Materials and Methods

4.1. Plant Material and Growth Conditions

Spores of *D. affinis* were obtained from sporophytes growing in Turón valley (Asturias, Spain), 477 m.a.s.l., 43º 12′ 10 N−5º 43′ 43 W. In the case of *D. oreades*, spores were collected from sporophytes growing in Burgos, Neila lagoons, 1.920 m a.s.l., 42º 02′ 48N−3º 03′ 44 W. Spores were released from sporangia, soaked in water for 2 h, and then washed for 10 min with a solution of NaClO (0.5%) and Tween 20 (0.1%). Then, they were rinsed three times with sterile, distilled water. Spores were centrifuged at 1,300 g for 3 min between rinses and then cultured in 500 mL Erlenmeyer flasks containing 100 mL of liquid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). Unless otherwise noted, media were supplemented with 2% sucrose (w/v), and the pH was adjusted to 5.7 with 1 or 0.1 N NaOH. The cultures were kept at 25 ºC under cool-white fluorescent light (70 µmol m-2s-1) with a 16 h photoperiod and put on an orbital shaker (75 rpm).

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Following spore germination, gametophytes develop as filamentous structures. Then, they were subcultured to 200 mL flask containing 25 mL of MS medium supplemented with 2% sucrose (w/v) and 0.7% agar. The gametophytes of *D. affinis* become two-dimensional, achieving the spatulate and heart shapes after 20 or 30 additional days, respectively. Gametophytes of *D. oreades* grow up slower and needed around six months to become cordate and reach sexual maturity (Fig. 7). By using light microscopy, apogamous and sexual gametophytes were collected. In the first case, they have only female reproductive organs (i.e. archegonia); and in the case of *D. affinis*, cordate gametophytes have visible signs of an evolving apogamic center, composed of smaller and darker isodiametric cells. Samples of apogamous and sexual cordate gametophytes were weighed before and after being lyophilized for 48h (Telstar-Cryodos), and stored in Eppendorf tubes in a freezer at -20 ºC until required.

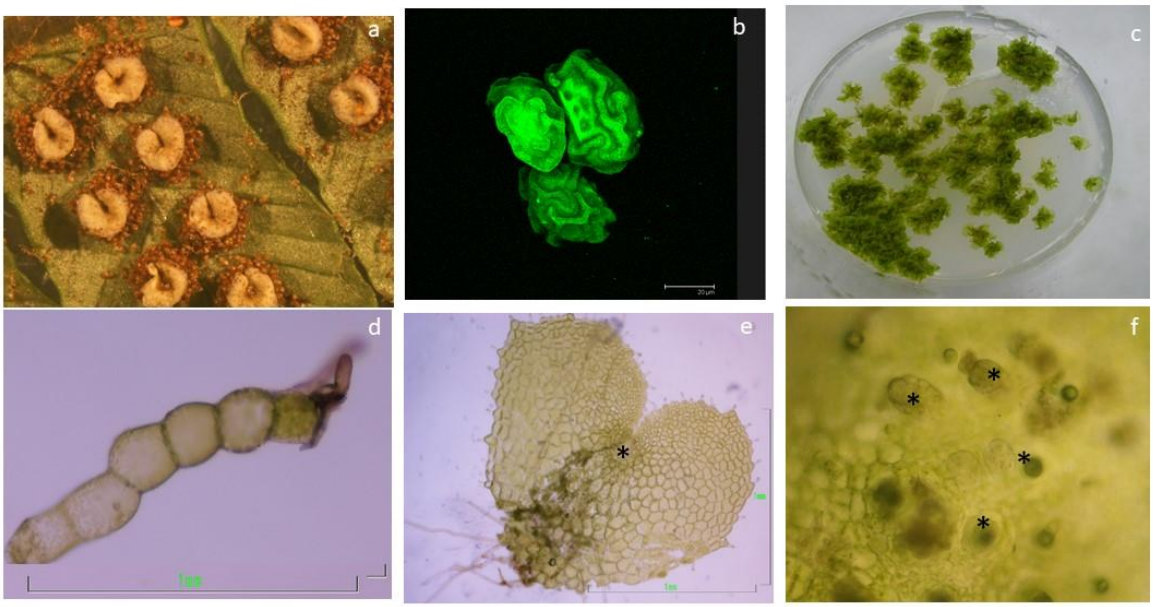

Figure 7. Morphological features in the apogamous fern *Dryopteris affinis* and its sexual relative *D. oreades*. From left to right, and from top to below **(a)** typical kidney sori on the leaf underside; **(b)** confocal image of spores; **(c)** gametophytes growing up in a Petri dish; **(d)** and **(e)** images under light microscope of one- and two-dimensional gametophytes of *D. affinis*; and **(f)** female sexual organs or archegonia in the gametophyte of *D. oreades*.

4.2. Protein Extraction, Separation, and In-Gel Digestion

From the cordate apogamous and cordate sexual gametophytes (three samples each), an amount of 20 mg dry weight of gametophytes was homogenized using a Silamat S5 shaker (Ivoclar Vivadent, Schaan, Liechtenstein). The protocol used for protein extract, separation, and in-gel digestion was reported by Fernández et al., (2021).

4.3. Protein Identification, Verification, and Bioinformatic Downstream Analyses

Mass spectrometry and peptide identification (Orbitrap XL) were performed accordingly (Grossmann et al., 2017). The peptide FDR and protein FDR were estimated at 2% and 1%, respectively, indicating the stringency of the analyses. To have a functional understanding of the identified proteins, we blasted the whole protein sequences of all identified proteins against *S. moellendorffii* and *A. thaliana* Uniprot sequences and we then retrieved the best matching identifier from each of them, along with the corresponding e-value, accepting blast-hits which e-value below 1E-7. These better-annotated orthologue identifiers are then used in further downstream analysis.

4.4. Protein Analysis Using the STRINGPlatform

The identifiers of the genes from the apogamous and sexual gametophyte samples were used as input for STRING platform version 11.5 analysis and a high threshold (0.700) was selected for positive interaction between a pair of genes.

5. Conclusions

The analysis of a set of 218 proteins shared by the gametophytes of the apomictic fern *D. affinis* and its sexual relative *D. oreades* reveals the presence of proteins involved in biological functions mostly associated with metabolism, and also the processing of genetic information. Some smaller groups were studied in detail: metabolism of carbohydrates, biosynthesis of amino acids, metabolism of energy and secondary compounds, transcription, translation, and transport. The interactions between the proteins were identified, the most common source of them derived from database and textmining information. The proteins involved in transcription and translation exhibit the strongest interactions. All this information about the description of the possible biological functions and the different protein-protein interactions expands the existing current knowledge about these species of ferns and plants in general.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Strongest STRING interactions of proteins extracted from gametophytes of *D. affinis* and *D. oreades* and classified into the following groups: metabolism of carbohydrates, biosynthesis of amino acids, metabolism of energy and secondary compounds, transcription, and translation, and transport.

Author Contributions: Conceptualization, H.F., and U.G.; methodology, H.F., J.G., V.G., J.M.A., and S.O.; formal analysis, J.G., and H.F.; writing—original draft preparation, H.F., and S.O.; writing—review and editing, U.G., J.G., V.G., L.G.Q. and J.M.A.; funding acquisition, H.F., and U.G.; resources, L.G.Q. and U.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the University of Zurich, University of Oviedo: Grant CESSTT1819 for International Mobility of Research Staff, and European Union's 7th Framework Program: PRIME-XS-0002520.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The concatenated dDB is available online at http://fgcz-r-021.uzh.ch/fasta/p1222_combo_NGS_n_Viridi_20160205.fasta (accessed on 9 November 2022).

Acknowledgments: We thank the University of Oviedo for a grant from International Mobility of Research Staff, according to the collaboration agreement CESSTT1819, and the Functional Genomics Center Zurich for access to its excellent infrastructure. We also thank Hanspeter Schöb for his logistics upport during visits to the Grossniklaus laboratory

Conflicts of Interest: The authors declare no conflict of interest.

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