Proteomic analysis demonstrates that Bidens pilosa root exudates differentially impact Pteris multifida gametophyte growth

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Abstract

The Novel Weapon Hypothesis (NWH) implicates root exudates as a primary factor for successful take over and destruction of native flora by invasive species. However, the precise mechanisms by which invasive species root exudates mediate this impact are unclear. This study compares and evaluates specific allelochemical impacts on native plants under invasive pressure. Specifically, after 10 days' exposure, a label-free proteomics was applied to analyze the proteins and responsive pathway in *Pteris multifida* gametophyte upon exposure to two *Bidens pilosa* root exudates, undecane and palmitic acid. And each treatment has three biological replicates. The data show that 2183 proteins were detected in the untreated *P. multifida* gametophyte; 1911 proteins in the gametophyte treated with the undecane, and 2148 proteins in the gametophyte treated with palmitic acid. After exposure for 10 days, undecane treated gametophytes exhibited morphological anomalies and exhibited chlorosis; palmitic acid exposure induced no such effects, although development was delayed relative to the control. Using GO functional protein analysis and KEGG pathways detection, we found that the root exudates played different roles on gammopathy growth. Undecane down regulated fatty acid biosynthesis, damaging the cell and chloroplast membrane, and ultimately leading to cell death. Palmitic acid down regulated flavonoid biosynthesis, compromising the gametophyte photosystem and increasing oxidative stress risk. These findings align with NWH, indicating that the exudate release profile is important to the invasion of non-native species, and have implications for the successful management and control of invasive plant species in agriculture and environment.

Highlights

1. Specific major root exudate presents different role on native plants under invasive pressure.

2. Undecane treated gametophytes exhibited morphological anomalies and exhibited

chlorosis;

3. Undecane down regulated fatty acid biosynthesis, damaging the cell and chloroplast

membrane;

4. Palmitic acid exposure induced no strong effects, but the development was delayed;

5. Palmitic acid down regulated flavonoid biosynthesis, compromising the gametophyte

photosystem and increasing oxidative stress risk.

1. Introduction

The identification of mechanisms that underlie successful invasion of exotic plant species is a critical unknown in terrestrial ecosystems (Liu et al., 2020; Ullrich et al., 2019). Several non-mutually exclusive

hypotheses, including the novel-weapons hypothesis (Golivets and Wallin, 2018), the enemy-release hypothesis (Hartshorn et al., 2022), and the shifting-defense hypothesis (Liu et al., 2019) have been proposed to explain plant invasiveness. The novel-weapons hypothesis (NWH) predicts that select plants are successful invaders because they release allelopathic compounds from their roots that are highly suppressive to native plant species in disturbed habitats (Yuan et al., 2021). According to the NWH, root exudates are the major determinant driving the success of invasive terrestrial plant species (Khashi u Rahman et al., 2019). Tian et al reported that flavonoids (quercetin and quercitrin) in the root exudates of the invasive plant *Triadica sebifera* can promote its invasion ability into new ecosystems (Tian et al., 2021). The colonization of *Ambrosia trifida* with its major root exudates, (1E,4E)-germacrdiene-6β,15-diol, (E)-4β,5α-epoxy-7αH-germacr-1(10)-ene-2β,6β-diol, and (2R)-δ-cadin-4-ene-2,10-diol, has been shown to be critical to invasion success in northeast China (Li et al., 2020). *Alternanthera philoxeroides* (Mart.) was shown to inhibit *Humulus scandens* (Lour.) Griseb. growth through the activity of its root allelochemicals, petroleum ether, ethyl acetate, and N-butanol (Wang et al., 2021). Given the complexity of the composition of root exudates and the dynamics of their production, the precise mechanisms by which NWH result in successful invasion remain unknown.

We previously reported that *Bidens pilosa* L. root exudates could promote antioxidant activity and slow down cell programmed death in *Pteris multifida* Poir. gametophytes (Zhang et al., 2019). These effects were mediated through the contributions on multiple root exuded allelochemicals. Root exudates typically include ions, carbon-based compounds, amino acids, sterols, and a broad range of other analytes (Badri and Vivanco, 2009). It has been reported that m-tyrosine, which is a non-protein amino acid, is a significant allelochemical and was identified as a causal agent when fescue grass interfered with the root development of neighboring plants (Bertin et al., 2007). Dayan et al. reported that sorghum released the root allelopathic compound sorgoleone which then inhibited the growth of surrounding weed species (Dayan et al., 2010). In addition, exogenous amendment of ferulic acid, a common exudate from the invasive species *Imperata cylindrica* L, inhibited rice seedling growth (Shen et al., 2020). Importantly, the underlying mechanisms by which these root exudated allelochemicals negatively impact surrounding plants are unclear.

Proteomic analysis is a powerful method that has been used to determine stress-related impacts on biological function (Kosová et al., 2018). Xiao et al. reported that abiotic stresses profoundly impacted plant proteomes, including alterations in protein relative abundance, cellular localization, post-transcriptional and post-translational modifications, protein interactions with other biological molecules, and protein functionality (Xiao et al., 2020). Wang et al. reported that upon exposure to the volatile compound 2-hydroxy-4-methoxy-benzaldehyde from *Periploca sepium* Bunge, 11 of 12 proteins were significantly downregulated, manty of which were cellular component expressed proteins that were located in the cell membrane and chloroplast of *Humulus scandens* (Lour.) Merr. (Wang et al., 2021). Chen et al used comparative proteomics to reveal that microcystin-LR, a kind of cycloheptapeptide, significantly upregulated ascorbate peroxidase and catalase content during the early stages of *Chlamydomonas reinhardtii* L. exposure (Chen et al., 2020). In addition, proteomic analysis demonstrated that six photosynthetic proteins in *Conyza canadensis* L., an invasive weed in agriculture, showed

significant abundance changes upon exposure to in caprylic acid (Li et al., 2019). Importantly, root exudates can have multiple effects on plants, although the role of combined allelochemicals working separately on other plants and the mechanisms by which those impacts are mediated is poorly understood.

Our central hypothesis is that different allelochemicals from an invasive plant would have different effects on local native species. Specifically, this study explores how the invasive plant *B. pilosa* affects the growth of the native plant *P. multifida*. *B. Pilosa*'s major root exudates, undecane and palmitic acid, were selected as the key allelochemicals for study. The fern gametophyte is known to be an environmentally sensitive receptor, and is particularly useful for environmental stress analysis (Zhang et al., 2017; Zhang et al., 2019). This study aims to: (1) understand the effects of allelochemical on fern gametophyte growth, (2) determine the functional proteins' response to the invasive species root exudates. This work increases our understanding of the mechanisms by which terrestrial invasive plant species outcompete native flora and can inform novel strategies of invasive species management.

2. Materials And Methods

2.1. Plant materials and root exudate exposure

P. multifida fronds with spores were collected at the West Lake Scenic Area (Hangzhou, China) in November, 2021. The fronds were placed in clean paper bags and air dried at room temperature. After 7 d, the spores were screened and isolated with a 0.088-mm diameter mesh (Zhejiang Shangyu Yarn and Sieve Factory, Shangyu, China), washed with 95%, 75%, and 50% alcohol every minute, and left to soak in DI water. The spores were then spread evenly on plastic dishes (measuring 25 cm × 20 cm × 5 cm) with MS solid media at an average density of 100–150 spores per cm^2 under a fluorescent light (photon flux density 1000 μ mol m^{-2} s^{-1}) at 25°C for a 12-h light photoperiod. Twenty days after spore sowing, healthy and uniform gametophytes (10 × 5 mm) were selected and rinsed carefully with 1/2 strength Hoagland solution.

Undecane and palmitic acid were previously reported as the major *B. pilosa* root exudates (Zhang et al., 2019) and were selected as treatments for this study; specifically, 8.15% (w/w) undecane and 13.96% (w/w) palmitic acid were used in the gametophyte treatments. The chemicals were first dissolved with (1%, v/v) methanol before adding to each treatment before the experiment. We selected 20-day old healthy and uniform gametophytes; approximately 0.2 g/dish of gametophytes for each treatment-and each treatment had three replicates.

2.2 Gametophyte biomass and phenotype

Forty full-grown gametophytes of similar size were collected from each plate on the 10th day after exposure. The biomass was expressed as fresh weight per gametophyte. Observations of three dishes from each treatment were made using a stereomicroscope (Nikon SMZ18, Japan). The growth in the control was normalized as 100%.

2.3 Proteomics sample preparation

Fresh gametophyte samples (0.20 g) were ground individually in liquid nitrogen and lysed with SDT lysis buffer (4% SDS, 100 mM DTT, 10 mM TEAB), followed by 5 min of ultrasonication on ice. After reacting at 95 ℃ for 8 min, the lysate was centrifuged at 12,000 g for 15 min at 4℃. The supernatant was alkylated with 500 mM iodoacetamide for 1 h at room temperature in the dark. The samples were completely mixed with four times the volume of precooled acetone by vortexing and incubated at -20 ℃ for at least 2 h. The samples were then centrifuged at 12,000 g for 15 min at 4 ℃, and the precipitate was collected. After washing with 1 mL cold acetone, the pellet was dissolved in a dissolution buffer (8 M urea, 100 mM TEAB, pH 8.5).

An aliquot of each protein was taken, and the volume was adjusted to 100 μL with DB lysis buffer (8 M urea, 100 mM TEAB, pH 8.5); trypsin and 100 mM TEAB buffer were then added, and the sample was mixed and digested at 37°C for 4 h. Trypsin and $CaCl_2$ were then added, and the samples were digested overnight. Formic acid was mixed with the digested sample, the pH was adjusted to less than 3, and the samples were centrifuged at 12,000 g for 5 min at ambient temperature. The supernatant was slowly loaded onto a C_{18} desalting column and rinsed with washing buffer (0.1% formic acid, 3% acetonitrile) three times, and then elution buffer was added (0.1% formic acid, 70% acetonitrile). The eluents of each sample were collected and lyophilized in Table S2 (Shen et al., 2019).

2.4 Proteomics Analysis

The mobile phase A (100% water, 0.1% formic acid) and B solutions (80% acetonitrile, 0.1% formic acid) were prepared. The lyophilized powder from each sample was dissolved in 10 μL of solution A and centrifuged at 14,000 g for 20 min at 4 ℃, and 1 μg of the supernatant was injected into a C18 Nano-Trap column (4.5 cm × 75 μm, 3 μm). The peptides were separated on a custom analytical column (15 cm × 150 μm, 1.9 μm) using a linear gradient elution as described in Table 1. The separated peptides were analyzed using a Q Exactive TM HF-X mass spectrometer (Thermo Fisher) with an ion source of Nanospray Flex™ (ESI), spray voltage of 2.1 kV, and ion transport capillary temperature of 320°C. The full scan ranges from m/z 350 to 1,500 had a resolution of 60,000 (at m/z 200), the automatic gain control (AGC) target value was 3×10^6, and the maximum ion injection time was 20 ms. The top 40 precursors of the highest abundance in the full scan were selected and fragmented using higher-energy collisional dissociation (HCD) and analyzed in MS/MS, where the resolution was 15,000 (at m/z 200), the automatic gain control (AGC) target value was 1 × 105, the maximum ion injection time was 45 ms, the normalized collision energy was set as 27%, the intensity threshold was 2.2 × 104, and the dynamic exclusion parameter was 20 s.

All spectra were searched against the UniPort database (https://www.uniprot.org/) using the search engine Proteome Discoverer 2.2 (PD 2.2, Thermo). The search parameters were set as follows: the mass tolerance for precursor ions was 10 ppm, and the mass tolerance for product ions was 0.02 Da. Carbamidomethyl was specified as the fixed modification, oxidation of methionine (M) was specified as

the dynamic modification, and acetylation was specified as the N-Terminal modification in PD 2.2. A maximum of two missed cleavage sites were allowed. To improve the quality of the analysis results, the software PD 2.2 further filtered the retrieval results: peptide spectrum matches (PSMs) with a credibility of more than 99% were identified as PSMs. The identified protein contained at least one unique peptide. The identified PSMs and proteins were retained and performed with an FDR of no more than 1.0%. The protein quantitation results were statistically analyzed using a *T*-test. The proteins whose quantitation was significantly different between the experimental and control groups were defined as differentially expressed proteins (DEPs) (Table S3).

Gene Ontology (GO) and InterPro (IPR) functional analysis were conducted using the interproscan program against the non-redundant protein database (including Pfam, PRINTS, ProDom, SMART, ProSite, and PANTHER) (Chu et al., 2019), and the COG (Clusters of Orthologous Groups) and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases were used to analyze the protein family and pathways. DEPs were used to construct a volcanic map analysis, cluster heat map analysis, and enrichment analysis of GO, IPR, and KEGG (Xia et al., 2021). The probable protein–protein interactions were predicted using the STRING-db server (http://string.embl.de/) (Crosara et al., 2018).

2.5 Statistical analysis

A one-way ANOVA was conducted on the biomass, with exudates serving as treatments. Descriptive statistics were used to analyze the central tendency and dispersion trends of the data. Mean differences were separated using the *Duncan*'s tests. The level of significance was fixed at $p < 0.05$. All statistical analyses were performed using SPSS v. 21.0 for Windows; graphs were constructed with Origin 9.0.

3. Results

3.1 Gametophyte phenotypic response under allelochemical treatment

After 10 days of treatment, untreated *P. multifida* gametophyte seedlings (Fig. 1-a) grew better than those under whole root exudates treatment, and the seedlings under undecane and palmitic acid treatment (Fig. 1-b, c). Specifically, the untreated gametophyte fronds were round and straight, while the rhizoids were clear and robust. When treated with undecane, the gametophyte shape was visually different, with fronds and rhizoids being deformed and chlorotic lesions were evident; other structures, such as the rhizoid and archegonia, were difficult to distinguish. Although the gametophyte shape and size seemed unaffected by 10 days of treatment with palmitic acid, development was delayed; the size of treated gametophyte was smaller than the control. In the controls, the gametophyte biomass increased by 183.2% over 10 days, whereas the biomass increased 120.4% and decreased 13.5% when upon treatment with undecane and palmitic acid, respectively (Table S1).

3.2 Response of clusters of orthologous genes (COG) under allelochemical treatment

Label-free proteomics was applied to analyze the gametophyte response to allelochemical treatment. An average of 2,183 proteins were isolated from the untreated gametophytes, 1,911 proteins from the undecane treated gametophytes, and 2,148 proteins from the palmitic acid treated gametophytes, respectively (Table S2). Through COG analysis, the largest protein number was 263, with functions being associated with translation, ribosomal structure, and biogenesis. The protein cluster of posttranslational modification, protein turnover, and chaperones was second with a value of 243. In addition, 192 proteins related to general function ranked third in the gametophyte cells (Fig. 2-a). Based on PCA of the enrichment and function data, it was shown that the three groups of gametophyte proteins were well separated (Fig. 2-b).

When evaluating the differences in proteins from the control and the two treatments, gametophytes treated with palmitic acid had significantly increased protein number enrichments with 1.5-, 1.3-, and 1.2-fold changes. The protein number increased by a lesser extent compared with the control and undecane and the control and palmitic acid (Fig. 2-c). With undecane treatment, the protein number decreased significantly compared treatment with palmitic acid. The enrichments of 526 proteins decreased 1.2-fold, 519 proteins decreased 1.3-fold, and 474 proteins decreased 1.5-fold between gametophytes treated with undecane and palmitic acid, respectively. The protein number enrichments that decreased by 1.2-fold were 413 and 329, 1.5-fold were 360 and 294, and 2.0-fold were 251 and 235, respectively (Fig. 2-d).

3.3 Gene Ontology (GO) functional protein response under allelochemical treatment

GO was used to analyze functional protein response upon allelochemicals exposure. Because the protein profile separated clearly, this suggests that biological function was significantly altered by allelochemical treatment (Grove et al., 2012). The percentage of protein change was characterized in the major functional categories of biological process (BP), cellular composition (CC), and molecular function (MF). With undecane exposure, the gametophyte experienced altered BP of fatty acid metabolic processes (protein number: 8); the CC of the extrinsic membrane components (10), the photosystem II oxygen-evolving complex (11), and the large ribosomal subunit (2); and the MF of protein dimerization activity (6), small protein-activating enzyme activity (5), and flavin mononucleotide (FMN) binding (4) (Fig. 3-a). With palmitic acid, the protein enrichments were altered in the BP of macromolecule methylation (2), the CC of the cell wall (6) and nucleosome (2), and the MF of nucleic acid binding (49), magnesium ion binding (9), and carboxy-lyase activity (8) (Fig. 1-b). In addition, two gametophyte proteins treated with the allelochemicals presented differences at the BP of vesicle-mediated transport (14), endoplasmic reticulum (ER) to Golgi vesicle-mediated transport (4), and cellular iron ion homeostasis (2); the CC of intracellular organelle (48), cytoplasm (47), and membrane (45) ; and the MF of small protein-activating enzyme activity (4), nutrient reservoir activity (3), and flavin adenine dinucleotide (FAD) binding (3) (Fig. 3-c).

3.4 Major pathway responses under allelochemical treatment

As the functional proteins showed differences as a function of allelochemical treatments, the pathways' response was characterized by the KEGG platform; a total of 196 pathways were detected in the data. Upon undecane exposure, the pathways of homologous recombination and brassinosteroid biosynthesis were slightly changed; autophagy, alpha − linolenic acid metabolism, and starch and sucrose metabolism were significantly downregulated (Fig. 4-a). With palmitic acid, all pathways were downregulated. Flavonoid biosynthesis was significantly downregulated with six proteins, along with most of the detected proteins related to the spliceosome pathway (18), pentose and glucuronate interconversions (9), and arginine biosynthesis (7) (Fig. 4-b). Interestingly, sphingolipid metabolism and base excision repair pathways were not significantly changed by exposure. Upon undecane treatment, the major down-regulated pathways were glycerophospholipid metabolism and plant − pathogen interactions. Most proteins detected were related to protein processing in the endoplasmic reticulum (18), aminoacyl − tRNA biosynthesis (9), and the mRNA surveillance pathway (9) (Fig. 4-c).

3.5 Structural domain responses under allelochemical treatment

To better understand protein response to allelochemical exposure, the structural domains of the differential proteins were analyzed. The data show that the Photosystem II PsbP oxygen-evolving complex was significantly downregulated upon gametophyte exposure to undecane. The structural domains of histone − fold (4), dehydrogenase, multi-helical (4), and 6 − phosphogluconate dehydrogenase, and C − terminal − like (6) were down-regulated with most detected proteins (Fig. 5-a). Upon palmitic acid exposure, the chalcone/stilbene synthase, N − terminal and chalcone/stilbene synthase, and C − terminal presented no changes; NmrA − like and initiation factor eIF − 4 gamma, MA3 were significantly downregulated. In addition, the structural domains of pectin lyase fold/virulence factor (13), Ubiquitin − conjugating enzyme/RWD − like (11), and ClpP/TepA (9) were downregulated with the most detected proteins (Fig. 5-b). When comparing the gametophyte protein structural domains treated with undecane and palmitic acid, oxoglutarate/iron − dependent dioxygenase was unchanged. The addition of undecane downregulated the protein domains of Peptidyl − prolyl cis − trans isomerase, FKBP − type (8), Sec23/Sec24, trunk and helical domains (4 and 4), initiation factor eIF − 4 gamma, MA3 (4), and the gelsolin domain (4) (Fig. 5-c).

4 Discussion

4.1 The mechanisms of response to allelochemical treatment

As described above, the exogenous application of major root exudates were harmful to gametophyte growth as demonstrated by both phenotype and proteomic analysis (Fig. 1, 2). In addition, the two allelochemicals induced different response mechanisms upon exposure to the native plant species.

The data show that *P. multifida* gametophyte was significantly damaged by undecane exposure, and the proteomic analysis demonstrates that fatty acid metabolism was the most significantly downregulated pathway. Fatty acid metabolism is a critical biological process, particularly for a plant is under environmental stress (Sanchez-Martin et al., 2018). It is known that 18:1 fatty acid plays a significant role in regulating defense signaling upon stress (Mandal et al., 2012). In addition, ω-3 fatty acid is an important plant signaling molecule; its content has been shown to be stress responsive, with decreasing activity under salt stress and increasing activity during recovery (Sui et al., 2018). In addition, fatty acids are the sole phospholipid species present in the thylakoid membrane, and as such, is a critical component of the membrane-bound photosynthetic apparatus, e.g., Photosystem II (Hernandez and Cejudo, 2021) that produces high-energy molecules for plant growth. Our findings align well with previous studies, as it has been reported that the proteins associated with fatty acid metabolism and pathways became active when gametophytes were exposed with *B. pilosa* root exudates (Zhang et al., 2016a). Thus, fatty acid downregulation is an important reason for membrane dysfunction and photosystem inefficiency under undecane exposure (Fig. 3-a). In addition, due to the decrease in photosystem efficiency, there is likely a shortage of energy molecules to support starch and sucrose metabolism and glycolysis.

Importantly, the data show the potential structural domains impacted by undecane-induced toxicity. The oxygen-evolving complex in the PsbP of Photosystem II was significantly downregulated; this system has two critical roles: electron denotation to $P680^{+}$ and the release of oxygen molecules (Wang et al., 2021a). We previously reported that under *B. pilosa* root exudate treatment, the electron transfer chain could be inhibited and energy generation disrupted in gametophyte photosystems (Zhang et al., 2016b). The downregulation of PsbP is directly linked to the loss in photosystem efficiency (Fig. 6-a). Consequently, with a shortage of energy, plant growth is significantly inhibited (Fig. 1-b).

Interestingly, few strong negative effects were evident on gametophyte growth upon exposure to palmitic acid (Fig. 1-c). Given the GO results, it appears likely that DNA and RNA methylation occur with palmitic acid treatment (Fig. 3-b). Nuclear acid methylation is an important biomarker for plant adaptation to environmental stress (Kristensen et al., 2020; Liu and He, 2020). Under salinity stress, Sun et al reported 4,402 differentially methylated region-associated genes were observed between stressed and control maize (Sun et al., 2018). CG DNA hypomethylation in wheat is known to impact the expression of metal detoxification transporters and to confer resistance against metal toxicity in soil contaminated with lead, zinc, and cadmium (Shafiq et al., 2019). It has also been reported that in zinc-deficient soil, Arabidopsis seedlings exhibited cytosine followed by guanine in the DNA sequence (CpG) methylation (Chen et al., 2018). However, gene methylation is typically evident through phenotype in later generations (Reik et al., 2001); this may explain the lack of significant changes in the first generation *P. multifida* gametophyte.

Under treatment with palmitic acid, flavonoid biosynthesis pathways were downregulated; in plant systems, flavonoids help combat oxidative stress and act as growth regulators (Kumar and Pandey, 2013). It has been reported that flavonoid peroxidase may act as a mechanism for H_2O_2 scavenging, and thus, flavonoids act as important detoxifying agents (Yamasaki et al., 1997). With this function, flavonoids have been suggested as a mechanism by which plants can survive soils contaminated with toxic metals such as aluminum (Barcelo and Poschenrieder, 2002). It has been hypothesized that antioxidant flavonoids increase of photosystem activity to confer protective functions during drought stress (Tattini et al., 2004). In addition, flavonoids accumulated under toxic metal exposure are not only able to detoxify ROS but potentially also the toxic metals directly by chelation. The accumulation of palmitic acid leads to oxidative stress in the gametophyte. Flavonoids have also been shown to silence hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase, which can strongly inhibit plant growth (Besseau et al., 2007). In our study, we observed that gametophyte growth was delayed under palmitic acid exposure, and this is likely a function of decreased flavonoid biosynthesis. In addition, flavonoids are involved in photosystem protection and have been reported to improve drought stress in *Oudeneya africana* through this mode of action (Talbi et al., 2020). Specifically, under UV-B radiation, flavonoid content increased 19.5% after exposure, with the chlorophyll content recovering from 2.15 mg/kg to 2.76 mg/kg (Piccini et al., 2020). It has also been demonstrated that flavonoid content could increase by 25% when involved in photo-protective processes in tomato leaves (Bednarczyk et al., 2020). Our results support the conclusion that lower flavonoid biosynthesis would affect plant growth (Fig. 4-b). This is a function of the N and C terminals on chalcone/stilbene synthases being downregulated, which serve as the catalysts for the initial step in flavonoid biosynthesis (Tropf et al., 1994). It has also been observed that most nuclear acid biosynthesis and expression are downregulated with the negative expression of spliceosome upon palmitic acid exposure.

4.2 The role of root exudates acting as “weapons” in invasion

The allelopathic effect is typically the final event and is caused by the combined contributions of many allelochemicals, making it difficult to ascribe mechanisms and to evaluate risk. We report that different allelochemicals have different negative effects on plants; undecane acts as a “membrane vandal” that downregulates fatty acid biosynthesis and compromises the cell and chloroplast membrane. Palmitic acid works by limiting flavonoid biosynthesis to reduce photosystem protection and increase oxidative stress, although this mode of toxicity is less impactful than that of undecane. This demonstrates the importance of identifying the major allelochemicals in the root exudates so as to design an effective control strategy for invasive plants.

Root exudates are known to be an important factor in the NWH, and the damaging effects caused by invasive species are a topic of intense interest in both agricultural and ecological research. However, root exudates are frequently considered collectively; for example, it has been shown that root exudates can change soil pH to solubilize nutrients into assimilable forms, chelating toxic compounds and attract beneficial microbiota in the rhizosphere (Vives-Peris et al., 2020). However, one root exudate, 6-methoxy-

benzoxazolin-2-one, was found to alter root-associated fungal and bacterial communities and decrease plant growth in wheat and maize fields (Hu et al., 2018), while other allelochemicals had no impact. Importantly, the co-effects of the root exudates are unclear; for example, exudates such as ethylene, strigolactones, jasmonic acid, (-)-loliolide, and allantoin mediate root detection and behavior, kin recognition, flowering, and also driving inter- and intra-specific facilitation in cropping systems and mixed-species plantations (Wang et al., 2021b). At the same time, individual analytes within root exudates are difficult to detect and quantify, and making this an area of much needed research (Preece and Penuelas, 2020). For example, the traits of the root exudates of wild crop relatives could be used to improve agricultural output and reduce environmental impacts.

5. Conclusion

To conclude, our data demonstrates that specific allelochemicals from invasive species can have different effects on native plant species. An understanding of the analyte-specific nature of allelochemical effects of root exudates on native plants will help to uncover the mechanism of NWH, which will further control and management efforts in agriculture and increase protections for native plants in the environment.

Declarations

Authors' Contributions

KZ and YS conducted the seedlings culture assay under exudates, extracted proteins from tissues and wrote the first manuscript draft. LF and KZ collected the *Pteris multifida* gametophyte spores. YS performed the protein purification and mass spectrometer analysis for peptide identification. KZ and YS conceived the study, YS performed the bioinformatic analyses and protein identification, KZ, JW, and YS performed manuscript writing and conceived the proteomic work and coordinated the experimental work. All authors contributed to edit the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available in the Supplementary Data repository, “GO.anno.go2protein.xls” in the Supplementary Material.

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Table

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Figures

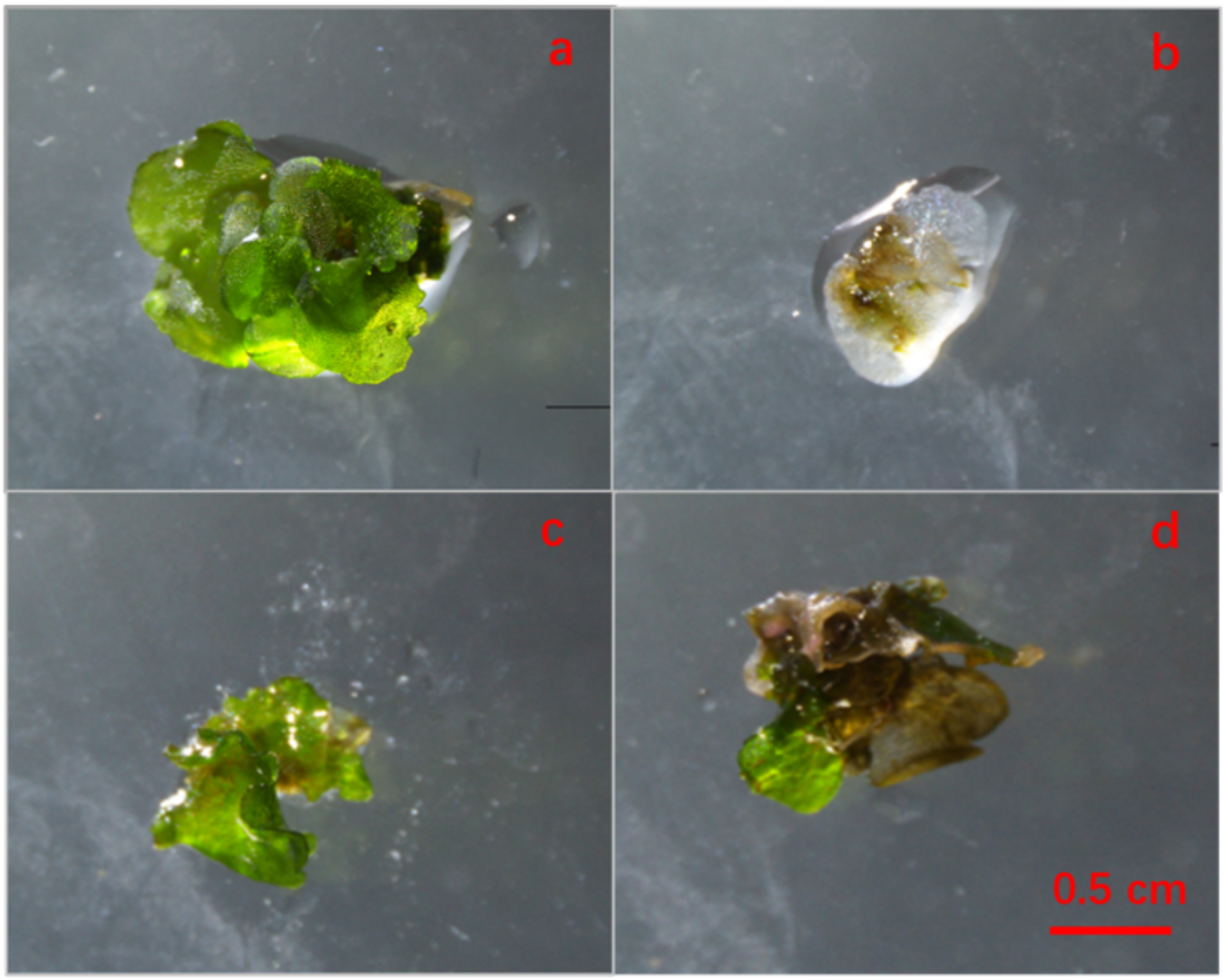

Figure 1

Gametophyte phenotype response under control (a), undecane (b), palmitic acid (c), and whole root exudates treatments at day 10, respectively.

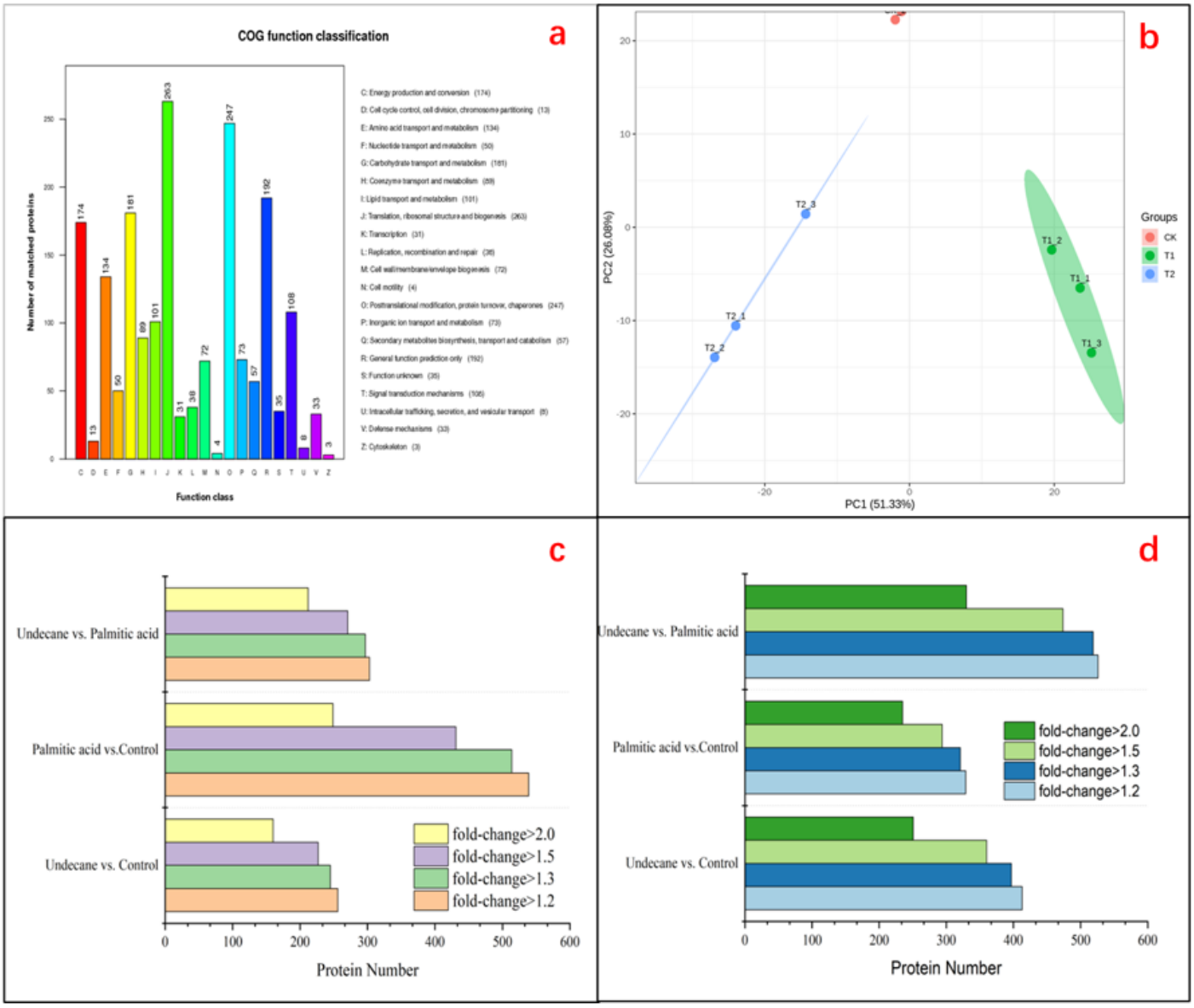

Figure 2

The proteomic analysis of clusters of orthologous genes (COG) (a) and PCA of the difference proteins under control, undecane and palmitic acid treatments (b). Difference protein analysis results (up-regulated change (c) and down-regulated change (d)) after 10-day treatment of control, undecane and palmitic acid, respectively.

(Note, T1, undecane treatment; T2, palmitic acid treatment; and CK, control.)

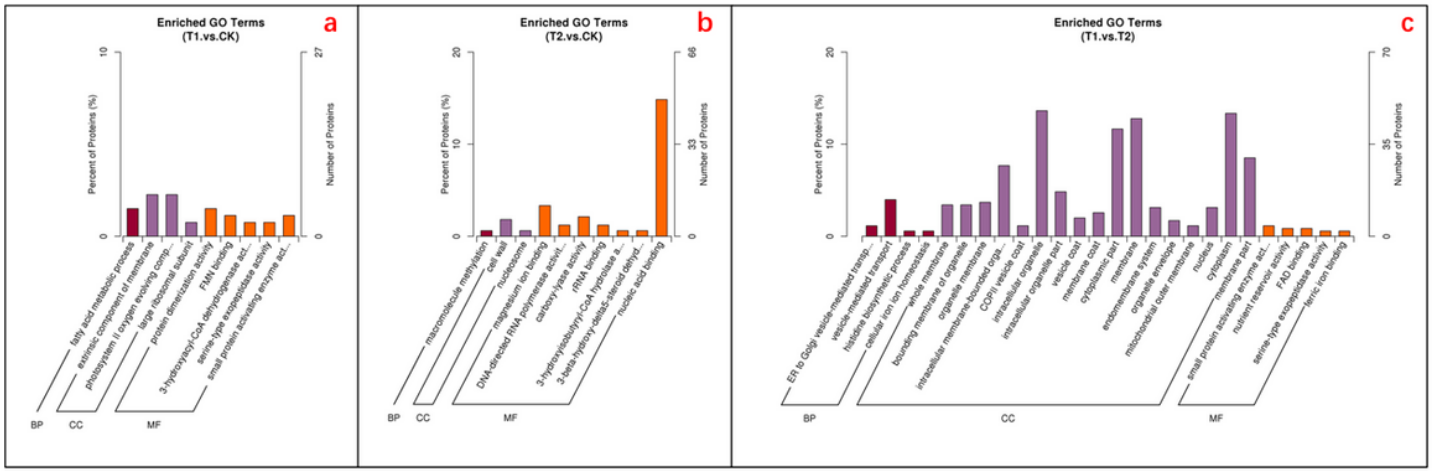

Figure 3

Gene Ontology (GO) functional proteins difference with treatments of undecane vs control (a); palmitic acid vs control (b); and undecane vs palmitic acid (c), respectively.

(Note, BP, biological process; CC, cellular composition; MF, molecular function; T1, undecane treatment; T2, palmitic acid treatment; and CK, control.)

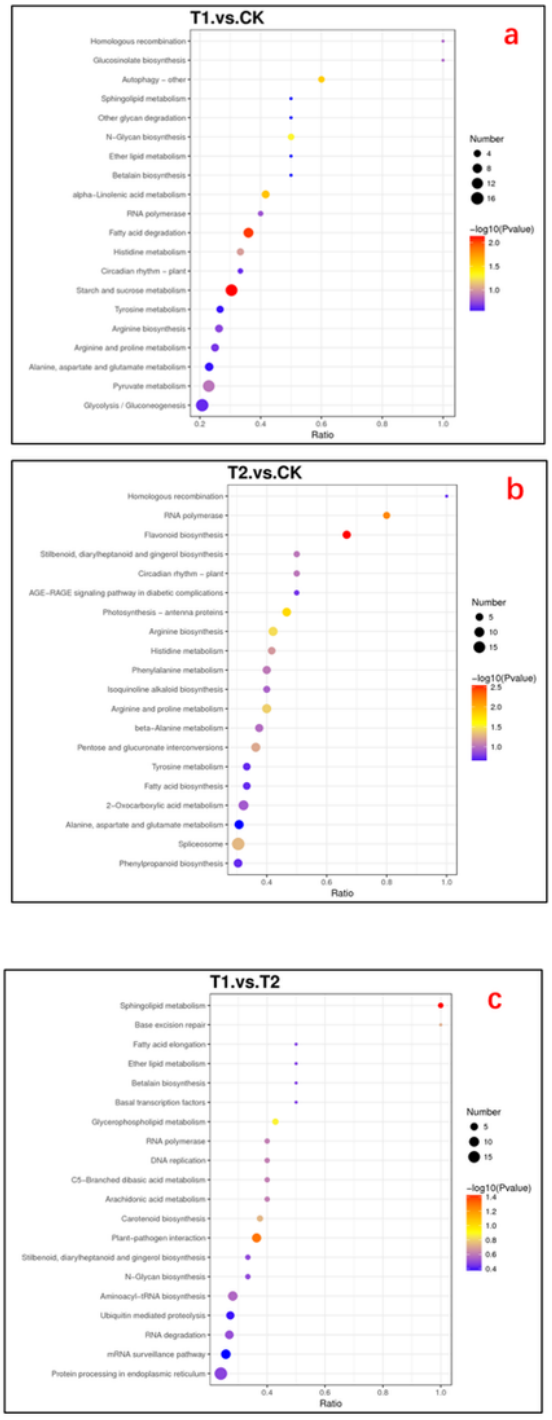

Figure 4

Enrichment bubble plot of KEGG metabolism pathways differences among treatments of undecane vs control (a); palmitic acid vs control (b); and undecane vs palmitic acid (c), respectively.

(Note, T1, undecane treatment; T2, palmitic acid treatment; and CK, control.)

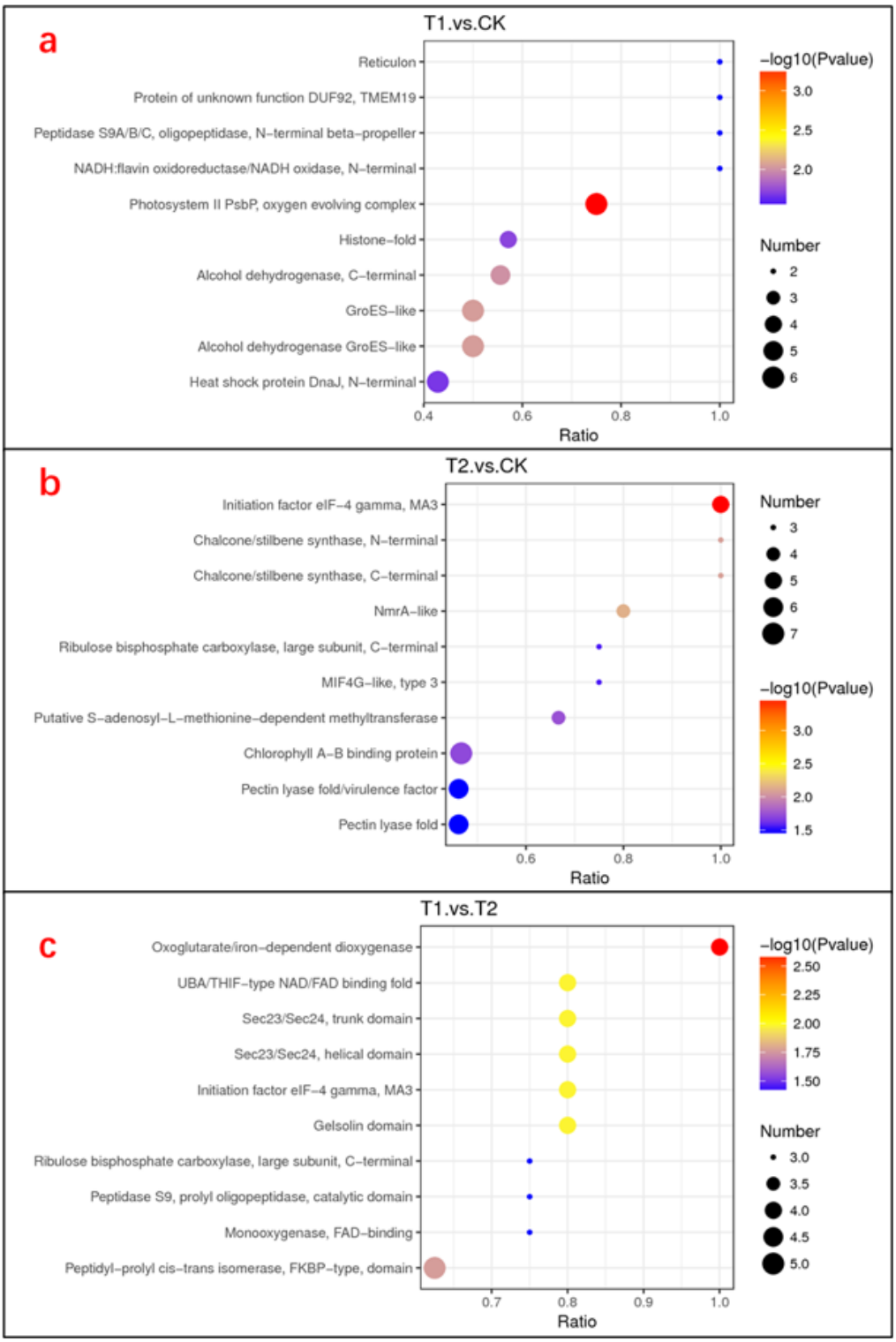

Figure 5

Enrichment bubble plot of structural domain differences among treatments of undecane vs control (a); palmitic acid vs control (b); and undecane vs palmitic acid (c), respectively.

(Note, T1, undecane treatment; T2, palmitic acid treatment; and CK, control.)

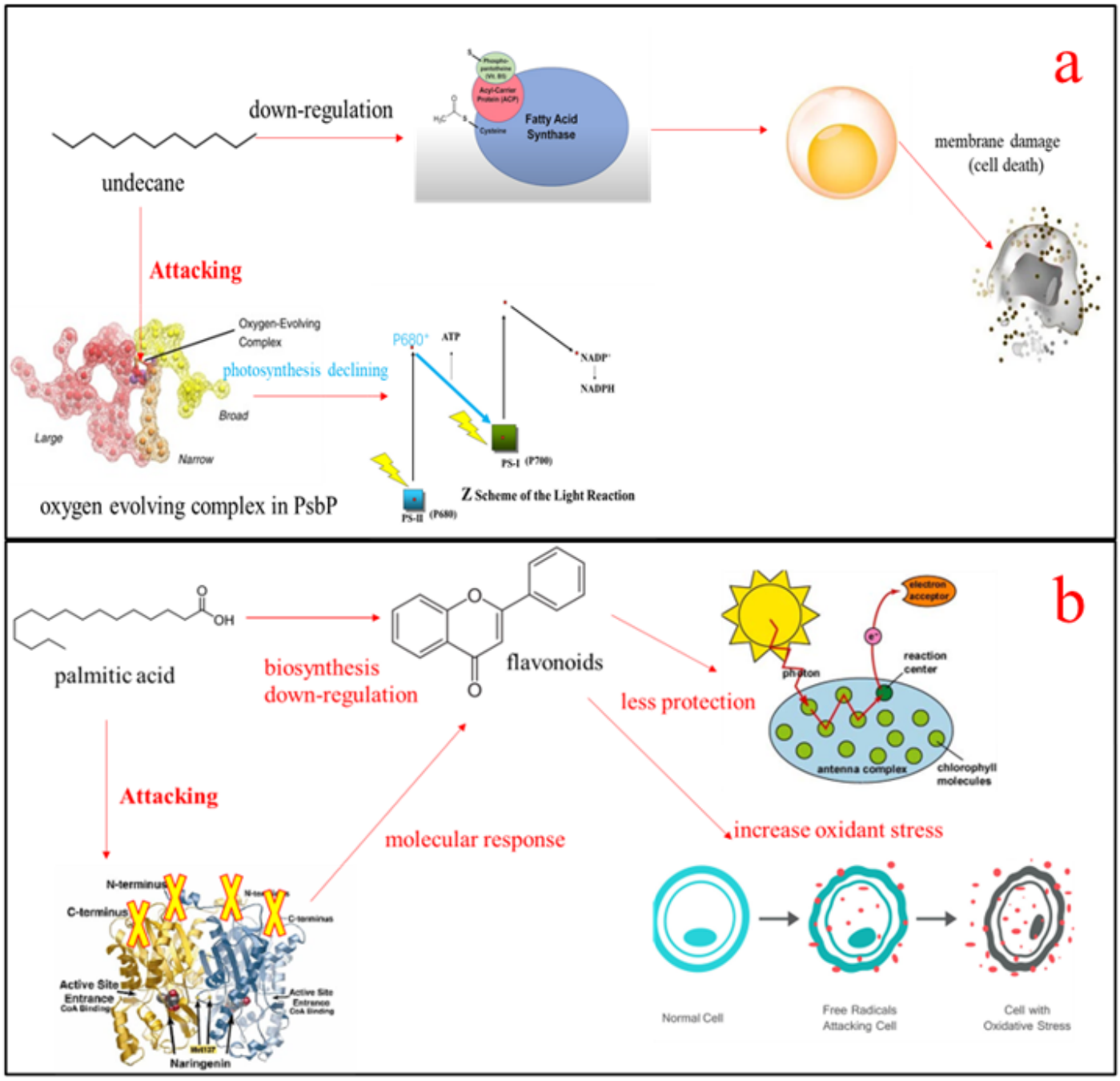

Figure 6

Potential Mechanisms of allelochemical specific toxicity pathways of major root exudates on native plants caused by invasive species.

Supplementary Files

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