



Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.journals.elsevier.com/spectrochimica-acta-part-a-molecular-and-biomolecular-spectroscopy

Use of ATR-FTIR spectroscopy for analysis of water deficit tolerance in *Physalis peruviana* L

Romeu da Silva Leite^{a,b,c,*}, Marilza Neves do Nascimento^a, Salvador Hernández-Navarro^b, Norlan Miguel Ruiz Potosme^d, Sivakumaran Karthikeyan^e

^a Biological Sciences Department, State University of Feira de Santana, 44036-900 Feira de Santana, Bahia, Brazil

^b Agriculture and Forestry Engineering Department, Universidad de Valladolid, 34004 Palencia, Castilla y Leon, Spain

^c Baiano Federal Institute of Science and Technology, Campus Xique-Xique, 47400-000 Xique-Xique, Brazil

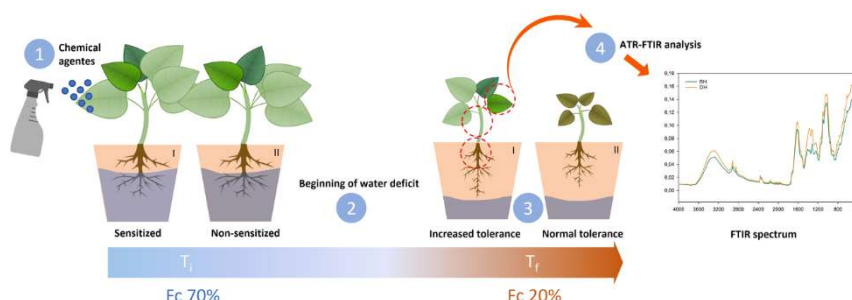
^d Superior Polytechnic School, European University Miguel de Cervantes, 47012 Valladolid, Castilla y Leon, Spain

^e Department of Physics, Dr. Ambedkar Government Arts College, 600039 Chennai, Tamil Nadu, India

HIGHLIGHTS

- ATR-FTIR spectral analysis was used in monitoring the biochemical changes due to *Physalis peruviana* L. under water deficit conditions.
- Chemical priming was used for increasing water deficit tolerance.
- Water deficit promotes alterations in the functional groups, mainly lipids, proteins and carbohydrates.
- The application of pretreatment with sodium nitroprusside results in lipids and protein changes under water deficit conditions which are studied from ATR-FTIR spectra.
- The ATR-FTIR technique is able to assisting in the selection of priming treatments to increase tolerance to water deficit.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Water stress
Water deficit mitigation
Chemical priming
Fourier transform infrared spectroscopy
Goldenberry

ABSTRACT

Treatments that allow plants to better tolerate water deficit become essential, such as the application of chemical priming. In addition, it is essential to use analyses capable of measuring these effects at the biomolecular level, complementing the other physiological evaluations. In view of the above, this study aimed to evaluate the use of attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy for analyses of water deficit tolerance in *Physalis peruviana* plants. For this, samples of leaves, stems and roots of plants subjected to different pretreatments with proline (10 mM and 20 mM), sodium nitroprusside (SNP 25 μ M and 50 μ M) and H₂O as control, aiming at increasing tolerance to water deficit, were evaluated. The chemical agents used attenuated water deficit in *P. peruviana* plants, influencing phenotypic characterization and spectral analyses. Analysis of FTIR spectra indicates that different functional groups present in leaves, stems and roots were influenced by water deficit and priming treatments. Changes in lipid levels contributed to reducing water losses by increasing the thickness of cuticular wax. Accumulation of proteins and carbohydrates promoted osmoregulation and

* Corresponding author at: Biological Sciences Department, State University of Feira de Santana, 44036-900 Feira de Santana, Bahia, Brazil.

E-mail address: romeu.silva@ifbaiano.edu.br (R. da Silva Leite).

<https://doi.org/10.1016/j.saa.2022.121551>

Received 21 March 2022; Received in revised form 14 June 2022; Accepted 20 June 2022

Available online 25 June 2022

1386-1425/© 2022 Elsevier B.V. All rights reserved.

maintenance of the water status of plants. Thus, water deficit causes changes in the functional groups present in the organs of *P. peruviana*, and the ATR-FTIR technique is able to detect these biomolecular changes, helping in the selection of priming treatments to increase tolerance to water deficit.

1. Introduction

Plants are sessile organisms and, as such, need to respond and adapt to severe environmental conditions to survive [1]. The main abiotic stresses that affect the performance of cultivated plants and plants in their natural environments are saline conditions, heavy metals, nutritional deficiency, temperature, pollution and excess or lack of water. Of these, water deficit is the main limiting factor of agricultural production in the world, especially in a scenario of constant climate change [2,3].

One of the mechanisms of protection from adverse conditions is the ability of plants to sense stress factors and respond appropriately, through the activation of signaling pathways [4]. As a final response to a certain stress, such as water deficit, there may be stomatal closure [5] and accumulation of osmoprotectants [6]. However, the reduction of photosynthesis [7] and the accumulation of reactive oxygen species [8] in stressed plants compromise their final performance.

Technologies have been developed to increase plant tolerance and/or reduce the effects of water deficit, such as chemical priming. This technique, which can be used in species considered underexploited, such as *Physalis angulata* L. [9], is based on the pretreatment of plants through the application of chemical compounds such as proline and nitric oxide donors. Priming in plants consists of prior exposure to an abiotic stressor, natural or induced, which activates defense responses more rapidly and better, making the plant more tolerant if exposed to the same stress in the future [10].

The attenuation of water deficit effects promoted by priming can be seen through different mechanisms, ranging from ionic and protein homeostasis to osmoregulation [11]. Other responses are measured to evaluate the degree of acclimatization of plants to stress, such as gas exchange, water potential and biomass accumulation. These analyses, although essential, require a large amount of inputs and labor, making the process time-consuming, requiring the search for other tools to evaluate or complement studies on tolerance to abiotic stresses. In this context, Fourier transform infrared (FTIR) spectroscopy may indicate chemical variability in plant organs in response to water deficit.

FTIR spectroscopy is a versatile tool that quickly and nondestructively determines the molecular composition of plant organs, where major functional groups can be identified, providing structural information on the chemical compounds present, such as lipids, proteins and

polysaccharides [12,13]. It is important to mention that attenuated total reflectance (ATR) with FTIR has been widely used to evaluate the chemical composition of biological samples from a small amount of material [14].

There are no reports of the use of the FTIR technique to evaluate the effect of chemical priming against water deficit in plants. Research shows that FTIR analyses can be used in studies on abiotic stresses [15,16]. In addition, it has already been possible to evaluate changes in biomolecular composition in organs of *Physalis angulata* L. plants grown under different nitrogen doses [17,18]. Plants of the genus *Physalis* are underexploited in Brazil, and the species *Physalis peruviana* L. stands out with growing cultivation and commercialization of fruits in the country, in addition to medicinal potentials [19].

The hypothesis is that *P. peruviana* plants respond differently to different priming treatments and part of these responses may be related to chemical and structural composition in their different organs. If these differences can be detected by ATR-FTIR, it will be possible to use the technique to investigate water stress in plants and identify efficient treatments to increase tolerance to water deficit. Thus, the objective of this study was to evaluate the use of ATR-FTIR spectroscopy for analyses of water deficit tolerance in *Physalis peruviana* plants.

2. Material and methods

2.1. Plant material

The analyses were carried out at the *Escuela Técnica Superior de Ingenierías Agrarias*, University of Valladolid, Palencia Campus, Spain. For this, plant samples from an experiment carried out at the State University of Feira de Santana were sent to the university of destination following the protocols for sending plant material abroad (art. 24, Decree 8.772/2016, which deals with access to genetic heritage and the distribution of benefits for conservation and sustainable use of biodiversity).

Samples of an experiment carried out in 2020 with the species *Physalis peruviana* were evaluated. This experiment was conducted to evaluate the use of sodium nitroprusside (SNP) and proline (Pro) as possible agents of chemical priming against water deficit, in addition to distilled water as control (H₂O). The following treatments were

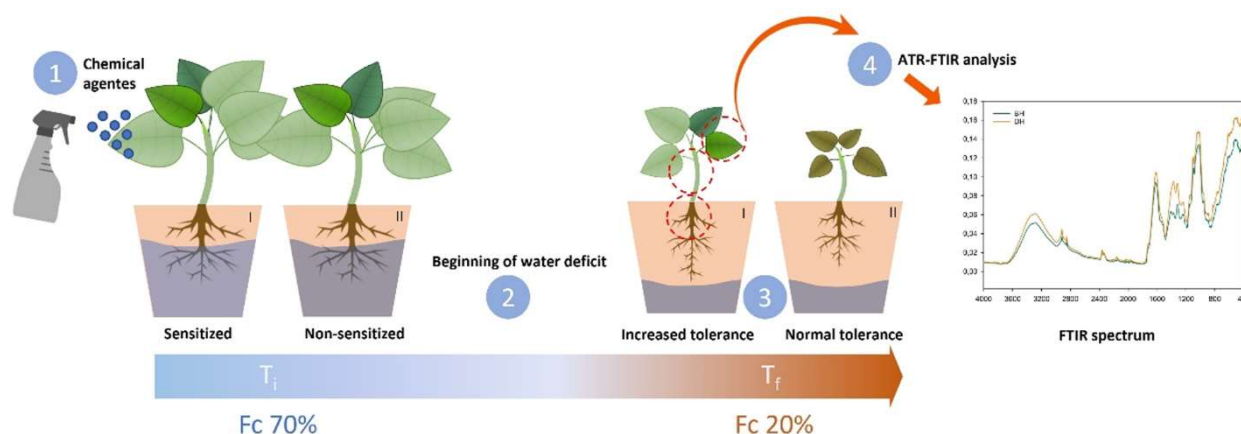


Fig. 1. Application of chemical priming in *Physalis peruviana* plants: (1) chemical agents were sprayed on plants grown at pot field capacity (Fc) of 70%, (2) followed by the beginning of water deficit; (3) chemical agents promoted increased tolerance to water deficit and (4) spectral analyses of different organs may identify biomolecular changes responsive to priming and tolerance to water deficit. Adapted from Leite et al. (2021 a).

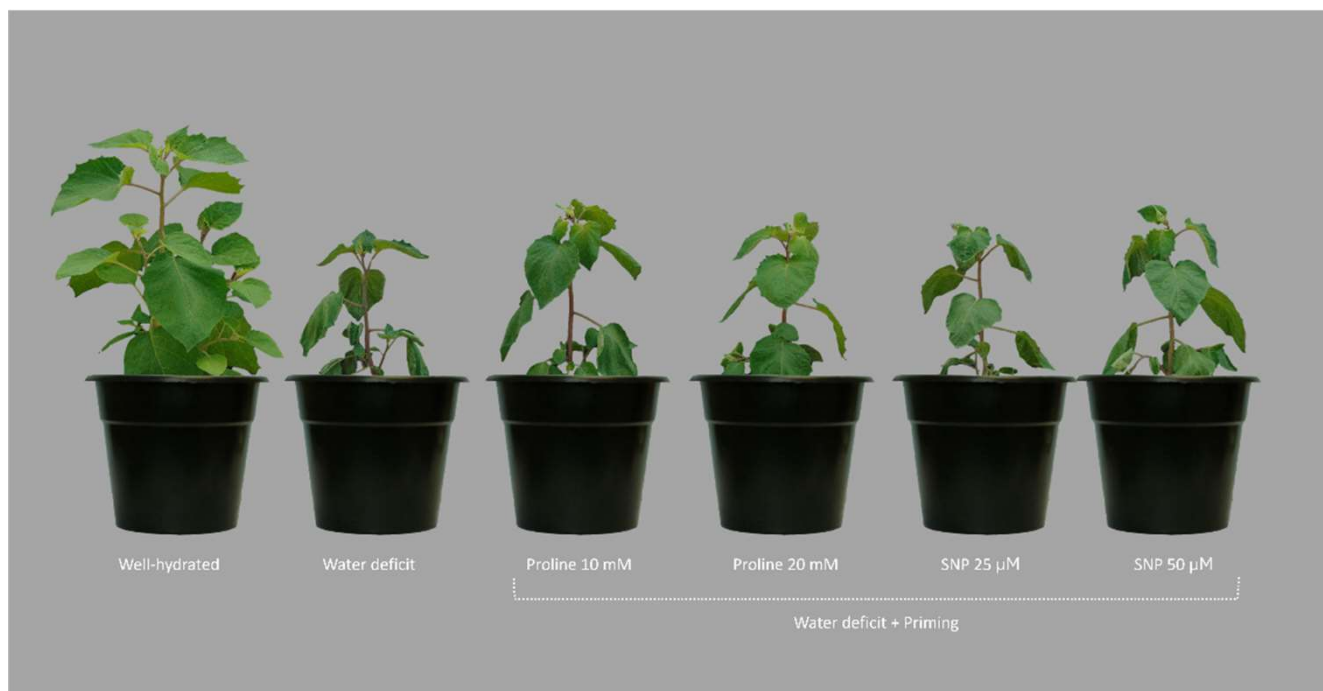


Fig. 2. Visual symptoms of the effects of treatments on *Physalis peruviana* plants.

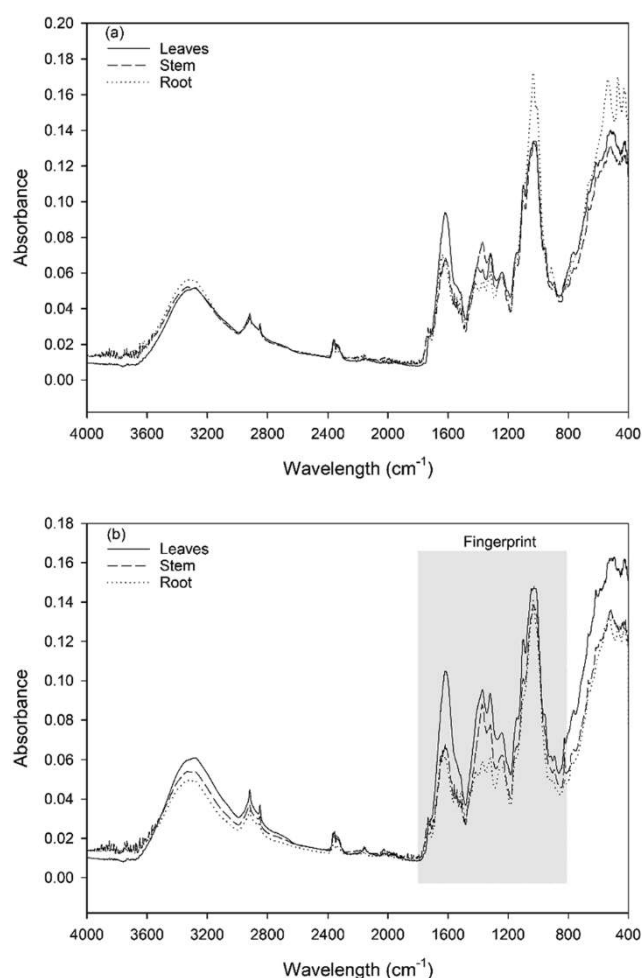


Fig. 3. Fourier transform infrared spectra for different organs of *Physalis peruviana*: (a) well-hydrated plants and (b) plants under water deficit.

evaluated in *P. peruviana* plants: plants grown at field capacity (Fc) of 70% + foliar spraying with H₂O (T1); Fc 20% + H₂O (T2); Fc 20% + Proline 10 mM (T3); Fc 20% + Proline 20 mM (T4); Fc 20% + SNP 20 μM (T5); Fc 20% + SNP 50 μM (T6), totaling 6 treatments.

Soil water storage capacity (Fc) was calculated based on a test described by Leite et al. [9] and Bonfim-Silva et al. [20]. The pots used were filled with 8 kg of air-dried soil and then saturated and suspended to drain water excess. The drainage time was 48 h, and maximum water storage capacity was determined based on the difference of weights. The evapotranspiration of each pot was determined based on weight, by using an electronic scale (5 g of precision).

The plants were sprayed with 100 mL m⁻² of the different substances at 10 days after transplantation (DAT) and were kept under 70 ± 5% Fc for more five days (Fig. 1). Then, water replacement was suspended until the soil reached Fc of 20 ± 5% after 15 days, when the effects of priming on plants exposed to water deficit were evaluated and the different organs (leaves, stem and roots) were collected. The samples were dried in a forced air circulation oven at 60 °C until reaching constant weight.

2.2. Spectral analysis

The samples from the different organs of plants subjected to the treatments were crushed in liquid nitrogen, homogenized in a 1-mm-mesh sieve and analyzed by Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy using the Thermo Nicolet iS50 spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA). The 1D spectra were recorded in the mid-infrared (4000–400 cm⁻¹) with spectral resolution of 4 cm⁻¹ [17] and 64 scans per sample. The 1D spectra were analyzed individually to identify the distinct absorption peaks of the functional groups present in the plant organs and the influence of the evaluated treatments.

For the leaf samples, the degree of methyl esterification and calcium oxalate were calculated from the band area according to Lionetti; Cervone; Bellincampi [21]. For the band area ratios of lipids, amide I and carbohydrates, the following values were used: lipid (3000–2800)/amide I (1705–1575), lipid (3000–2800)/Carbohydrates (1200–900).

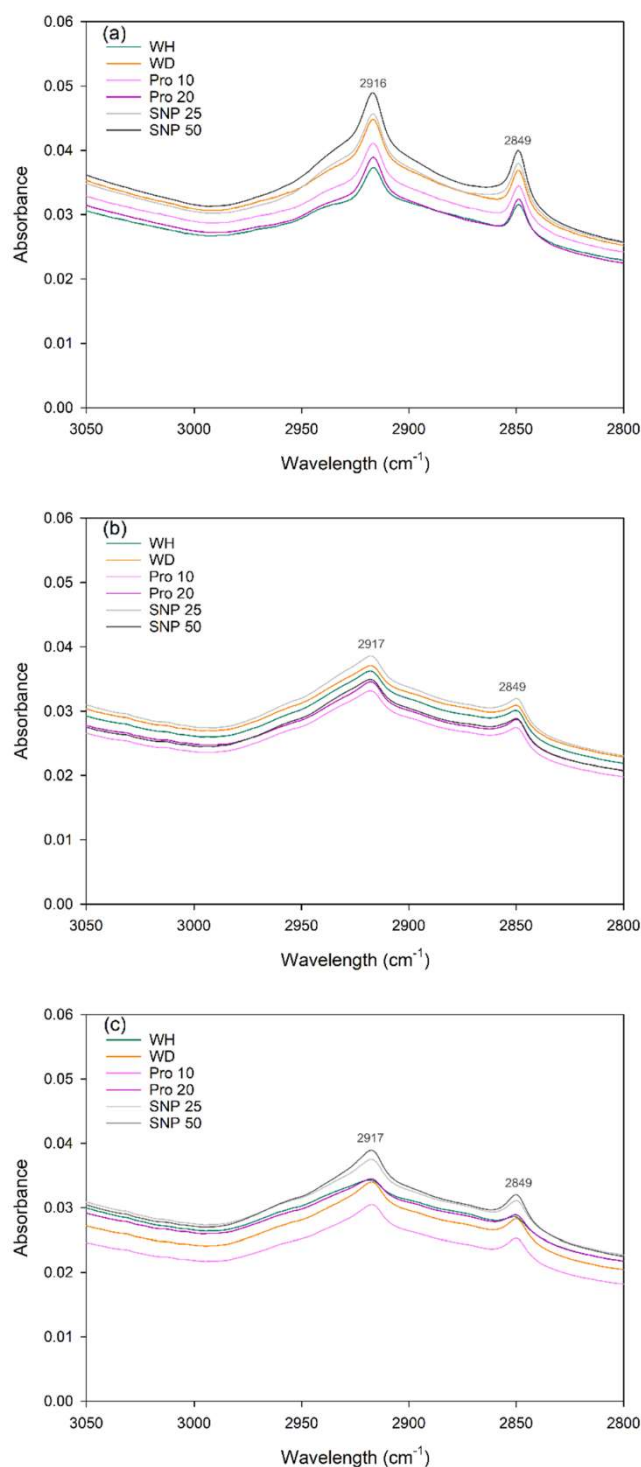


Fig. 4. Fourier transform infrared spectra in different organs of *Physalis peruviana* in the lipid region: (a) leaves, (b) stem and (c) roots. WH – well-hydrated; WD – water deficit.

2.3. Statistical analysis

Four replicates of the plant organs (root, stem and leaf) were evaluated for each treatment used. The vibrational data were analyzed using SIGMAPLOT 14.0 software (Systat Software Inc., Chicago, USA). The comparison was performed relative to spectral peaks and the analysis to the corresponding functional group.

For the area band ratios, the data were subjected to analysis of variance at 5% significance level and expressed with mean $\pm$ standard

error (SE). Tukey test was used, and the data obtained from the analyzed parameters were statistically evaluated using Sisvar 5.6 statistical software.

Principal component analysis (PCA) was performed with SPSS 16.0 software. FTIR data were centered on the mean, with second derivative and vector normalized before being subjected to PCA analysis. PCA output consisted of scores and loadings and explains the maximum variation of the data present in the samples. The scores were plotted to show the variation of FTIR spectra [22].

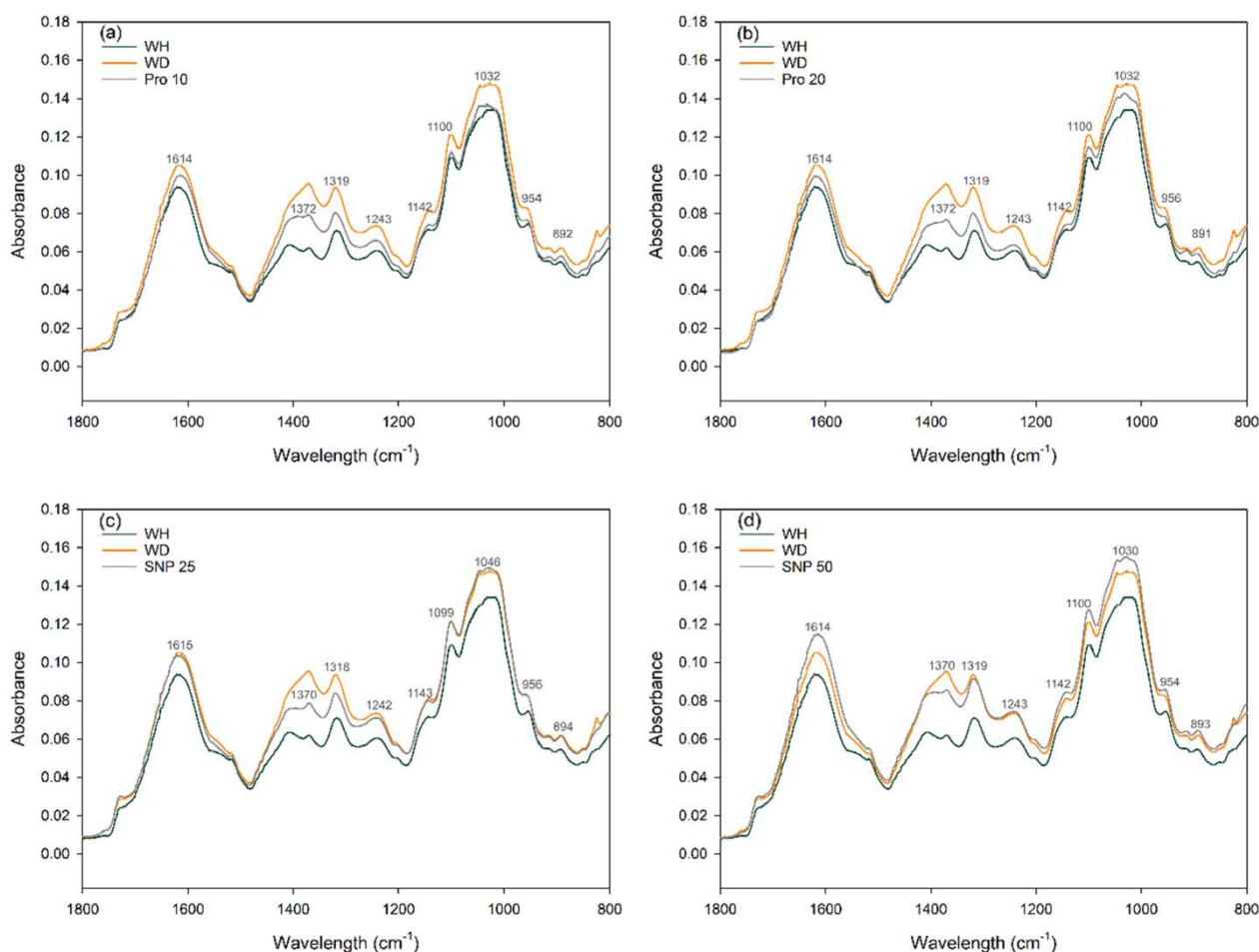


Fig. 5. Fourier transform infrared spectra of *Physalis peruviana* leaves in the fingerprint region: (a) proline 10 mM, (b) proline 20 mM, (c) SNP 25 μ M and (d) SNP 50 μ M. WH – well-hydrated; WD – water deficit.

3. Results and discussion

3.1. Visual symptoms

The chemical agents used attenuated water deficit in *Physalis peruviana* plants, influencing phenotypic characterization. In plants not sensitized by chemical agents, water deficit resulted in wilting and leaf abscission, highlighting the negative effects characteristic of this stress. Nevertheless, mitigating effects were observed in plants pretreated with Pro 20 mM, SNP 25 μ M and SNP 50 μ M, evident by the maintenance of leaf turgor and color. It is important to mention that there was no expression of phenotypic characteristics of toxicity of the substances at the concentrations used (Fig. 2).

Maintenance of hydration of plant tissues under water deficit conditions is a desired characteristic for increasing the efficiency of agricultural crops. Recent research shows that SNP acts on leaf water potential and accumulation of sugars in leaves [7], increasing plant tolerance to water deficit through osmoregulation and antioxidant system [23]. Similarly, the application of proline acts to improve water use efficiency [24] and photosynthetic activity [25]. In addition, SNP spraying promotes an increase in chlorophyll content in *Physalis angulata* plants [9], which supports the phenotypic observations.

3.2. Spectral analysis

The analysis of FTIR spectra indicates that different functional groups present in leaves, stems and roots were influenced by water deficit and priming treatments. Fig. 3 shows the spectra of the organs of

well-hydrated plants and plants under water deficit without pre-treatments. The highest absorption peaks for leaves, stems and roots occurred between wavelengths from 3500 cm^{-1} to 3000 cm^{-1} , related to the OH and NH stretching [26], between 2920 cm^{-1} and 2800 cm^{-1} , corresponding to polysaccharides and lipids [27,28] and between 1800 cm^{-1} and 800 cm^{-1} , the fingerprint region [17]. The observed peaks are shared between well-hydrated plants (Fig. 3a) and plants under water deficit (Fig. 3b).

The use of infrared spectra to evaluate biomolecular changes in plants under water deficit is still scarce. However, FTIR spectroscopy is a sensitive and nondestructive analytical tool that is useful for investigating the chemical composition of biological systems [29] such as the different organs of plants. It is possible to notice variations in absorbance between the spectra of leaves, stems and roots, as well as between the conditions of soil water availability (Fig. 3). The technique has also been used in other studies with abiotic stresses, such as in different doses of nitrogen in the cultivation of *Physalis angulata* L. and *Physalis peruviana* L. [18], water deficit in *Arabidopsis thaliana* L. genotypes [16] and salinity in *Glycine max* L. [30].

Lower absorbance peaks at wavelengths of 2916 cm^{-1} and 2849 cm^{-1} were observed in leaves of well-hydrated plants and are attributed to asymmetric and symmetric stretching vibrations of CH_2 [31], corresponding to lipids. On the other hand, there was an increase in the average absorbance of the spectra of plants under water deficit and when the pretreatments were applied (Fig. 4 a), which may indicate greater thickness of cuticular wax [32] and, consequently, less possibility of water losses. Both treatments with proline (10 mM and 20 mM) remained with intermediate levels of absorbance between well-hydrated

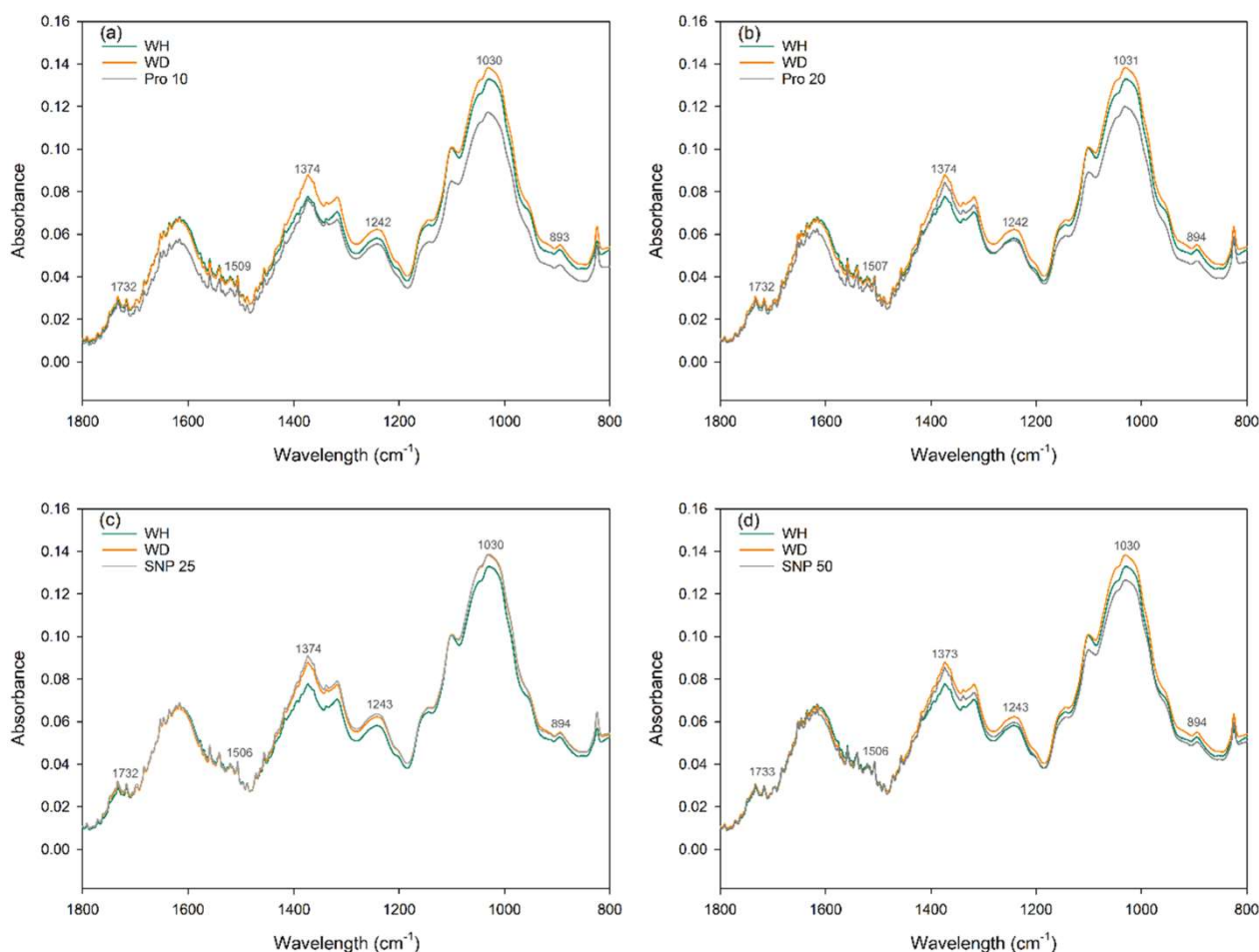


Fig. 6. Fourier transform infrared spectra of *Physalis peruviana* stems in the fingerprint region: (a) proline 10 mM, (b) proline 20 mM, (c) SNP 25 μM and (d) SNP 50 μM . WH – well-hydrated; WD – water deficit.

plants and plants under water deficit without pretreatment. Plants sprayed with SNP (25 μM and 50 μM) showed higher absorption peaks for this spectral region.

Both proline doses and the SNP dose of 80 μM promoted changes in the stem spectra (2917 cm^{-1} and 2849 cm^{-1}), leading to the lowest mean absorptions (Fig. 4 b) for the lipid region. The highest absorption peaks were observed in plants sprayed with SNP 25 μM . For root spectra (2917 cm^{-1} and 2849 cm^{-1}), the highest absorbances were observed in plants sprayed with SNP 25 μM and 50 μM , respectively (Fig. 4 c). Spectral changes at lipid level have been reported in *Zea mays* L. plants [28] and *Triticum aestivum* L. [33] under water deficit conditions.

The use of the different priming treatments altered the spectra of leaves in the fingerprint region (Fig. 5). In the region of the functional group amide I (1614 cm^{-1}) [18], strong peaks were observed for the absorbance values in the treatments Pro 10 mM, Pro 20 mM and SNP 25 μM , which remained intermediate between the values for well-hydrated plants and plants under water deficit without pretreatment. The highest peak of absorbance was observed in plants sprayed with SNP 50 μM (Fig. 5 a). In mid-infrared spectra, the mode amide I (C=O stretching) is attributed to proteins [26].

The peaks observed at the wavelengths 1370 cm^{-1} , 1318 cm^{-1} , 1242 cm^{-1} , or close to them, are attributed to the carboxylate and pectin group [18] and the spectra showed similar performance to that of the functional group of amide I. Other peaks related to pectin appeared close to 956 cm^{-1} , corresponding to galactose [34]. Peaks located near 1099 cm^{-1} (C-O stretching vibration) are characteristic of all carbohydrates [35]. The highest peak of absorbance for this functional group was observed in the spectrum of plants sprayed with SNP 50 μM , which may

indicate greater accumulation of carbohydrates in leaves (Fig. 5 d).

Polysaccharides exhibit a band around 1030 cm^{-1} due to O-H and C-OH stretching, such as cellulose [26,32]. Weak peaks were observed at the wavelength of 893 cm^{-1} , also characteristic of cellulose [36] (Fig. 5). The accumulation of solutes within the cell, such as carbohydrates and amino acids, reduces the osmotic component of the water potential and consequently maintains turgor pressure [37], in a physiological process called osmoregulation. Carbohydrates accumulate in plants in the form of soluble molecules such as sucrose, hexoses, alcohols and oligosaccharides [38] and, in addition, structural carbohydrates such as cellulose and lignin accumulate [39].

Cellulose and lignin are the two main structural carbohydrates of cell walls in vascular plants and are components responsible for stem resistance [40]. The application of priming treatments also altered the infrared spectra of stems (Fig. 6). The peaks observed at wavelengths of 1732–1733 cm^{-1} are attributed to the ester C=O stretching, such as phospholipids, hemicellulose, pectin, lignin, and suberin and cutin esters [26], with higher absorbance for plants treated with SNP 25 μM and SNP 50 μM (Fig. 6 c; 6 d). These results are similar to those observed for the peak at 1506 cm^{-1} , attributed to lignin.

Peaks at wavelengths of 1374 cm^{-1} , 1030 cm^{-1} and 893 cm^{-1} are characteristic of cellulose [26,32,36]. The highest absorbance values were observed for plants under water deficit and for those pretreated with SNP 25 μM (Fig. 6 c). The other treatments had spectra with intermediate absorbance between plants under water deficit and well-hydrated plants, or values lower than theirs. Peaks close to 1242 cm^{-1} were attributed to the C=O stretching, relative to the pectic substances, lignin, hemicellulose, and suberin and cutin esters [26]. Thus, even

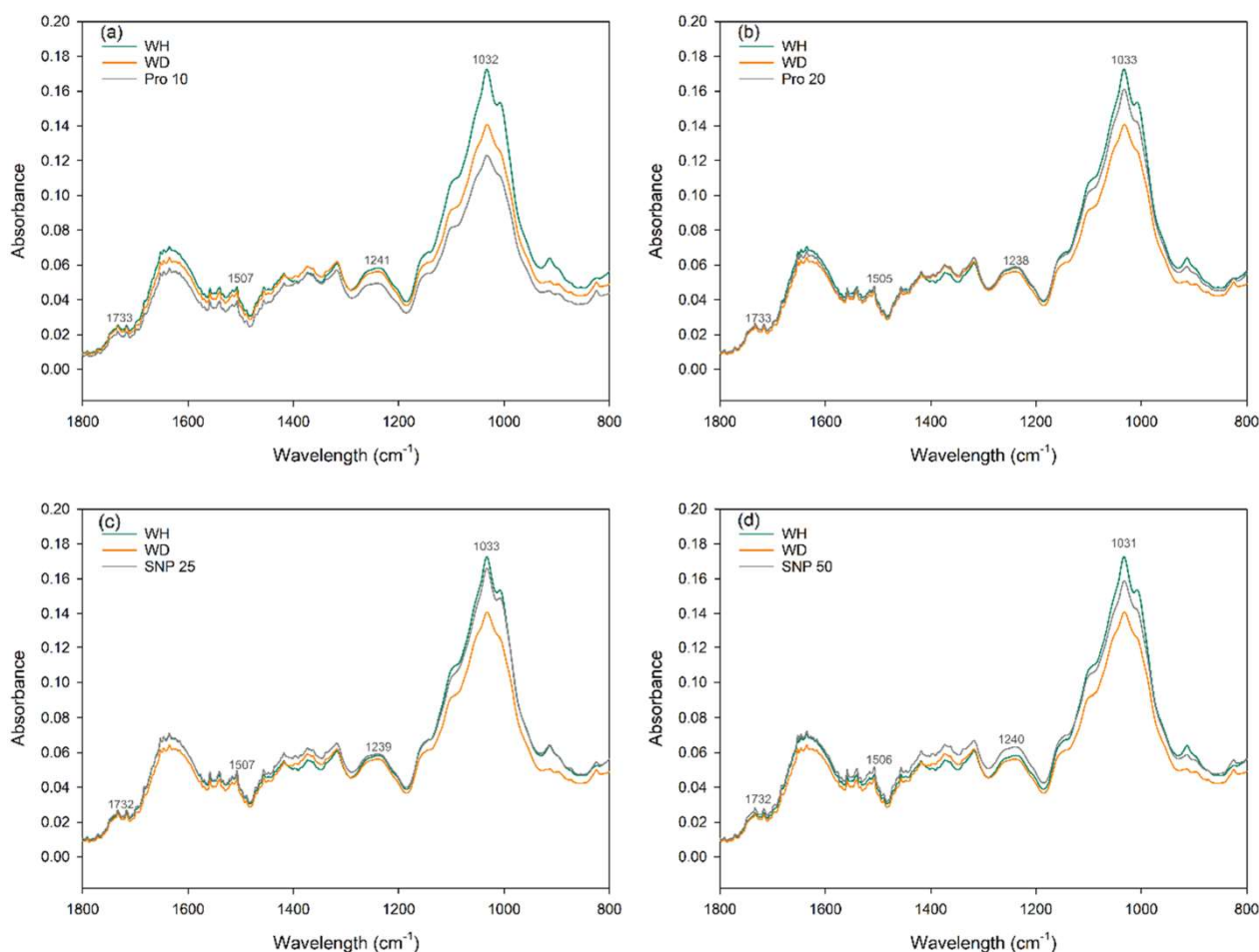


Fig. 7. Fourier transform infrared spectra of *Physalis peruviana* roots in the fingerprint region: (a) proline 10 mM, (b) proline 20 mM, (c) SNP 25 μ M and (d) SNP 50 μ M. WH – well-hydrated; WD – water deficit.

under water deficit conditions, the *Physalis peruviana* genotype used was able to allocate structural carbon (C), pointing to a possible tolerance characteristic.

The infrared spectra obtained from root samples showed different responses from those found in leaves and stems (Fig. 7). The highest absorbances were observed in roots of well-hydrated plants or in those under water deficit with application of priming treatments. For the peaks of average intensity around 1733 cm^{-1} , 1507 cm^{-1} and 1241 cm^{-1} or close to them, which are characteristic of lignin, the highest absorbance values were observed in roots of plants treated with Pro 20 mM, SNP 25 μ M and SNP 50 μ M, with reduction of these values in Pro 10 mM and in plants under water deficit without pretreatment.

Strong peaks at wavelength of 1032 cm^{-1} are responsive to cellulose, with higher values for plants grown under well-hydrated conditions (Fig. 7). The lowest absorbance values were recorded for plants under water deficit without priming and sprayed with Pro 10 mM (Fig. 7 a). The treatments Pro 20 mM, SNP 25 μ M and SNP 50 μ M remained with intermediate values, indicating that they acted in the improvement of tolerance to water deficit (Fig. 7 b; 7c; 7 d), since the reduction in absorbance relative to lignins and cellulose may be related to the reduction of C allocation for structural growth, such as reduction in the length of plant organs [41]. Root growth reduction has been reported in studies with plants under water deficit conditions [42,43].

The peaks found for the different organs of *Physalis peruviana* at 824 cm^{-1} can be attributed to the epoxy group [44] and those found at 780 cm^{-1} to N-H oscillation, primary and secondary amide and nitrate group (Fig. 8). It can be noted that for the functional group of epoxides the highest peaks were observed in stem spectra (Fig. 8 b). Nitrate peaks

alternated between weak peaks for leaves and medium peaks for stem and roots. For roots, the absorbance values of plants pretreated with Pro 20 mM, SNP 25 μ M and SNP 50 μ M were intermediate between those of well-hydrated plants, with higher average absorbance value, and plants under water deficit without pretreatment (Fig. 8 c). The medium peaks for the nitrate group in the roots may be related to the fact that this organ is the main site of nitrate reductase activity (NRA), as observed in *Physalis angulata* plants [45].

3.3. Frequency assignment and the band area of leaves

Table 1 shows the measured band area for peak assignments of *Physalis peruviana* leaves with various treatments. It is observed that significant reduction in CH_2 asymmetric stretching lipids due to SNP treatments (SNP 25 μ M and SNP 50 μ M) with respect to both T1 (well hydrated plants) and T2 (plants under water deficit). The CH_2 symmetric stretching lipids show similar trends having a significant reduction due to both SNP treatments. This shows disordering in lipids and fatty acids due to changes in the hydrocarbon chain [46].

Amide I bands dominating in 1635 cm^{-1} are used for protein change conformation. A decrease/increase in the band area is related to protein changes. Here significant ($p < 0.01$) increase in band area is observed due to SNP treatments. In the case of Pro 10 mM and 20 mM treatments, the reduction in proteins is observed showing SNP treatments (SNP 25 μ M and SNP 50 μ M) better improved the plant productivity. The cell wall polysaccharides have better improvement for the SNP 50 μ M compared to water deficit plant treatment (T2). The band 1249 cm^{-1} due to pectin shows a significant reduction due to treatments. Pectin is a

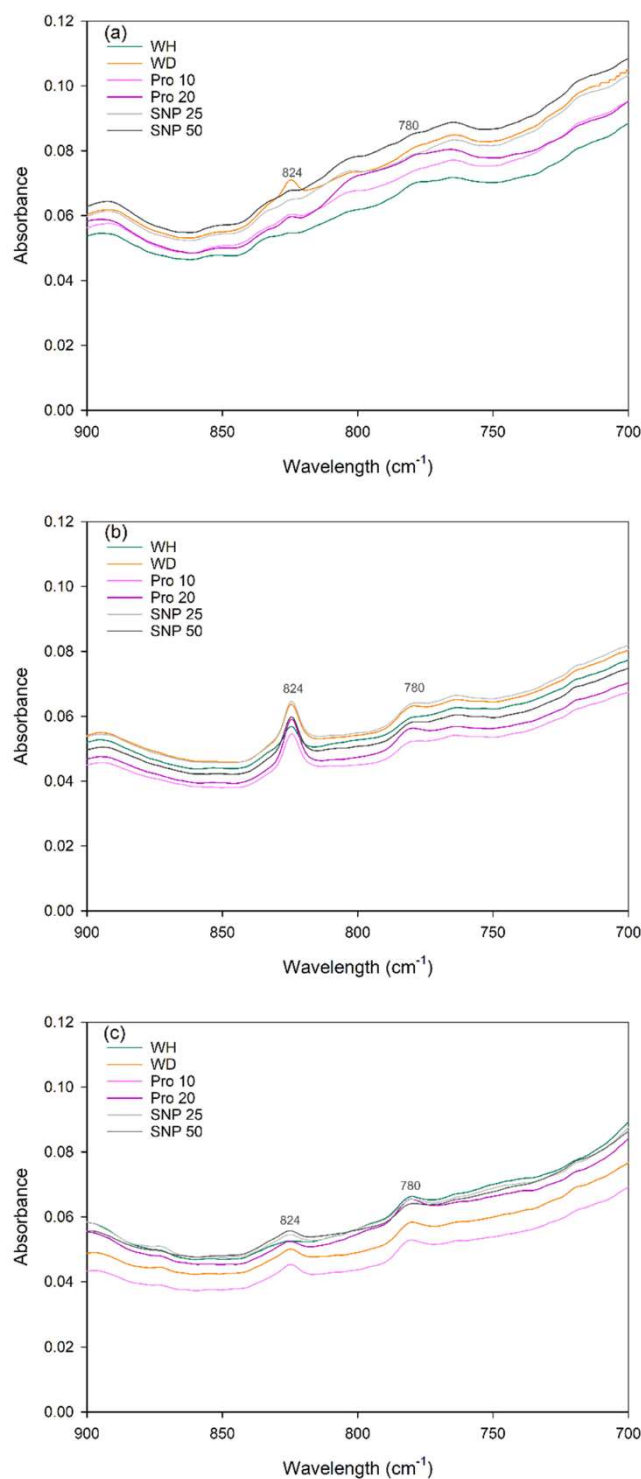


Fig. 8. Fourier transform infrared spectra in different organs of *Physalis peruviana* in the region from 900 to 700 cm^{-1} : (a) leaves, (b) stem and (c) roots. WH – well-hydrated; WD – water deficit.

component of the cell walls of plants, it functions in cell adhesion and wall hydration, and pectin crosslinking influences wall porosity and plant morphogenesis [47]. Also, the C = O stretch of pectin substances, lignin, and hemicellulose correspond to 1249 cm^{-1} has an increase in band area due to SNP 25 μM (T5) and SNP 50 μM (T6) treatments. These are a large family of cell wall compounds that binds to cellulose microfibrils which leads to the strength of cell plant cell walls [48]. The increase in band area supports our finding as shown in Table 1. In

addition, the accumulation of carbohydrates in plants under water deficit may be related to osmoregulation, which was potentiated with treatments with SNP.

The band at 1098 cm^{-1} and 1019 cm^{-1} due to cell wall polysaccharides and Pyranose ring has a significant reduction in band area showing the utilisations of carbohydrate metabolism as an immediate resource of energy due to water deficit during plant growth. But SNP treatments ($P < 0.01$) have better improvement and have good

Table 1

Frequency assignment and the band area of *Physalis peruviana* leaves well-hydrated (T1), water deficit (T2) with proline 10 mM (T3), proline 20 mM (T4) SNP 25 μ M (T5) and SNP 50 μ M (T6).

Wavenumber cm^{-1}	Functional group	Treatments					
		WW	WD	Pro 10 Mm	Pro 20 mM	SNP 25 μ M	SNP 50 μ M
2925	CH ₂ asymmetric stretching lipids	2.49 $\pm$ 0.04	2.49* $\pm$ 0.03	2.45* $\pm$ 0.03	2.8 ^{ac} $\pm$ 0.08	2.19 ^{bc} $\pm$ 0.03	1.24 ^{bd} $\pm$ 0.02
2850	CH ₂ symmetric stretching; mainly lipids	0.35 $\pm$ 0.01	0.36* $\pm$ 0.02	0.36 ^a $\pm$ 0.01	0.24 ^{ab} $\pm$ 0.02	0.27 ^b $\pm$ 0.01	0.33 ^d $\pm$ 0.02
1635	Amide I	22.48 $\pm$ 0.94	20.81 ^a $\pm$ 0.38	19.66 ^{ac} $\pm$ 0.09	19.73 ^{ac} $\pm$ 0.05	20.49 ^b $\pm$ 0.01	21.28 ^d $\pm$ 0.09
1373	C–H symmetric bending of CH ₂ and CH ₃ ; cell wall polysaccharides	0.53 $\pm$ 0.05	0.35 ^a $\pm$ 0.01	0.62 ^d $\pm$ 0.01	0.75 ^{ad} $\pm$ 0.01	0.46 ^c $\pm$ 0.46	0.63 ^{cd} $\pm$ 0.01
1324	Pectin	3.14 $\pm$ 0.06	2.51 ^b $\pm$ 0.07	2.29 ^{bc} $\pm$ 0.01	2.06 ^{bd} $\pm$ 0.01	2.03 ^{bd} $\pm$ 0.01	2.29 ^{bc} $\pm$ 0.01
1249	C = O stretch: pectin substances, lignin, hemicellulose	1.58 $\pm$ 0.04	1.5 ^a $\pm$ 0.05	1.68 ^{ac} $\pm$ 0.02	1.66 ^{ac} $\pm$ 0.03	2.04 ^{bd} $\pm$ 0.02	1.75 ^{bd} $\pm$ 0.04
1098	O–H and C–OH stretch: cell wall polysaccharides	1.60 $\pm$ 0.02	1.21 ^b $\pm$ 0.03	1.44 ^{ac} $\pm$ 0.04	2.17 ^{bd} $\pm$ 0.02	0.96 ^{bd} $\pm$ 0.02	0.65 ^{bd} $\pm$ 0.03
1019	Pyranose ring vibrations Saccharides	26.02 $\pm$ 0.83	18.49 ^b $\pm$ 0.26	18.87 ^b $\pm$ 0.09	23.75 ^d $\pm$ 0.37	22.90 ^{ac} $\pm$ 0.07	20.19 ^{bc} $\pm$ 0.06

^a $p < 0.05$; ^b $p < 0.01$ w.r.t to T1 treatments; ^c $p < 0.05$; ^d $p < 0.01$ w.r.t to T2 treatments; * Not statistically significant; The values are the means $\pm$ S.E for each group (n = 4).

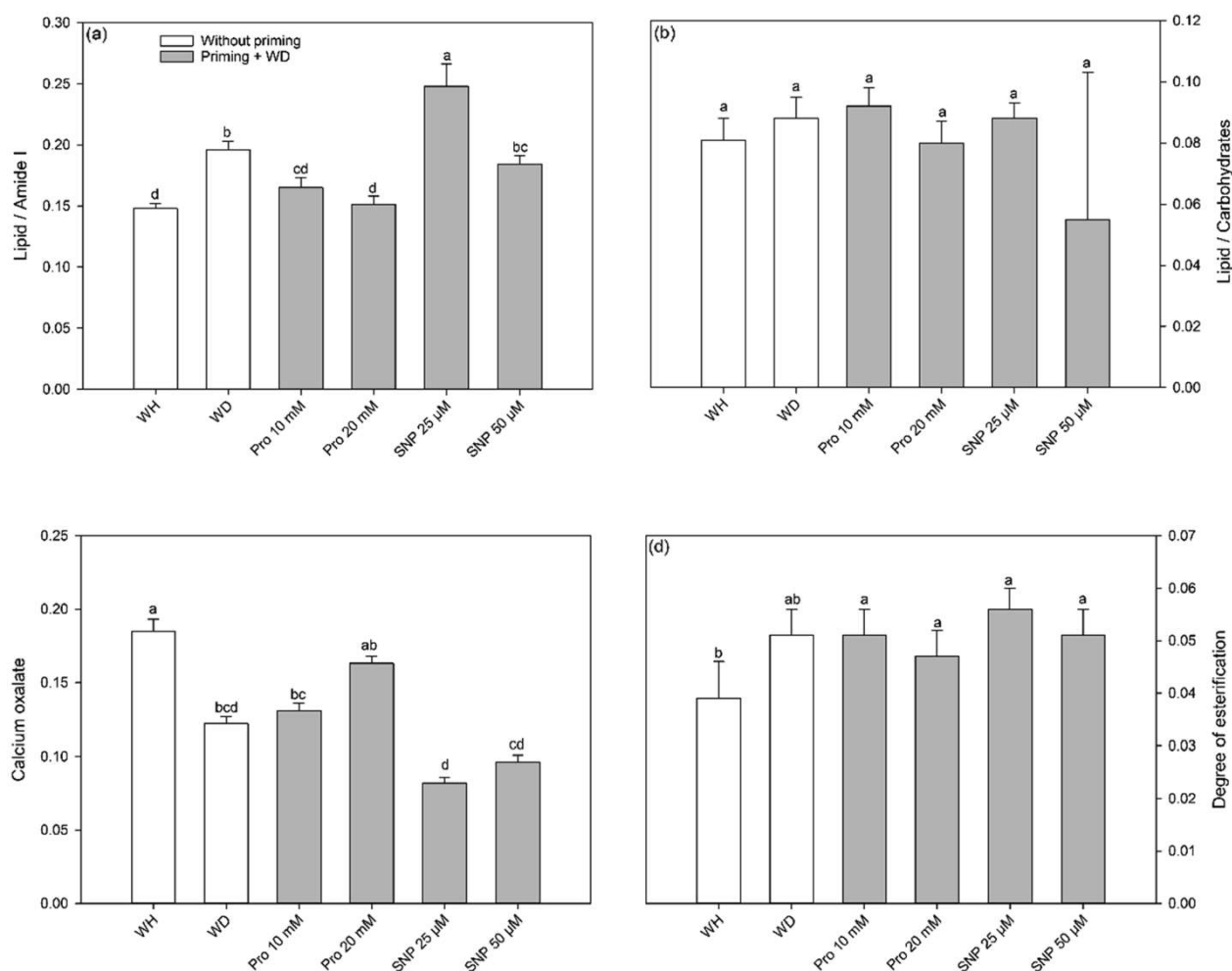


Fig. 9. Band area ratios of lipid/amide I (a), lipid/carbohydrates (b), calcium oxalate (c) and esterification degree (d). WH – well-hydrated; WD – water deficit.

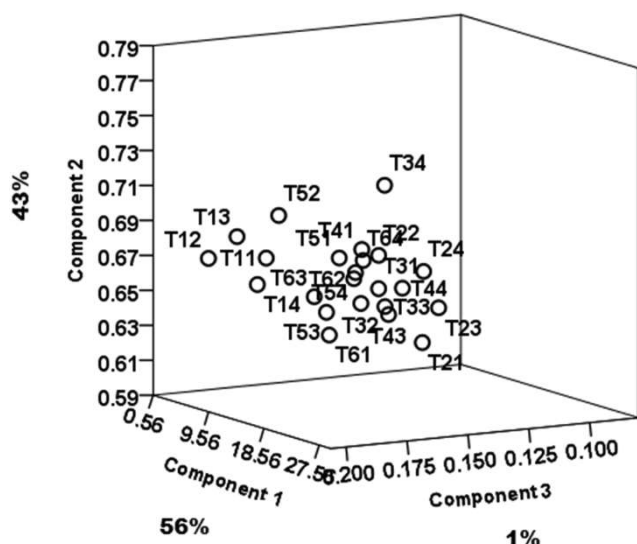


Fig. 10. Principal components analysis of *Physalis peruviana* plants under well-hydrated conditions (T1), under water deficit without pretreatment (T2) and after spraying with proline at 10 mM (T3) and 20 mM (T4) and sodium nitroprusside at 25 μ M (T5) and 50 μ M (T6).

carbohydrate reserves compared with Pro 10 mM and 20 mM treatments. Hence band area measurement clearly shows SNP treatments result in lipid reductions, and increased protein and carbohydrates compared with Pro 10 mM and 20 mM treatments.

Water deficit increased the lipid/amide I ratio in leaves when compared to plants grown under well-hydrated conditions (Fig. 9 a). Application of SNP 25 μ M led to the highest mean value for this ratio, followed by pretreatment with SNP 50 μ M, indicating that the use of this substance at the concentrations mentioned in pretreatment affects the production of lipids and proteins (such as amide I) under water deficit conditions, probably as energy storage products [49]. On the other hand, there were no statistical differences for the lipid/carbohydrate ratio between the treatments evaluated ($p = 0.1769$) (Fig. 9 b).

The highest mean values for calcium oxalate band area were observed in well-hydrated plants and in plants sprayed with Pro 20 mM (Fig. 9 c). Calcium absorption and oxalic acid synthesis are important for ionic balance in the plant and for osmoregulation, through the regulation of excess calcium ions by precipitation with oxalic acid in the form of CaOx crystals [50], which provide an internal reservoir of Ca, as they can be reabsorbed [51]. Changes at the calcium oxalate level from FTIR spectra have been reported in other studies [18,52]. For the degree of esterification, plants subjected to priming treatments showed higher values compared to plants grown under well-hydrated conditions (Fig. 9 d).

3.4. Principal components analysis

Fig. 10 presents the results of the principal components analysis (PCA) obtained for *Physalis peruviana* plants grown under well-hydrated conditions and water deficit treatments. The output of PCA results had three principal components. The first component (PC1) corresponded to 56% variation, the second component (PC2) had 43% variation and the third component (PC3) had at least 2% variation. The results in the graph show that the highest value was obtained for the T1 treatment (well-hydrated plants) followed by the T5 treatment (water deficit + SNP 25 μ M). The lowest values were obtained for treatments T3 (water deficit + Pro 10 mM) and T4 (water deficit + Pro 20 mM). The difference shows that the samples were separated due to biochemical changes of *Physalis peruviana* plants in response to priming treatments against water deficit.

4. Conclusions

Water deficit promotes alterations in the functional groups present in the different organs of *Physalis peruviana*, mainly lipids, proteins and carbohydrates. The use of sodium nitroprusside at 25 μ M and 50 μ M is efficient to mitigate the negative effects of water deficit. The application of pretreatment with SNP results in lipids and protein changes under water deficit conditions which are studied from FTIR spectra. The computed calcium oxalate band area measured from FTIR spectra in plants sprayed with Pro 20 mM shows calcium absorption and oxalic acid synthesis which are important for ionic balance. The ATR-FTIR technique is able to detect changes in the chemical composition of leaves, stems and roots of *Physalis peruviana* plants, assisting in the selection of priming treatments to increase tolerance to water deficit.

CRediT authorship contribution statement

Romeu da Silva Leite: Investigation, Methodology, Data curation, Formal analysis, Validation, Writing – original draft. **Marilza Neves do Nascimento:** Resources, Investigation, Supervision. **Salvador Hernández-Navarro:** Resources, Investigation, Supervision. **Norlan Miguel Ruiz Potosme:** Data curation, Validation, Supervision. **Siva-kumaran Karthikeyan:** Writing – review & editing.

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.

Acknowledgements

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

References

- [1] F. Takahashi, K. Shinozaki, Long-distance signaling in plant stress response, *Curr. Opin. Plant Biol.* 47 (2019) 106–111, <https://doi.org/10.1016/j.pbi.2018.10.006>.
- [2] M. Hussain, S. Farooq, W. Hasan, S. Ullah, M. Tanveer, M. Farooq, A. Nawaz, Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives, *Agric. Water Manag.* 201 (2018) 152–166, <https://doi.org/10.1016/j.agwat.2018.01.028>.
- [3] P. Langridge, M.P. Reynolds, Genomic tools to assist breeding for drought tolerance, *Curr. Opin. Biotechnol.* 32 (2015) 130–135, <https://doi.org/10.1016/j.copbio.2014.11.027>.
- [4] N. Sewelam, K. Kazan, P.M. Schenk, Global plant stress signaling: Reactive oxygen species at the cross-road, *Front. Plant Sci.* 7 (2016) 1–21, <https://doi.org/10.3389/fpls.2016.00187>.
- [5] M. Simontacchi, A. Galatro, F. Ramos-Artuso, G.E. Santa-María, Plant survival in a changing environment: The role of nitric oxide in plant responses to abiotic stress, *Front. Plant Sci.* 6 (2015) 1–19, <https://doi.org/10.3389/fpls.2015.00977>.
- [6] M. Pintó-Marijuan, S. Munne-Bosch, Ecophysiology of invasive plants: Osmotic adjustment and antioxidants, *Trends Plant Sci.* 18 (12) (2013) 660–666, <https://doi.org/10.1016/j.tplants.2013.08.006>.
- [7] R. da Silva Leite, M.N. do Nascimento, T.T. Tanan, L.P. Gonçalves Neto, C.A. da Silva Ramos, A.L. da Silva, Alleviation of water deficit in *Physalis angulata* plants by nitric oxide exogenous donor, *Agric. Water Manag.* 216 (2019) 98–104, <https://doi.org/10.1016/j.agwat.2019.02.001>.
- [8] A. Carverzan, A. Casassola, S.P. Brammer, Antioxidant responses of wheat plants under stress, *Genet. Mol. Biol.* 39 (2016) 1–6, <https://doi.org/10.1590/1678-4685-GMB-2015-0109>.
- [9] R. da Silva Leite, M.N. do Nascimento, A.L. da Silva, R. de Jesus Santos, Chemical priming agents controlling drought stress in *Physalis angulata* plants, *Sci. Hortic.* 275 (2021) 109670, <https://doi.org/10.1016/j.scienta.2020.109670>.
- [10] G.J.M. Beckers, U. Conrath, Priming for stress resistance: from the lab to the field, *Curr. Opin. Plant Biol.* 10 (4) (2007) 425–431, <https://doi.org/10.1016/j.pbi.2007.06.002>.
- [11] A. Savvides, S. Ali, M. Tester, V. Fotopoulos, Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends Plant Sci.* 21 (2016) 329–340, <https://doi.org/10.1016/j.tplants.2015.11.003>.
- [12] L. Corte, P. Rellini, L. Roscini, F. Fatichenti, G. Cardinali, Development of a novel, FTIR (Fourier transform infrared spectroscopy) based, yeast bioassay for toxicity testing and stress response study, *Anal. Chim. Acta.* 659 (1–2) (2010) 258–265, <https://doi.org/10.1016/j.aca.2009.11.035>.

- [13] B. Ribeiro da Luz, Attenuated total reflectance spectroscopy of plant leaves: A tool for ecological and botanical studies, *New Phytol.* 172 (2) (2006) 305–318, <https://doi.org/10.1111/j.1469-8137.2006.01823.x>.
- [14] C. Berthomieu, R. Hiennerwadel, Fourier transform infrared (FTIR) spectroscopy, *Photosynth. Res.* 101 (2–3) (2009) 157–170, <https://doi.org/10.1007/s11120-009-9439-x>.
- [15] N.A. Liu, C. Karunakaran, R. Lahlali, T. Warkentin, R.A. Bueckert, Genotypic and heat stress effects on leaf cuticles of field pea using ATR-FTIR spectroscopy, *Planta.* 249 (2) (2019) 601–613, <https://doi.org/10.1007/s00425-018-3025-4>.
- [16] N.A. Liu, L. Zhao, L. Tang, J. Stobbs, I. Parkin, L. Kunst, C. Karunakaran, Mid-infrared spectroscopy is a fast screening method for selecting *Arabidopsis* genotypes with altered leaf cuticular wax, *Plant Cell Environ.* 43 (3) (2020) 662–674.
- [17] R.S. Leite, S. Hernández-Navarro, M.N. Nascimento, N.M.R. Potosme, P. Carrión-Prieto, E.S. Souza, E. Nitrogen fertilization affects Fourier Transform Infrared spectra (FTIR) in *Physalis L.* species, *Comput. Electron. Agric.* 150 (2018) 411–417, <https://doi.org/10.1016/j.compag.2018.05.021>.
- [18] R. da Silva Leite, K. Sivakumaran, S. Hernández-Navarro, M. Neves do Nascimento, N.M.R. Potosme, P. Carrión-Prieto, E.D.S. Souza, Nitrogen influenced biomolecular changes on *Physalis L.* species studied using 2DCOS spectral analysis coupled with chemometric and Receiver operation characteristics analysis, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 249 (2021) 119220, <https://doi.org/10.1016/j.saa.2020.119220>.
- [19] H.S. El-Beltagi, H.I. Mohamed, G. Safwat, M. Gamal, B.M.H. Megahed, Chemical composition and biological activity of *Physalis peruviana L.* *Chemische Zusammensetzung und biologische Aktivität von *Physalis peruviana L.** *Gesunde Pflanzen.* 71 (2) (2019) 113–122, <https://doi.org/10.1007/s10343-019-00456-8>.
- [20] E.M. Bonfim-Silva, T.J.A. Silva, C.E.A. Cabral, B.E. Kroth, D. Rezende, Desenvolvimento inicial de gramíneas submetidas ao estresse hídrico, *Rev. Caatinga.* 24 (2011) 180–186.
- [21] V. Lionetti, F. Cervone, D. Bellincampi, Methyl esterification of pectin plays a role during plant-pathogen interactions and affects plant resistance to diseases, *J. Plant Physiol.* 169 (16) (2012) 1623–1630, <https://doi.org/10.1016/j.jplph.2012.05.006>.
- [22] B. Velmurugan, L. Devaraj Stephen, S. Karthikeyan, S. Binu Kumari, Biomolecular changes in gills of *Gambusia affinis* studied using two dimensional correlation infrared spectroscopy coupled with chemometric analysis, *J. Mol. Struct.* 1262 (2022) 132965, <https://doi.org/10.1016/j.molstruc.2022.132965>.
- [23] S. Farouk, A.A. Al-Huqail, Sodium nitroprusside application regulates antioxidant capacity, improves phytopharmaceutical production and essential oil yield of marjoram herb under drought, *Ind Crops Prod.* 158 (2020) 113034, <https://doi.org/10.1016/j.indcrop.2020.113034>.
- [24] A. Gholami Zali, P. Ehsanzadeh, Exogenously applied proline as a tool to enhance water use efficiency: Case of fennel, *Agric. Water Manag.* 197 (2018) 138–146, <https://doi.org/10.1016/j.agwat.2017.11.023>.
- [25] M. Zouari, C.B. Ahmed, W. Zorrig, N. Elloumi, M. Rabhi, D. Delmail, B.B. Rouina, P. Labrousse, F.B. Abdallah, Exogenous proline mediates alleviation of cadmium stress by promoting photosynthetic activity, water status and antioxidative enzymes activities of young date palm (*Phoenix dactylifera L.*), *Ecotoxicol. Environ. Saf.* 128 (2016) 100–108, <https://doi.org/10.1016/j.ecoenv.2016.02.015>.
- [26] S. Türker-Kaya, C.W. Huck, A review of mid-infrared and near-infrared imaging: Principles, concepts and applications in plant tissue analysis, *Mol.* 22 (2017) 168, <https://doi.org/10.3390/molecules22010168>.
- [27] Z. Cao, Z. Wang, Z. Shang, J. Zhao, Classification and identification of *Rhodoryum roseum* Limpr. and its adulterants based on Fourier-transform infrared spectroscopy (FTIR) and chemometrics, *PLoS One.* 12 (2017) 1–14, <https://doi.org/10.1371/journal.pone.0172359>.
- [28] C.O. Chukwuma, A.E.M. Matthew, A. Habib-ur-Rehman, N.J. Giles, Fourier transform infrared spectroscopic analysis of maize (*Zea mays*) subjected to progressive drought reveals involvement of lipids, amides and carbohydrates, *Afr. J. Biotechnol.* 16 (18) (2017) 1061–1066, <https://doi.org/10.5897/AJB2017.15918>.
- [29] S. Agatonovic-Kustrin, E. Doyle, V. Gegechkori, D.W. Morton, High-performance thin-layer chromatography linked with (bio)assays and FTIR-ATR spectroscopy as a method for discovery and quantification of bioactive components in native Australian plants, *J. Pharm. Biomed. Anal.* 184 (2020) 113208, <https://doi.org/10.1016/j.jpba.2020.113208>.
- [30] S. Akyuz, T. Akyuz, O. Celik, C. Atak, FTIR Spectroscopy of protein isolates of salt-tolerant soybean mutants, *J. Appl. Spectrosc.* 84 (6) (2018) 1019–1023, <https://doi.org/10.1007/s10812-018-0580-1>.
- [31] E.N. Dubis, A.T. Dubis, J.W. Morzycki, Comparative analysis of plant cuticular waxes using HATR FT-IR reflection technique, *J. Mol. Struct.* 511–512 (1999) 173–179, [https://doi.org/10.1016/S0022-2860\(99\)00157-X](https://doi.org/10.1016/S0022-2860(99)00157-X).
- [32] C.M. Topalá, I. Rusea, Analysis of leaves using fir spectroscopy and principal component analysis discrimination of different plant samples, *CTNS.* 7 (2018) 286–291.
- [33] J. Liu, J. Zhang, G. Zhu, D. Zhu, Y. Yan, Y. Yan, Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality, *Crop Sci.* 10 (1) (2022) 216–223, <https://doi.org/10.1016/j.cj.2021.04.006>.
- [34] A. Alonso-Simón, A.E. Encina, P. García-Angulo, J.M. Álvarez, J.L. Acebes, FTIR spectroscopy monitoring of cell wall modifications during the habituation of bean (*Phaseolus vulgaris L.*) callus cultures to dichlobenil, *Plant Sci.* 167 (6) (2004) 1273–1281, <https://doi.org/10.1016/j.plantsci.2004.06.025>.
- [35] M. Chylińska, M. Szymańska-Chargot, A. Zdunek, FT-IR and FT-Raman characterization of non-cellulosic polysaccharides fractions isolated from plant cell wall, *Carbohydr. Polym.* 154 (2016) 48–54, <https://doi.org/10.1016/j.carbpol.2016.07.121>.
- [36] X. Liu, C.M.G.C. Renard, S. Bureau, C. Le Bourvellec, Revisiting the contribution of ATR-FTIR spectroscopy to characterize plant cell wall polysaccharides, *Carbohydr. Polym.* 262 (2021) 117935, <https://doi.org/10.1016/j.carbpol.2021.117935>.
- [37] P. Guicherd, J.P. Peltier, E. Gout, R. Bligny, G. Marigo, Osmotic adjustment in *Fraxinus excelsior L.*: malate and mannitol accumulation in leaves under drought conditions, *Trees.* 11 (3) (1997) 155–161, <https://doi.org/10.1007/PL00009664>.
- [38] F. Tardieu, C. Granier, B. Muller, Water deficit and growth. Co-ordinating processes without an orchestrator? *Curr. Opin. Plant Biol.* 14 (3) (2011) 283–289, <https://doi.org/10.1016/j.pbi.2011.02.002>.
- [39] J. Zhang, G. Li, Y. Song, Z. Liu, C. Yang, S. Tang, C. Zheng, S. Wang, Y. Ding, Lodging resistance characteristics of high-yielding rice populations, *Field Crops Res.* 161 (2014) 64–74, <https://doi.org/10.1016/j.fcr.2014.01.012>.
- [40] L. Wu, W. Zhang, Y. Ding, J. Zhang, E.D. Cambula, F. Weng, Z. Liu, C. Ding, S. Tang, L. Chen, S. Wang, G. Li, Shading contributes to the reduction of stem mechanical strength by decreasing cell wall synthesis in japonica rice (*Oryza sativa L.*), *Front. Plant Sci.* 8 (2017) 881, <https://doi.org/10.3389/fpls.2017.00881>.
- [41] L. Perrier, L. Rouan, S. Jaffuel, A. Clément-Vidal, S. Roques, A. Soutiras, C. Baptiste, D. Bastianelli, D. Fabre, C. Dubois, D. Por, D. Luquet, Plasticity of sorghum stem biomass accumulation in response to water deficit: a multiscale analysis from internode tissue to plant level, *Front. Plant Sci.* 8 (2017) 1516, <https://doi.org/10.3389/fpls.2017.01516>.
- [42] M. Durand, B. Porcheron, N. Hennion, L. Mauroussat, R. Lemoine, N. Pourtau, Water deficit enhances C export to the roots in *Arabidopsis thaliana* plants with contribution of sucrose transporters in both shoot and roots, *Plant Physiol.* 170 (3) (2016) 1460–1479, <https://doi.org/10.1104/pp.15.01926>.
- [43] X. Nxele, A. Klein, B.K. Ndimba, Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants, *S. Afr. J. Bot.* 108 (2017) 261–266, <https://doi.org/10.1016/j.sajb.2016.11.003>.
- [44] F. Zhang, Y. Hu, J. Zhang, J. Huang, R. Liu, C. Liu, X. Ren, R. An, Y. Zhou, High-performance UV-curable polyurethane acrylate resins derived from low-iodine woody plant oils, *Mater. Today Commun.* 25 (2020) 101526, <https://doi.org/10.1016/j.mtcomm.2020.101526>.
- [45] T.T. Tanan, M.N. nascimetro, A.L. Silva, D.S. Guimarães, R.S. Leite, L.S. Pereira, F. Santos Neto, Characterization of nitrate reductase activity (NR) in foliar and radicular tissues of *Physalis angulata L.*: Diurnal variations and protocol optimization, *Aust. J. Crop Sci.* 13 (2019) 1120–1125, <https://doi.org/10.21475/ajcs.19.13.07.p1644>.
- [46] A.D. Gupta, S. Karthikeyan, Individual and combined toxic effect of nickel and chromium on biochemical constituents in *E. coli* using FTIR spectroscopy and Principle component analysis, *Ecotoxicol. Environ. Saf.* 130 (2016) 289–294, <https://doi.org/10.1016/j.ecoenv.2016.04.025>.
- [47] C. Xiao, C.T. Anderson, Roles of pectin in biomass yield and processing for biofuels, *Front. Plant Sci.* 4 (2013) 67, <https://doi.org/10.3389/fpls.2013.00067>.
- [48] M. Szymanska-Chargot, A. Zdunek, Use of FT-IR spectra and PCA to the bulk characterization of cell wall residues of fruits and vegetables along a fraction process, *Food Biophys.* 8 (2013) 29–42, <https://doi.org/10.1007/s11483-012-9279-7>.
- [49] Y. Jiang, T. Yoshida, A. Quigg, Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae, *Plant Physiol. Biochem.* 54 (2012) 70–77, <https://doi.org/10.1016/j.plaphy.2012.02.012>.
- [50] C.S.S. Gouveia, J.F.T. Ganança, V. Lebot, M.Á.A.P. de Carvalho, Quantitation of oxalates in corms and shoots of *Colocasia esculenta (L.) Schott* under drought conditions, *Acta Physiol. Plant.* 40 (12) (2018), <https://doi.org/10.1007/s11738-018-2784-7>.
- [51] A. Gaberščik, M. Grašič, K. Vogel-Mikuš, M. Germ, A. Golob, Water shortage strongly alters formation of calcium oxalate druse crystals and leaf traits in *Fagopyrum esculentum*, *Plants.* 9 (2020) 1–19, <https://doi.org/10.3390/plants9070917>.
- [52] A. Kayabaş, E. Yildirim, New approaches with ATR-FTIR, SEM, and contact angle measurements in the adaptation to extreme conditions of some endemic *Gypsophila L.* taxa growing in gypsum habitats, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 270 (2022) 120843, <https://doi.org/10.1016/j.saa.2021.120843>.

