


Monitoring a metabolic profile of wheat by using FTIR spectroscopy and chemometric methods – concept studies

Aleksandra Borek-Dorosz¹ B-F, Rafał Kurczab² A-D,G 

¹Jagiellonian University, Faculty of Chemistry, Gronostajowa 2, 30-387 Krakow, Poland

²University of Applied Sciences in Tarnow, Faculty of Mathematics and Natural Sciences, Department of Chemistry, Mickiewicza 8, 33-100 Tarnów, Poland

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Abstract

Changes in plants under the influence of a variety of chemical and physical factors are reflected in metabolomic changes. To date, there are very few methods that would allow studying metabolic changes occurring in single cells. Spectroscopic methods especially combined with the chemometrics methods are a very good tool to investigate such changes in metabolomics. Tracking changes in plants is of particular importance in industry, especially when studying how the use of fertilizers affects plants. In this paper, we present preliminary research as concept of proof to examine whether the use of FTIR (Fourier Transform Infrared Spectroscopy) helps to monitor the changes in the metabolomic profile of the plants. For preliminary research, four species of cereals and cuckooflower were used. In this step, it was possible to verify the differences in metabolites that are produced by plants belonging to different families. Then one species of grain was selected and subjected to eleven different physical and chemical factors. Next, the research was expanded to determine the optimal concentration of hydrogen peroxide. FTIR spectra of leaves and extracts of the plants were obtained for all experimental groups and then analyzed with the use of chemometric methods: HCA (Hierarchical Component Analysis) and PCA (Principal Component Analysis). Those methods were used to help in the interpretation of metabolic changes resulting in the plant in response to external factors.

Keywords

- metabolic fingerprint
- FTIR
- HCA
- PCA
- stress factors

Authors contributions

- A – Preparation of the research project
B – Assembly of data for the research undertaken
C – Conducting of statistical analysis
D – Interpretation of results
E – Manuscript preparation
F – Literature review
G – Revising the manuscript

Corresponding author

Rafał Kurczab

e-mail: r_kurczab@atar.edu.pl
Akademia Tarnowska
Katedra Chemii
ul. Mickiewicza 8
33-100 Tarnów, Poland

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ul. Mickiewicza 8, 33-100 Tarnow, Poland

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Conflict of interest

None declared.

Introduction

Metabolomics is the study and analysis of chemicals (metabolites) present in cells and tissues, which are products or intermediates formed during metabolism [1]. Thanks to these studies, it is possible to detect physiological changes in the body caused by external and toxic factors, it is also possible to determine metabolites that are characteristic of particular disease syndromes, e.g. tumors. Toxicity assessment of metabolites is used in pharmacology, the appropriate dose is assessed, which will be non-toxic and its effect on physiology is easily investigated. Substances produced in plants can be divided into two basic groups: primary and secondary metabolites [2]. Metabolomics could also contribute to the study of defining the product of stress metabolism or molecules that can be assigned to the acclimation of plants [3,4].

The world of plants is very diverse. Currently, it has been described around 500,000 species [5], which differ in structure, origin, climate zone in which they grew, and many other factors. Due to their diversity in structure, a large variety of metabolites – chemical compounds are present in plants. They are mainly involved in the primary and secondary metabolism of the plant, and due to this feature, they are divided into two main groups: basic substances (primary metabolites) and specific substances (secondary metabolites) [6,7]. Primary metabolites are formed by primary metabolism and are present in all plants, while secondary metabolites are produced by specific enzymes associated with primary metabolic pathways. Specific substances occur only in specific plant groups in response to environmental conditions for better adaptation [8]. Their metabolic pathways include photosynthesis, hydrocarbon conversion, respiration, amino acid, protein, fat synthesis, and fat utilization [8]. These substances are involved in growth and plant development, the process of respiration, the process of photosynthesis, and the synthesis of hormones and proteins. They can be divided into four basic groups: carbohydrates, protein, nucleic acid, and fatty acid [7].

Secondary metabolites are produced with the participation of specific enzymes in conjunction with the main metabolic pathways. Secondary metabolites only occur in specific groups of plants in response to environmental conditions to better adapt [8]. Secondary metabolites are not involved in plant growth and development [7]. They are responsible for the colour of plants and protect against herbivores, microorganisms, and UV radiation. They also attract insects to pollinate the plant [9]. British Nutrition Foundation has adopted a division of these metabolites into four

groups: phenolic compounds, terpenes, alkaloids, and sulfur compounds [7]. Within those two basic groups, there is considerable variation. Several criteria divided them into subgroups, due to the unclear meaning of many of these compounds. A very large number of lesser-known so far, substances are used in medicine, although some are harmful to human health.

Metabolomics in plant research has many applications. It enables the improvement of genetically modified plants and the development of new safer pesticides. Plant metabolomics is of particular interest because it allows for determining the range and function of both first and secondary metabolites [3,10]. Fourier Transformed Infrared Spectroscopy (FTIR) in combination with chemometric methods is ideal for the analysis of metabolites. FTIR spectroscopy is a non-invasive method that is relatively fast [11], has a low percentage of error [12], and the bands on the spectra can be assigned to specific groups of metabolites produced by the plant [13], hence its widespread use in both plant and human tissue metabolomic studies. The disadvantage of FTIR-ATR (Attenuated Total Reflectance) is high water absorption and the bands that come from it have high intensity [11]. Spectrum, which is obtained by spectroscopic method contains a lot of data, to reduce multidimensionally, minimization of variations between the groups, and maximize differences apply chemometric methods [12]. There are many examples in the literature of FTIR being used in this type of research. The analysis of fingerprint region allows to distinguish between plants belonging to different types [14] to detect mechanical damage [12] and specify differences in phenotypic and genotypic. It also allows to determine metabolic changes, identify changes in the major metabolic pathways [13], and monitor changes in metabolic or structural [14–16]. Yeliana et al. [17] show that with the use of FTIR spectroscopy, it was possible to distinguish the total content of phenols and flavonoids in propolis from different geographical regions in Indonesia. Hussian et al. [18] have shown that with the use of FTIR spectroscopy combined with PCA (Principal Component Analysis), it is possible to determine the metabolic changes in fruit caused by climate changes.

Plants require the proper condition for the correct development i.e. temperature, irrigation, lighting, soil pH, fertilization, and the lack of interaction with the stress factors. At a time when at least one factor is in deficiency or excess, it causes changes in the metabolism of the plant. Plant stress can be caused by physical factors such as the lack of water, intense lighting, and low temperature (i.e. abiotic stress). A distinction is made between biotic stress, which is called biological agents such as the onslaught of pathogens or the presence of

other plants. Both types of stress are associated with the uncontrolled growth of active oxygen species (ROS) in a cell, to which the hydrogen peroxide belongs. The cumulative H_2O_2 can react with a variety of biomolecules, some of these reactions are irreversible and cause their inactivation (e.g. organelles dysfunction or necrosis). There are also reversible redox reactions, which protect the biomolecules to preserve their biological functions and modulate their activity. Hydrogen peroxide affects the expression of genes, the activity of phytohormones, sugar metabolism, and photoperiod (duration of light on plants daily) to obtain long-term resilience of the plants on a wide range of pathogens. It also contributes to the activation of PR proteins (called Pathogenesis Related), the antioxidant enzyme system, and the synthesis of secondary metabolites [19].

In this work, we present the preliminary research which shows the potential of the FTIR-ATR method combined with chemometric methods to distinguish the metabolic profile of plants belonging to different rows and to monitor the changes in the metabolic profile of plants induced by physical and chemical stress factors. The extract and leaves of plants were analyzed after 14 and 28 days after seeding and treated with selected factors and measured with the FTIR-ATR method. To analyze the metabolomic profile two chemometric methods: HCA (Hierarchical Cluster Analysis) and PCA (principal component analysis) were performed.

Materials and methods

To determine metabolic profiles in plants belonging to different rows; we planted 4 species of cereals: oats, wheat, barley, rye (Granum company), and cuckooflower (POLAN company) (Figure S1 in Supporting Information). Then the wheat was subjected to both chemical and physical stress. The reference sample was watered and kept at 20°C. One of them was grown at a lower temperature of about 5°C, another was kept without access to the light (in the following description this sample was called shadow), and at a lower temperature. Those plants were watered with normal water. The other plants were watered with additions of selected substances: coffee grounds (Tchibo®), extract of black tea (Remsey Earl Grey®), 3% sucrose solution (sugar – Slodka Lyzeczka®), 1% H_2O_2 solution (pharmaceutical laboratory AV-ENA®), 2% salicylic spirit solution (pharmaceutical plant Amara®), 1% NaOH solution (POCh), 1% H_2SO_4 solution (POCh), fertilizer used for orchids (Planta company), solid fertilizer with eight component (POLIFOSKA 8, Grupa Azoty S.A.). The plants were watered with about 50 ml of the substance, every two days.

All plants were grown for 30 days and counted after germination. From each pot (experimental group) after 14 and 28 days the leaves were harvested. Then part of the collected leaves was crushed in a mortar and subjected to extraction in waterless ethanol, another part was retained unchanged.

In addition, the effect of H_2O_2 concentration on metabolic changes was determined. The wheat was watered and treated with hydrogen peroxide solutions at concentrations of 0.5%, 1.5%, 2.0%, 2.5%, and 3.0%. From these plots, the leaves and extract were obtained.

Infrared spectroscopy measurements

Extracts and leaves were measured with the use of FTIR-ATR spectroscopy (Thermo Scientific FTIR Nicolet iS5). All spectra were collected with 34 scans with 2 cm^{-1} spectral resolution. For each experimental group, one spectrum of leaves and extracts was collected after 14 and 28 days. Then the spectra were preprocessed which includes: trimming scale ($4000\text{--}600\text{ cm}^{-1}$), automatic baseline correction, and ATR correction. To facilitate the analysis and readability of images the abbreviation was used (Figure S2 in SI).

Collection of plant material

Preprocessed spectra were subjected to the RStudio program and chemometric analyses were performed (PCA and HCA) using the ChemoSpec library [20]. Due to a low number of data points for each experimental group the PCA shows only a graphical representation in scores plots.

To perform chemometric analysis, recorded spectra have been encoded according to the following algorithm: the first symbol denotes the plant type (p – wheat, o – oats, j – barley, r – cuckooflower, and z – rye), the second symbol indicates whether the spectrum was recorded for extract or leaves and how many days after planting they were harvested (source: e – extract, l – leaves, time: 14 and 28 days), and the last position in the name refers to the conditions with which the plantings were treated at the time of farming (w – watered, t – kept at a lower temperature, ts – kept at a lower temperature and without exposure to light, f – watered with coffee grounds, h – watered with tea extract, c – watered with 3% sucrose solution, uX – watered with X% H_2O_2 solution, n – watered with 1% NaOH solution, k – watered with 1% H_2SO_4 solution, s – watered with orchid fertilizer, and a – watered with Polifoska 8 fertilizer) (Figure S2 in SI).

Results and discussion

Organoleptic analysis

The changes caused by different stress factors were first measured based on visual inspection. The plants were photographed after 14 and 28 days after sowing (Figure 1). Significant differences in high, density, and leaf thickness of the plants were detected.

During the breeding of seedlings, the following observations were made. The first plants that sprouted were watering with 1% H_2O_2 solution and supplied with fertilizer for orchids, whereas, the last pierced-out seeds watering with 1% H_2SO_4 solution. In pot

watering by salicylic spirit solution, no seed ever germinated. The plant watered with H_2O_2 solution has the coarsest leaves and the most seeds had sprouted. During the watering the hydrogen peroxide has been decomposed which resulted in loosening the ground. After some time near the pot powered by sucrose, the fruit fly gathered and the plant was the lowest in comparison with the rest. The seedling supplied by fertilizers has got long but thin leaves which caused lodging, the plant watering by H_2SO_4 solution has got also thinner leaves and some of them wrap off the soil. The quickset kept without access to sunlight has got yellow and thin leaves, and only part of the seed has to be pierced out.

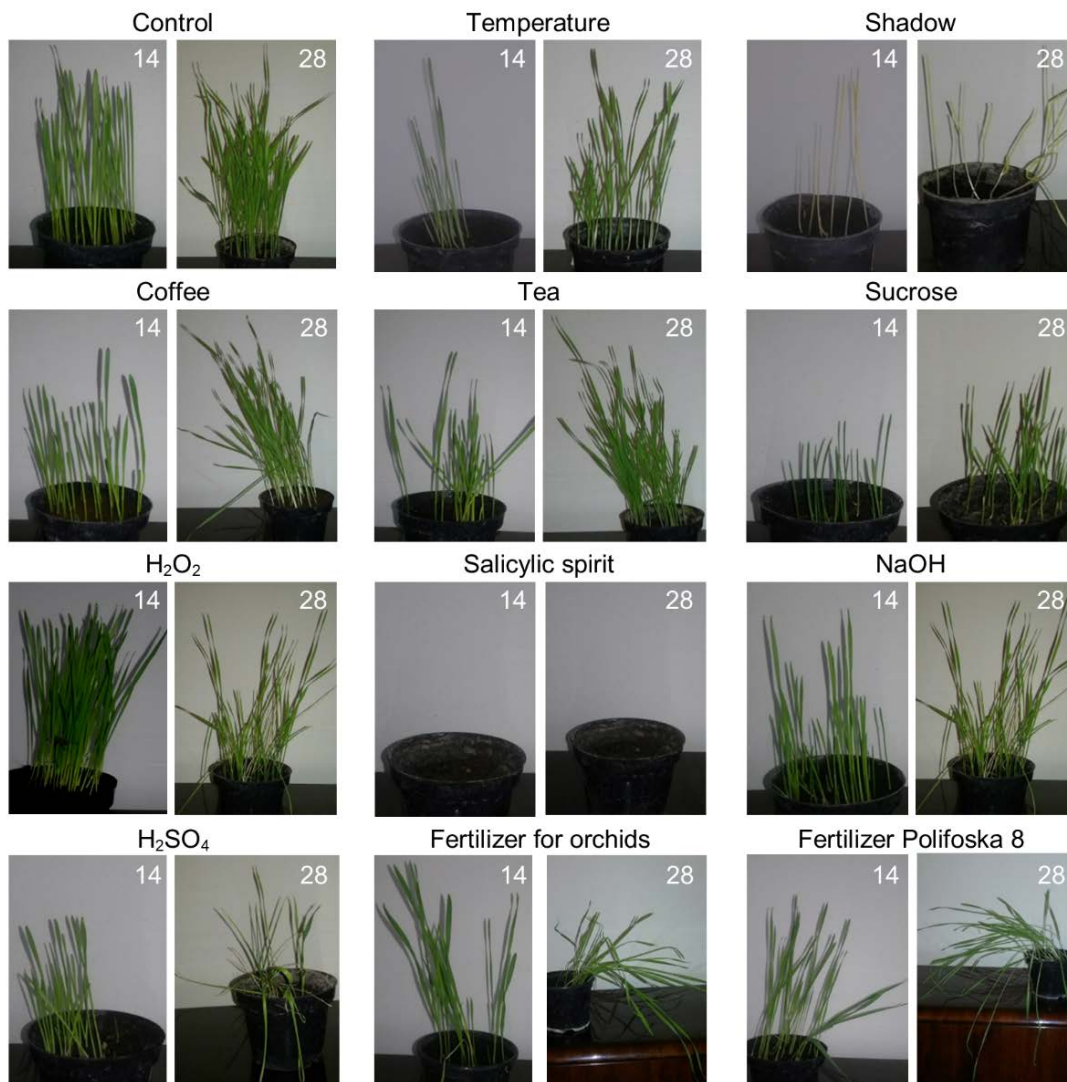


Figure 1. The illustration of the changes in tested oats after 14 and 28 days as a result of different external stress factors

Identification of metabolomic markers

Analysis of the infrared spectrum can cause a lot of trouble, mainly due to the very large number of bands that occur. An additional difficulty is the presence of water in the analyzed samples. Therefore, spectral analyses were undertaken in terms of the presence or absence of selected bands on the spectrum, which may contribute to the differentiation of the experimental

groups studied. The upsent of selected bands was marked as a minus sign (-) and presented in Table 1.

As can be seen in Table 1 more differences can be withdrawn based on the spectra of leaf extracts. Comparing the spectra of the extracts of leaves and some dependencies can be seen, some of the peaks are shown in only one of these groups, which is due to that some of the compounds are only visible after the extraction while others only analyze if pure leaf. Some peak appears after 4 weeks, which is probably because

Table 1. Analysis of infrared spectra – a summary of the bands that distinguish individual seedlings

Wave number at which the peak attains maximum value [cm ⁻¹]	Jw	Ow	Zw	Rw	Pw	Pt	Pts	Pf	Ph	Pc	Pu	Pn	Pk	Ps	Pa
Extracts / 14 days after sowing															
1646	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
1630	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
1490	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
1472	+	+	+	+	+	-	+	+	-	+	+	+	-	+	+
1464	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+
1274	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
1206	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-
1164	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
730	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
710	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Extracts / 28 days after sowing															
1649	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
1630	-	-	-	+	-	+	+	-	+	+	+	+	+	+	-
1616	-	+	-	+	+	+	+	+	+	+	-	+	+	+	-
1490	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+
1472	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1206	+	+	+	-	+	+	-	-	+	-	-	-	+	-	+
1180	-	-	+	-	+	+	-	-	-	-	-	-	+	-	+
1161	+	-	+	-	+	+	-	-	-	-	-	-	+	-	+
730	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
710	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+
Leaves / 14 days after sowing															
2330	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
1668	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Leaves / 28 days after sowing															
1668	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-
1616	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-

Jw – barley watering with water, Ow – oats watering with water, Zw – rye watering with water, Rw – cuckooflower watering with water, Pw – wheat watering with water, Pt – wheat kept in a lower temperature, Pts – wheat kept without access to light, Pf – wheat watering with coffee grounds, Ph – wheat watering with tea extract, Pc – wheat watering with 3% sucrose solution, Pu – wheat watering with 1% H₂O₂ solution, Pn – wheat watering with 1% NaOH solution, Pk – wheat watering with 1% H₂SO₄ solution, Ps – wheat watering with fertilizer for orchids, Pa – wheat supplied with fertilizer Polifoska, (+) – peak occurs, (-) – the peak is absent.

the metabolites which correspond to the wavelengths have been produced by the plant in response to environmental conditions. But others disappeared because they have been used for the production of other metabolites. There are also differences between the spectra and their first derivatives (data not shown) some peaks can be seen after examination of the first derivative. Further analyses were performed on both leaves and their extracts, but due to this fact, only extracts were present in this manuscript. The assignment of spectra presented in Table 1 was made based on the literature

(Table T1 in SI.) data about primary and secondary metabolites groups to wavenumbers (Table 2, and Table T2 in SI). It is not possible to accurately determine which of the individual metabolites derived data bandwidth because within groups of metabolites is enormous structural diversity and limitations in the methods of detection. The number of bands after 28 days is less than after 14 days for both the extract and leaves. Analyzing the band for all the examined plants it is noted that there are small shifts of the bands compared to a reference sample.

Table 2. Suggested metabolites for wheat extracts (1,11,27,12,13,21–26)

Wavenumber at which the peak attains maximum value [cm ⁻¹]				Band	Suggested group of metabolites
extract after 14 days		extract after 28 days			
Spectra	FDa	spectra	FDa		
3363	3363	3411	3411	vOH	Water, T
2975	2982	2977	2985	vNH; vOH associates	T
2928	2944	2929	2941	vOH associates; vCH ₃ ,as; vCH ₂ ,as	L, N, T
2894	2904	2900	2905	vCH	L, N, P, T
1647	1648	1645	1645	vC=O associates; vC=C; vC=O; vNO ₂ ,as; δNH; vC=C; vC=C;	B, W
1455	1459	1455	1456	δNH ₃ ,sym; δCH ₂ ; δCH ₃ ; δO-C-O,sym;	A(A), Ar, B, L
1381	1387	1382	1387	δCH ₃ ,sym; vSO ₂ ,as; vC-O	A(L), L, S
1088	1096	1088	1096	vP-O-C,as; vSi-O-Si; vC-F; vC-O-C,as	A(K), Ar, F, P, W
1048	1055	1047	1054	vS=O; vC=S; vP-O-C,as; vSi-O-Si; vC-F	Ar, L, N, P, S, W
880	885	803	809	vC-O-C,as	Ar, I, N, W
666	667	667	668	δCH; vC-Br; vC-S; vC-Cl	Ar, I, S

FDa first-order derivative of FTIR spectra; v – stretching vibration, δ – deformation vibration, sym- symmetric, as – antisymmetric, T – fatty acids, L – lipids, N – nucleic acids and nucleotides, P – polysaccharides, B – proteins, W – hydrocarbons, A – free amino acids, Ar – aromatic, S – sulfur compounds, K – alkaloids, F – phenolic, I – terpenes.

Analysis of different plant species

Each species of plant has a preference condition for the best growth, including a temperature suitable for germination, or the temperature in which occurs the biggest weight gain of the plant. The spectra of different cereal species and cress (Figure 2) differ only in the intensity of the peaks, so it can be concluded that the metabolites produced by these plants do not differ significantly. After 14 days, cuckooflower showed the highest intensity for extracts ranging from 3700–3000 and 1750–1500 cm^{-1} and wheat extract showed the lowest intensity. Barley also showed high intensity, while oats and rye are more similar to wheat. In the ranges not mentioned above, the intensities are arranged oppositely. After 28 days,

the order of intensity in the ranges 3700–3000 and 1750–1500 cm^{-1} is as follows: barley shows the highest intensity, followed by cress. Another extract is wheat and rye extract. The least intensity is characterized by oat extract. In the ranges not mentioned above, the situation is similar to that of extracts after 14 days – the intensities of extracts are reversed. Comparing the spectrum of leaves after 14 days also the greatest intensity in the ranges 3700–3000 and 1750–1500 cm^{-1} shows cuckooflower, followed by oats, barley, rye, and wheat. However, after 28 days the order changes. The greatest intensity is shown by cress. Next are the leaves of rye, oats, wheat, and barley. Both for leaves after 14 days and 28 in ranges except 3700–3000 and 1750–1500 cm^{-1} the order of the given leaves is reversed.

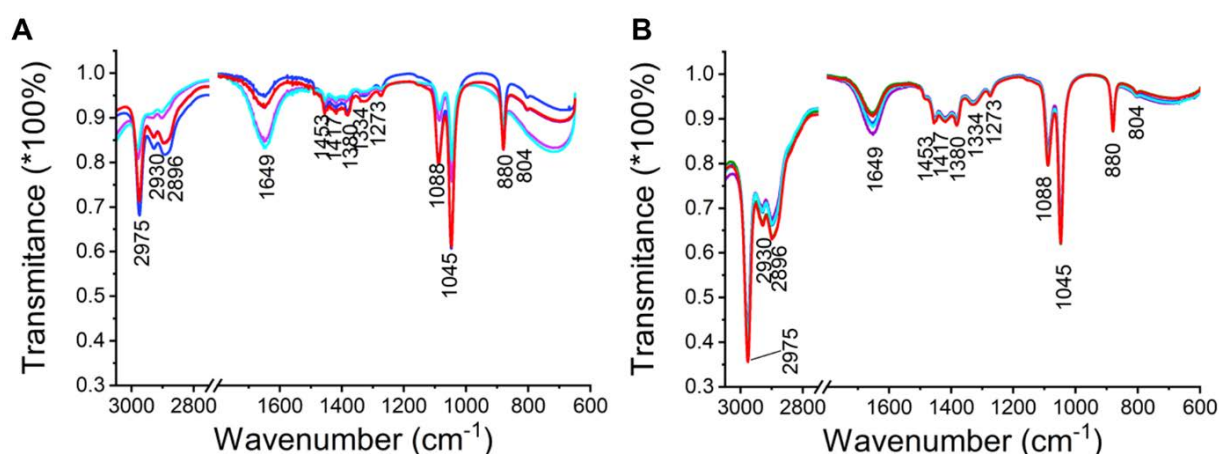


Figure 2. Comparison of the FTIR spectra of extracts for wheat (navy blue), oat (green), barley (purple), cuckooflower (blue), and rye (red) after 14 (A) and 28 (B) days of breeding

Based on Table T1 in SI and Table 1, it can be noted that some metabolites are not present in all plants. In cress leaves after 14 days there are no peaks at wavelengths of about 880 and 720 cm^{-1} . The distinguishing feature of cress from the rest of cereals is the presence of a strand in extracts after 14 days at a wavelength of about 1634 cm^{-1} . Cuckooflower and oat extracts show peaks after 14 days at wavelengths of about 1156, 1127, and 1121 cm^{-1} , these peaks are not present in the rest of the studied species. Wheat and oats are distinguished by the absence of peaks for extracts after 28 days at wavelengths of about 705 and 730 cm^{-1} . Among cereals, barley is the most diverse species.

HCA analysis (Figure 3A) shows a division of spectra first according to the collection time of leaves (14 – marked in green and 28 – marked in purple) then they are divided into two groups: leaves (blue frames) and extracts (yellow frames). Among the lower

levels, cress leaves after 28 days appear on a separate branch, which means that they differ most from the rest samples, the same situation is visible for wheat after 28 days. At a Euclidean distance of about 100 the branch was divided into pairs: barley leaf and wheat leaf and oat leaf and rye leaf. Among the extracts, oats occur on one branch as rye, and wheat on one branch with cress. Among the samples, 14 days after planting, the dendrogram also divides into two branches at a level of about 1300. On one of them, there are extracts of wheat, oats, and rye, the last two of which are similar to each other (as for extracts after 28 days). On the second branch at a height of about 250 can be extracted pairs: barley leaf and oat leaf and wheat leaf and rye leaf. The leaves of the cress are located on a separate branch, from which it follows that it is not homogeneous with the rest samples.

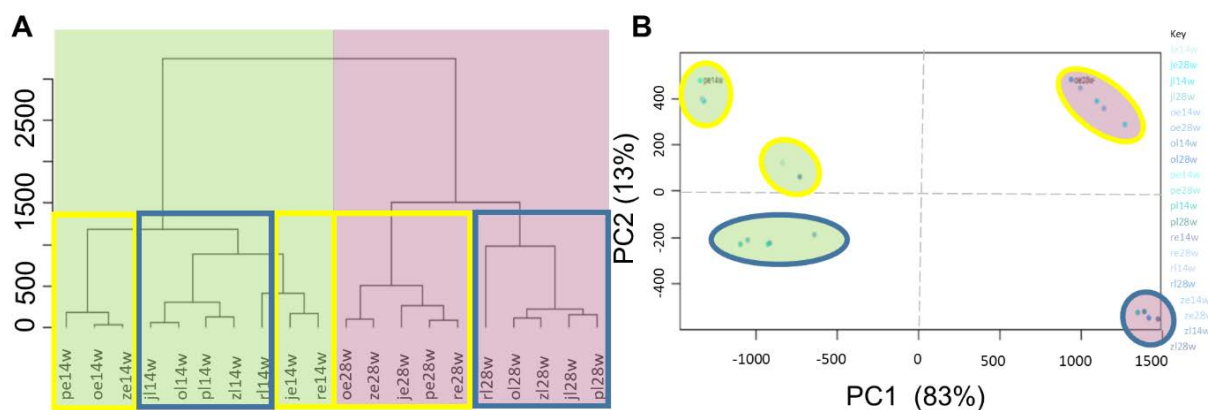


Figure 3. Chemometric analysis of leaves and extracts across different species. HCA analysis of extracts and leaves from different plant species (A). PCA scores of extract and leaves after 14 and 28 days (B)

In a further step, the PCA analysis was applied (Figure 3B). It is visible that all samples tested have been grouped into 5 main clusters. The first in the lower right corner of the chart (purple circle) shows the leaves analyzed after 28 days: wheat, barley, oats, and cress. The second cluster is located in the upper right corner (orange circle) and contains data on extracts after 28 days of different cereal species and cress. The next cluster is about -1500 PC1 and there are extracts after 14 days of oats, wheat, and cuckooflower. A little higher and to the right are extracts after 14 days of barley and watercress. On the other hand, the last group contains leaves of all plant species after 14 days and a cress leaf after 28 days from planting.

Metabolomics changes in plants due to chemical and physical factors

To study metabolic changes under the influence of various chemical and physical factors, wheat that grew rapidly was selected for experiments. The physical methods resulted in a reduced temperature and lack of access to sunlight. The chemical agents were: coffee grounds, tea extract, sugar solution, solution of H_2O_2 , NaOH, H_2SO_4 , and two types of fertilizers liquid (fertilizer for orchids) and solid (Polifoska 8). From each plant, the leaves were collected and subjected to FTIR measurements, and also their extracts after 14 days (Figure 4A) and 28 (Figure 4B) days of spreading.

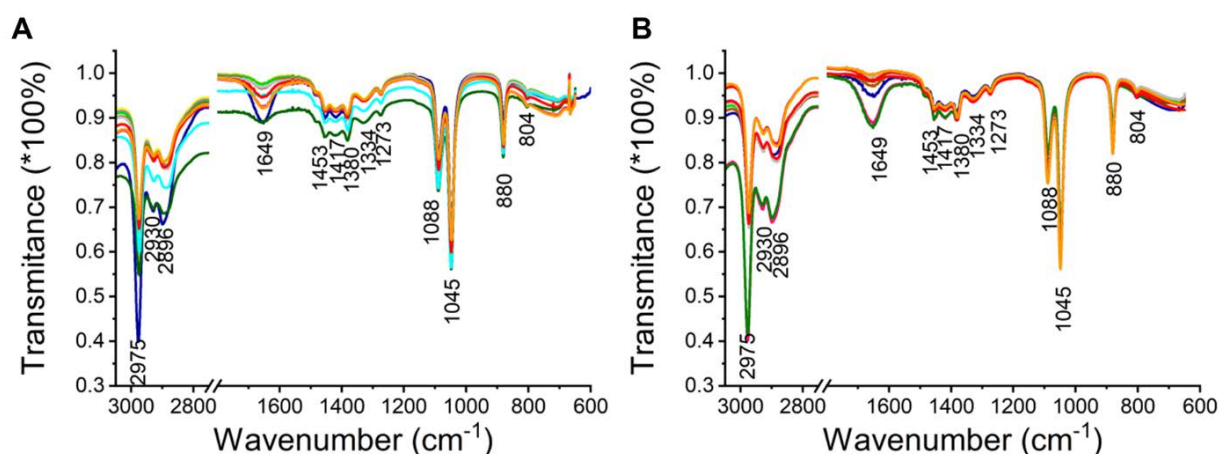


Figure 4. Comparison of the spectra of extracts after 14 (A) and 28 (B) days – wheat treated with various chemical and physical factors: water (navy blue), temperature (pink), shadow (green), coffee grounds (yellow), tea (purple), sucrose (light green), H_2O_2 (brown), NaOH (grey), H_2SO_4 (blue), fertilizer for orchids (red) and fertilizer Polifoska 8 (orange)

The visual analysis of changes was very difficult, due to the richness of different metabolites inside plants. A summary of the range from which it was possible to infer plant changes is presented in Tables 1 and 2. The biggest differences occur between the extract after 14 days after the sowing of individual seedlings. Frequency response at wavelength 1646 and 1274 cm^{-1} occur only in pe14k, and the band corresponding wavelength 1630 cm^{-1} is absent only in pe14f. The peak at 1490 cm^{-1} is not present in spectra of pe14k, pe14f, and pe14h. The peak at a wavelength of 1472 cm^{-1} is absent in the spectra of the extracts mentioned above and also in pe14t, the peak at 1464 cm^{-1} is not visible in pe14k and pe14ts. There can be also distinguished peaks that only occur in certain extracts of seedlings after 14 days. A peak at wavelength 1206 cm^{-1} occurs in pe14w, pe14k, and 1164 cm^{-1} is present in spectra of pe14t, while a peak at 703 cm^{-1} is absent only in spectra of pe14ts. Out of all examined spectra, the extracts after 14 days are the most similar to the extracts of the control seedlings. The only difference is the absence of a peak at a wavelength of 1206 cm^{-1} . Between the extracts of 28 days after sowing there is also a difference in the occurrence of peaks. The band at a wavelength 1630 cm^{-1} is absent in pe28f, pe28w, and pe28a, the peak at 1616 cm^{-1} did not occur at pe28s. In the spectra of pe28u1_0 the peak at 1490 cm^{-1} is not present, while the band at 1472 cm^{-1} is not noticeable in the spectra of pe28w and pe28c. In the spectra of the control sample, seedlings are not apparent in the peaks at wavelength 707 cm^{-1} while in the rest spectra of extracts, this band is present. Some of the peaks occur in the specific spectra of the extract after 28 days. Peak at wavelength 1206 cm^{-1} is evident in: pe28k, pe28h, pe28w, pe28t and pe28a. However, the peaks at wavelengths 1180 and 1161 cm^{-1} are demonstrable in spectra of pe28k, pe28w, and pe28t.

On the spectrum of wheat extracts after 14 days (Figure 4A) under different stressful factors can be noticed that the physical factors such as reduction of temperature and lack of light have different spectra profiles from the profiles of chemical factors. The most visible difference is a disparity in the intensity of bands. Plants subjected to physical factors accumulated large amounts of water in their leaves. In the spectra of leaves after 14 days this fact is not well seen, in both types of analysis, the bands are close to each, have similar intensity, and the spectra almost overlap each other. The spectrum of the control plants watering with water and kept at a normal temperature

differs from the other, it can be seen as a separate cluster on both HCA and PCA results. It can be easily seen from both types of analysis that both chemical and physical factors, can change the metabolomic profile of the plant.

Based on the FTIR spectra measured for extracts of all seedlings treated with different chemical and physical factors, the chemometric analysis was performed. HCA plot shows (Figure 5A) that the dispersion between samples is very large. One of the most striking results is the close grouping of plants watered with different concentrations of H_2O_2 , which may indicate that the concentration has no major effect on the resulting changes. There is also no clear separation between 14 and 28 days of growing seedlings on the dendrogram. PCA on the other hand shows (Figure 5B) the grouping of spectra of plants watered with water. The number of spectra subjected to PCA in this study is too low to draw exact conclusions, and for this reason, no loadings were shown and conclusions were drawn only based on graphs (scores).

Dependence of hydrogen peroxide concentration on changes occurring in plants

In the last step, the wheat was watered with different concentrations of hydrogen peroxide (0–3.0%). The FTIR spectra of leaf extracts after 14 and 28 days are presented in Figure 6.

As can be seen, the spectrum profiles of leaf extracts after 14 and 28 days are similar, which can mean that the plants have similar metabolomic profiles, and in the spectra, there are no significant differences in the intensity of bands. The spectra of wheat watered with 1% of H_2O_2 seedlings in November are not compared to the others which were bred in February, due to their large diversity. Between these two months, there is a big difference in light intensity and probably it was the cause of so different spectra profiles of these plants. By comparing the spectra of plants watered with different concentrations of hydrogen peroxide (except 1%) there are small differences in the intensity of individual bands. There are no offsets of bands or they are so small that almost invisible. The chemometric analysis (HCA and PCA – data not shown) also confirms that the differences between the spectra are small, these plants always have been grouped as one class.

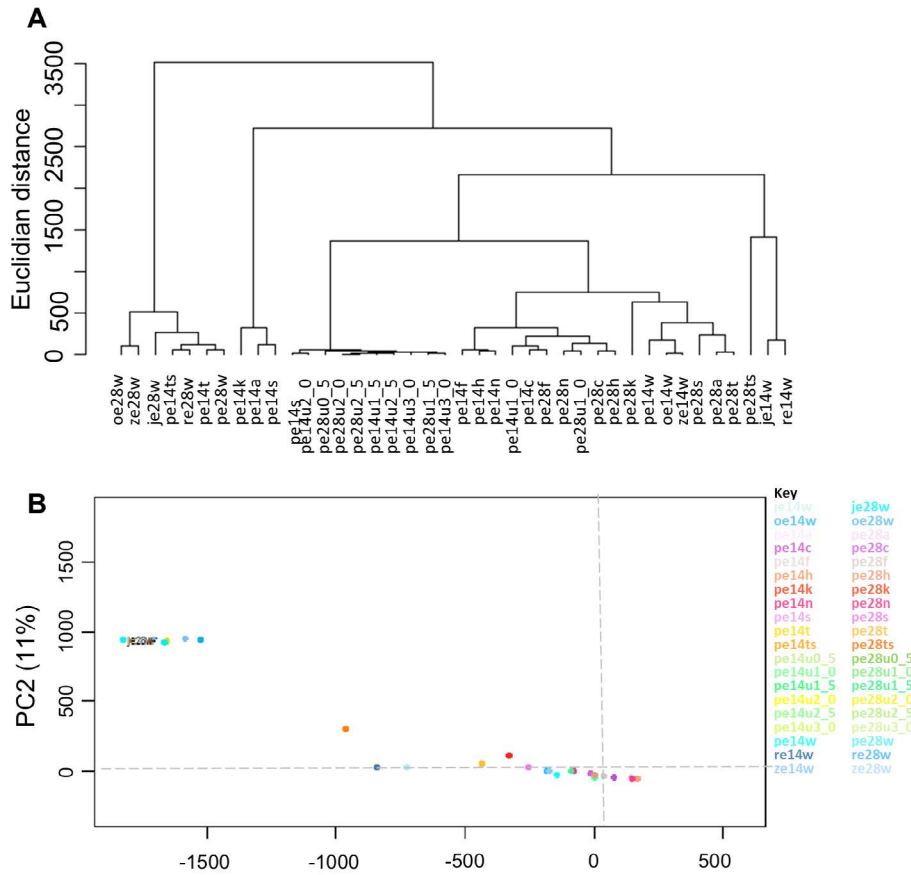


Figure 5. HCA (A) and PCA (B) results of extracts of all seedlings treated with different chemical and physical factors

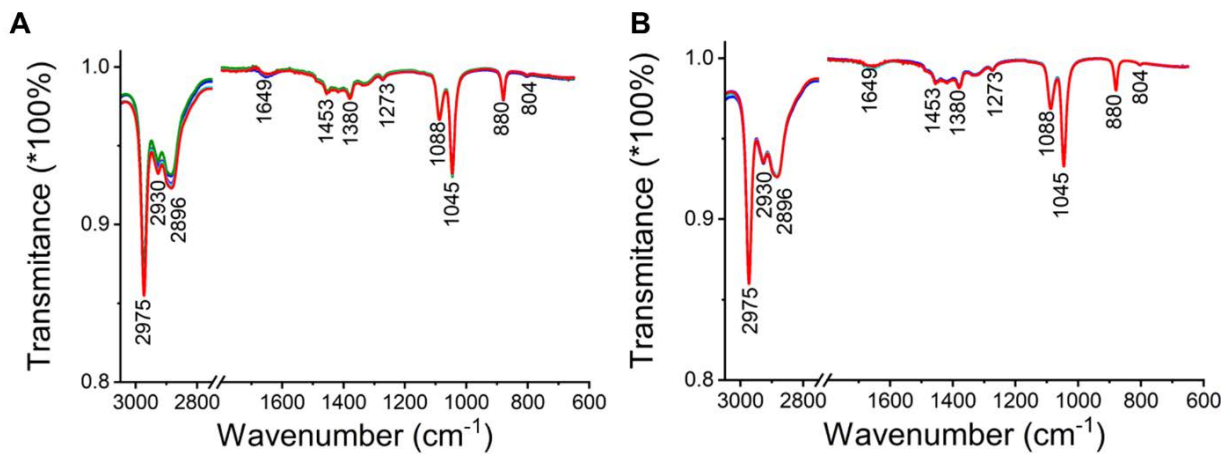


Figure 6. Comparison of the spectra of extracts – wheat watering with various H₂O₂ concentrations: 0.5% (navy blue), 1.5% (purple), 2.0% (green), 2.5% (blue), 3.0% (red) after 14 (A) and 28 (B) days

Conclusions

FTIR-ATR spectroscopy can be used to monitor the changes in the metabolic profile of plants, whereas chemometric analysis may be used as a complementary method. Based on conducted experiments it was shown that the analysis of only leaves does not provide enough information to identify the metabolomic differences caused by external factors, on the contrary the analysis of leaf extracts brings more information. What is interesting the changes in plants caused by external physical or chemical factors are visible after 14 days and could decrease after 28 days which may be caused by the activation of repair systems in plant cells. With the use of FTIR, based on the spectra, it is possible to distinguish the plants belonging to different families. Changes in the composition of plant metabolite can result from a variety of external sources like chemical and physical factors. Of naturally, it is also essential to emphasize that different environments can result in a range of metabolomic alterations. Both agriculture and other disciplines of life must comprehend the variation that exists in plants and how it will affect the metabolism of plant cells in the future. The uncontrolled increase in ROS in the cell containing hydrogen peroxide is an indicator of both types of stress. A variety of biomolecules can react with accumulated H_2O_2 , some of which are irreversible and render them inactive. It contributes also to activating PR (Pathogenesis Related) proteins, enzymes of the system antioxidant, and the synthesis of secondary metabolites, which can also be related to crop quality.

Acknowledgments

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Appendix 1

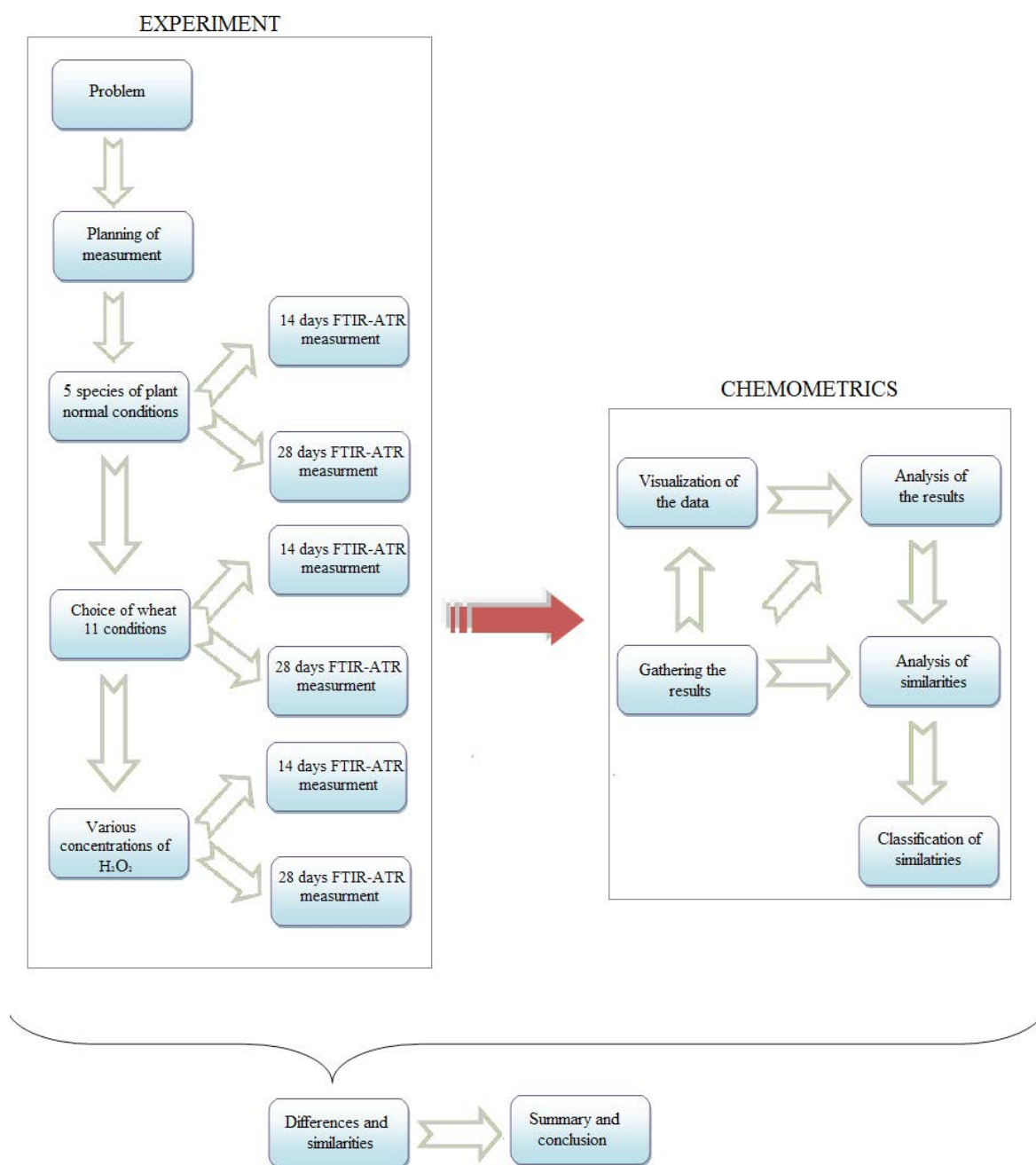


Figure S1. Schematic representation of methodology used in the experiments

Section II

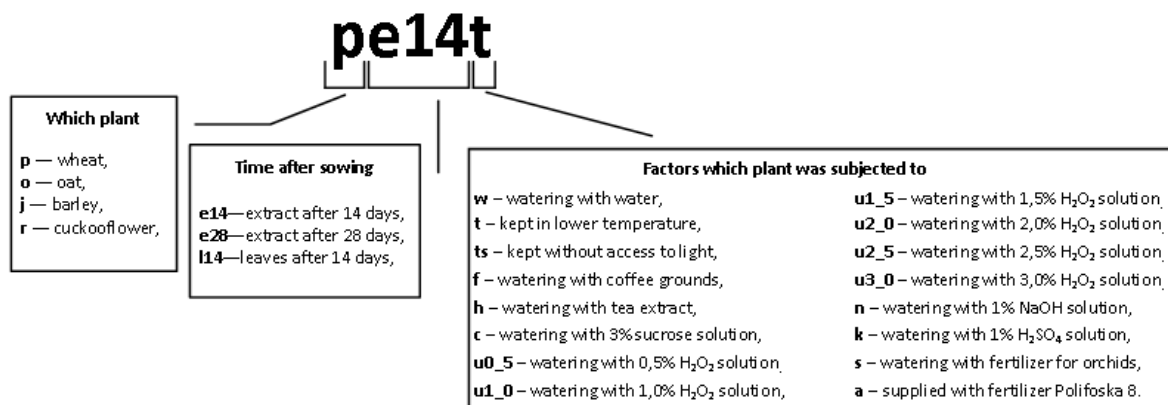


Figure S2. Schema of abbreviations used in chemometrics and spectral analysis

Table T1. Ranges of wavenumbers corresponding to given groups of metabolites [1,11–13,21–26]

Metabolite group	Wavenumber [nm]
Aminoacids ^[21]	1250–1500
	1250–1350 (S), 1375 (L), 1450 (V), 1465 (A), 1615(Q), 1650 (N)
	1720 (D)
	1415 & 1560 (E)
	1100, 1160, 1485–1550, 1610–1640 (K)
	700, 760, 1450, 1602 (F)
	1450, 1600 (Y) 1586, 1608 (R)
Aromatic compounds	617 ^[22]
	650–910 ^[13]
	690–900, 1000–1275, 1430–1600, 1700–2000, 3000–3100 ^[21]
Proteins	1568, 1639 ^[22]
	1550, 1650 ^[1]
	1065, 1080, 1489, 1500–1700 ^[13]
	1560, 1650 ^[12]
	1454–1460, 1500–1750, 1548–1550, 1648–1652, 1705 ^[11]
	600, 627, 725, 1299, 1567, 1653, 3110, 3300 ^[21] 1454–1460, 1548–1550, 1648–1652, 1705, 1735/1745 ^[23] 1299, 1548–1567, 1653 ^[24]
Phenols	900, 1200–1242 1500, 1714, 3000, 3100 ^[22]
	1000–1300, 3500–3550 ^[21]
Terpenes ^[25]	<1000
Alkaloids ^[26]	2100–2500
Lipids	1662, Phospholipids: 1257, 1267, 1277 ^[13]
	718, 720, 730, 820, 972, 1047, 1070, 1085, 1170, 1128, 1200–1400, 1378, 1405, 1460, 1463, 1468, 1472, 1473, 1485, 1730, 2850, 2870, 2920, 2956, 3010 ^[21]
	1401, 1460, 1468, 1470, 2850, 2870, 2920, 2956 ^[24]

Metabolite group	Wavenumber [nm]
Nucleic acids and nucleotides	1320 ^[1] 2850-2960 RNA: 996, 10150, 1038, 1084, 1120, 1160, 1218, 1244, 1690-1705 DNA: 916, 970, 1015, 1089, 1230, 1655-1660 ^[21] 804 ^[13]
Polysaccharides	923, 979, 1085, 1144, 1236, 2924 ^[22] 1090 ^[1] 1335 ^[13] 1200-1250 ^[12] 900-1200, 1052, 1085 ^[12] 1052, 1085 ^[23]
Sulfur compounds	450-550, 600-700, 1020-1060, 1120-1190, 1290-1390, 2500 ^[21]
Fatty acids	2800-3050 ^[1] 1730 ^[13] 3400, 2956, 2920, 2870, 2850, 1745/1735 ^[12] 2850, 2870, 2920, 2956 ^[23] 1743 ^[24]
Carbohydrates	770-922 ^[22] 750-1200 ^[13] 840, 845, 850, 869, 873, 900, 908, 910, 915, 916, 955, 1030, 1050, 1150-1170, 1240, 1260, 1510, 1590, 1600-1680, 1732 ^[21]

Group of amino acids and their abbreviations in brackets: S - serine, L - leucine, V - valine, A - alanine, Q - glutamine, N - asparagine, D - aspartic acid, E - glutamic acid, K - lysine, F - phenylalanine, Y - tyrosine, R - arginine.

Table T2. Metabolite groups for spectra of seedlings by spectrum and its first derivative [1,11-13,21-26]

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1) Water-soaked barley								
3382	3383	3422	3422	3371	3371	3422	3422	Water, T
2979	2986	2978	2986	-	-	-	-	T
2934	2935	2930	2948	2955	2958	2955	2966	L, N, T
2903	2904	2900	2909	2917	2922	2919	2932	L, N, P, T
-	2849	-	2843	2849	2851	2850	2853	L, N, T
2373	2377	2372	2379	2371	2382	2370	2362	K
2328	2325	2328	2321	2323	2325	2321	2333	K
2316	2317	2314	2315	2313	2309	2317	2319	K
2194	2195	2201	2196	2161	2156	2164	2171	K
1923	1924	1923	1924	1943	1943	1942	1935	Ar
1737	1738	1734	1735	1731	1739	1734	1739	Ar, B, L, T, W
1698	1699	1700	1699	1697	1684	1701	1699	Ar, B, N
1668	1667	-	1669	-	1668	-	1669	B, L, W
1651	1652	1651	1652	1651	1652	1652	1652	A(N), B, N, W
1645	1645	1645	1646	1645	1645	1644	1646	B, W
1634	1635	-	1635	1634	1635	1634	1635	A(K), B, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1615	1616	-	1616	1615	1616	1616	1616	A(K,Q), B, W
1569	1569	1571	1561	1558	1560	1550	1551	A(E,K), Ar, B
1489	1487	1481	1490	1489	1491	1489	1491	A(K), Ar, B
1472	1473	1478	1472	1473	1474	1473	1474	A, Ar, L
1455	1459	1455	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1420	A(E)
1386	1383	1383	1387	1367	1380	1374	1388	A(L), L, S
1339	1341	1330	1341	1317	1322	1317	1322	A(S), L, N, S
1274	1281	1278	1279	1255	1273	1255	1266	A(S), Ar, F, L
1205	1201	-	1206	1202	1206	1201	1206	Ar, F, L, P
1156	1164	1154	1162	1158	1169	1159	1168	A(K), Ar, F, N, P, S, W
1127	1129	-	1130	-	1125	-	1128	Ar, F, L, P, S, W
1121	1121	-	1122	-	-	-	-	Ar, F, P, S, W
1086	1094	1088	1196	1104	1112	1103	1111	A(K), Ar, F, P, W
1045	1052	1047	1154	1051	1079	1051	1067	Ar, L, N, P, S, W
878	883	879	884	896	904	897	905	Ar, I, F, W
-	810	802	808	-	-	-	-	Ar, I, N, W
730	729	-	-	731	733	731	732	Ar, I, B, L
-	-	704	706	718	721	720	721	Ar, I, L
667	668	668	668	667	668	668	668	Ar, I, S
2) Water-soaked oats								
3371	3371	3402	3402	3371	3371	3425	3425	Water, T
2975	2983	2977	2996	-	-	-	-	T
2928	2941	2929	2941	2956	2955	2954	2958	L, N, T
2894	2908	2900	2907	2917	2917	2919	2932	L, N, P, T
-	2846	-	2843	2849	2849	2850	2852	L, N, T
2373	2381	2370	2379	2369	2369	2372	2386	K
2328	2331	2328	2321	2323	2323	2326	2335	K
2313	2315	2314	2315	2311	2311	2314	2309	K
2194	2198	2200	2202	2162	2164	2146	2161	K
1923	1924	1926	1927	1943	1943	1943	1935	Ar
1738	1734	1734	1738	1732	1732	1734	1738	Ar, B, L, T, W
1698	1699	1700	1699	1697	1697	1701	1699	Ar, B, N
1668	1669	-	1669	-	-	-	-	B, L, W
1655	1652	1651	1652	1651	1651	1652	1652	A(N), B, N, W
1647	1648	-	1648	1644	1645	1645	1646	B, W
1634	1635	-	1634	1634	1634	1634	1635	A(K), B, W
1615	1616	1615	1615	1615	1615	1616	1616	A(K,Q), B, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1560	1560	1564	1590	1558	1558	1575	1557	A(E,K), Ar, B
1489	1491	1507	1490	1489	1489	1489	1491	A(K), Ar,B
1472	1477	1480	1472	1473	1473	1473	1474	A, Ar, L
1455	1459	1455	1457	1462	1462	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1418	1418	1420	A(E)
1382	1388	1382	1387	1369	1369	1374	1380	A(L), L, S
1339	1341	1328	1340	1320	1318	1317	1322	A(S), L, N, S
1274	1280	1274	1279	1256	1255	1249	1271	A(S), Ar, F, L
1206	1207	1176	1206	1201	1201	1202	1206	Ar, F, L, P
-	1151	-	1159	1160	1160	1159	1168	A(K), Ar, F, N, P, S, W
-	1129	-	1130	-	-	-	-	Ar, F, L, P, S, W
-	1121	-	1122	-	-	-	-	Ar, F, P, S, W
1088	1096	1089	1096	1104	1105	1104	1111	A(K), Ar, F, P, W
1047	1054	1048	1054	1054	1054	1055	1074	Ar, L, N, P, S, W
880	884	880	884	880	896	896	904	Ar, I, F, W
803	808	804	808	-	-	-	-	Ar, I, N, W
-	-	-	-	731	731	731	732	Ar, I, B, L
-	-	-	711	720	720	720	721	Ar, I, L
667	668	668	668	667	667	668	668	Ar, I, S
3) Water-soaked rye								
3374	3374	3408	3408	3383	3383	3423	3423	Water, T
2975	2982	2977	2985	-	-	-	-	T
2928	2932	2929	2941	2959	2963	2955	2958	L, N, T
2890	2890	2900	2908	2917	2920	2929	2925	L, N, P, T
-	2850	-	2842	2849	2952	2850	2852	L, N, T
2373	2377	2372	2373	2372	2381	2368	2362	K
2328	2326	2329	-	2323	2325	2326	2329	K
2316	2317	2315	2319	2313	2308	2317	2319	K
2194	2195	2193	2199	2162	2173	2168	2161	K
1923	1924	1926	1933	1942	1943	1943	1946	Ar
1733	1734	1734	1736	1734	1735	1734	1744	Ar, B, L, T, W
1698	1699	1700	1699	1698	1684	1701	1699	Ar, B, N
1668	1667	-	1669	-	1662	-	1684	B, L, W
1651	1652	1651	1652	1651	1652	1651	1652	A(N), B, N, W
1644	1645	1645	1646	1644	1645	1645	1646	B, W
1634	1635	-	1634	1634	1635	1634	1635	A(K), B, W
1615	1616	-	1616	1615	1616	1616	1616	A(K,Q), B, W
1569	1569	1570	1561	1574	1575	1575	1575	A(E,K), Ar, B

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1490	1490	1507	1490	1489	1491	1489	1491	A(K), Ar,B
1472	1473	1475	1472	1473	1473	1473	1474	A, Ar, L
1455	1459	1455	1457	1462	1456	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1420	A(E)
1382	1384	1382	1387	1368	1375	1373	1380	A(L), L, S
1339	1341	1330	1340	1317	1320	1339	1321	A(S), L, N, S
1274	1280	1275	1280	1260	1262	1250	1250	A(S), Ar, F, L
1202	1202	1196	1200	1201	1206	1201	1206	Ar, F, L, P
-	1164	1177	1178	1158	1166	1158	1168	A(K), Ar, F, N, P, S, W
-	1129	-	1120	-	-	-	1125	Ar, F, L, P, S, W
-	1121	-	1112	-	-	-	-	Ar, F, P, S, W
1088	1096	1089	1096	1104	1115	1104	1112	A(K), Ar, F, P, W
1047	1054	1048	1053	1053	1074	1052	1067	Ar, L, N, P, S, W
880	884	880	884	896	834	897	902	Ar, I, F, W
803	809	804	809	-	-	-	-	Ar, I, N, W
730	729	-	-	731	733	731	732	Ar, I, B, L
-	-	724	721	720	723	720	721	Ar, I, L
668	668	668	668	667	668	668	668	Ar, I, S
4) Water-soaked cuckooflower								
3382	3382	3417	3417	3383	3383	3446	3446	Water, T
2981	2989	2977	2986	-	-	-	-	T
2946	2949	2930	2941	2959	2963	2956	2957	L, N, T
2905	2912	2900	2901	2917	2920	2918	2922	L, N, P, T
-	2849	-	2842	2849	2852	2849	2852	L, N, T
2373	2381	2373	2374	2372	2381	2371	2378	K
2328	2330	2330	2338	2320	2326	2322	2328	K
2313	2313	2314	2315	2313	2314	2313	2315	K
2194	2197	2193	2195	2162	2174	2160	2161	K
1923	1924	1925	1923	1943	1919	1943	1943	Ar
1731	1734	1734	1735	1734	1750	1735	1752	Ar, B, L, T, W
1698	1698	1700	1699	1697	1695	1701	1699	Ar, B, N
1668	1667	1660	1662	1686	1684	-	1669	B, L, W
1651	1652	1651	1652	1651	1652	1652	1652	A(N), B, N, W
1645	1648	1645	1645	1645	1645	1645	1646	B, W
1634	1635	1634	1635	1634	1635	1634	1635	A(K), B, W
1615	1616	1616	1616	1615	1616	1616	1616	A(K,Q), B, W
1569	1560	1559	1575	1559	1560	1575	1575	A(E,K), Ar, B
1489	1491	1507	1490	1490	1491	1490	1491	A(K), Ar,B

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1472	1477	1481	1472	1472	1477	1474	1474	A, Ar, L
1455	1459	1455	1457	1455	1459	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1417	1420	A(E)
1387	1388	1382	1387	1374	1375	1374	1380	A(L), L, S
1339	1341	1329	1340	1339	1341	1319	1341	A(S), L, N, S
1274	1280	1274	1279	1244	1247	1242	1266	A(S), Ar, F, L
1206	1206	-	1205	-	1206	1205	106	Ar, F, L, P
1156	1157	-	1160	-	1166	1149	1167	A(K), Ar, F, N, P, S, W
1127	1129	-	1128	1148	-	-	-	Ar, F, L, P, S, W
1121	1121	-	1118	-	-	-	-	Ar, F, P, S, W
1083	1093	1088	1096	1104	1114	1105	1115	A(K), Ar, F, P, W
1045	1051	1047	1054	1052	1079	1066	1080	Ar, L, N, P, S, W
878	882	880	884	-	856	890	834	Ar, I, F, W
-	806	803	809	-	-	-	-	Ar, I, N, W
-	-	-	-	-	733	729	732	Ar, I, B, L
-	-	719	721	719	723	720	721	Ar, I, L
667	668	668	668	669	667	668	668	Ar, I, S
5) Water-soaked wheat								
3363	3363	3411	3411	3355	3355	3424	3424	Water, T
2975	2982	2977	2985	-	-	-	-	T
2928	2944	2929	2941	2954	2958	2955	2966	L, N, T
2894	2904	2900	2905	2918	2922	2920	2932	L, N, P, T
-	2946	-	2849	2849	2851	2850	2852	L, N, T
2373	2383	2370	2379	2373	2374	2370	2377	K
2328	2331	2330	2339	2341	2344	2348	2342	K
2313	2313	2314	2316	2322	2326	2328	2315	K
2206	2192	2197	2198	2162	2164	2164	2161	K
1923	1923	1923	1931	1943	1943	1918	1918	Ar
1733	1734	1730	1738	1735	1741	1735	1744	Ar, B, L, T, W
1698	1699	1700	1699	1698	1699	1701	1699	Ar, B, N
1668	1662	1660	1669	-	-	-	1684	B, L, W
1651	1652	1651	1652	1651	1652	1652	1652	A(N), B, N, W
1647	1648	1645	1645	1645	1645	1645	1646	B, W
1634	1635	-	1635	1635	1635	1634	1635	A(K), B, W
1615	1616	1615	1616	1615	1616	1616	1616	A(K,Q), B, W
1568	1560	1559	1560	1575	1575	1575	1575	A(E,K), Ar, B
1489	1490	1480	1490	1494	1491	1497	1491	A(K), Ar,B
1472	1473	-	1472	1472	1474	1473	1474	A, Ar, L

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1455	1459	1455	1456	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1420	A(E)
1381	1387	1382	1387	1369	1380	1374	1380	A(L), L, S
1339	1341	1330	1340	1317	1322	1317	1321	A(S), L, N, S
1274	1259	1274	1280	1260	1272	1256	1274	A(S), Ar, F, L
1206	1206	1213	1213	1201	1206	1202	1206	Ar, F, L, P
-	1164	1154	1162	1158	1169	1159	1170	A(K), Ar, F, N, P, S, W
-	1129	-	1134	-	1125	-	1126	Ar, F, L, P, S, W
-	1120	-	1123	-	-	-	-	Ar, F, P, S, W
1088	1096	1088	1096	1104	1112	1104	1112	A(K), Ar, F, P, W
1048	1055	1047	1054	1051	1080	1052	1080	Ar, L, N, P, S, W
880	885	880	884	897	903	897	904	Ar, I, F, W
803	808	803	809	-	-	-	-	Ar, I, N, W
-	-	-	-	731	732	731	732	Ar, I, B, L
-	-	-	708	720	721	720	721	Ar, I, L
666	667	667	668	667	668	668	668	Ar, I, S
6) Wheat kept in lower temperature								
3416	3416	3378	3378	3429	3429	3393	3393	Water, T
2977	2986	2976	2982	-	-	-	-	T
2928	2941	2929	2944	2955	2968	2955	2966	L, N, T
2900	2908	2900	2906	2919	2924	2918	2922	L, N, P, T
-	2845	-	2846	2850	2852	2849	2851	L, N, T
2372	2373	2374	2376	2368	2380	2374	2376	K
2331	2334	2333	2339	-	2334	2327	2347	K
2314	2316	2317	2318	2314	2319	2317	2318	K
2197	2201	2199	2209	2149	2150	2161	2170	K
1923	1924	1925	1929	1943	1919	1930	1921	Ar
1734	1738	1734	1735	1735	1744	1735	1752	Ar, B, L, T, W
1698	1699	1695	1696	1701	1699	1701	1696	Ar, B, N
1668	1669	1664	1669	-	1674	1685	1669	B, L, W
1651	1652	1652	1652	1652	1652	1652	1652	A(N), B, N, W
1645	1645	1645	1646	1645	1646	1645	1646	B, W
1634	1635	1632	1633	1634	1635	1634	1635	A(K), B, W
1615	1616	1614	1614	1615	1616	-	1617	A(K,Q), B, W
1560	1671	1576	1574	1575	1575	1550	1577	A(E,K), Ar, B
1481	1490	1496	1490	1490	1491	1496	1497	A(K), Ar, B
-	1472	1473	1470	1473	1474	1473	1474	A, Ar, L
1455	1456	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1418	1419	1419	1417	1414	1420	1420	1420	A(E)
1382	1387	1382	1387	1374	1388	1378	1388	A(L), L, S
1329	1340	1330	1339	1338	1341	1317	1337	A(S), L, N, S
1274	1280	1274	1283	1260	1266	1257	1264	A(S), Ar, F, L
-	1206	1196	1202	1202	1206	1202	1206	Ar, F, L, P
-	1162	1175	1161	1159	1168	1159	1169	A(K), Ar, F, N, P, S, W
-	1128	-	1128	-	1125	-	1125	Ar, F, L, P, S, W
-	1122	-	1123	-	-	-	-	Ar, F, P, S, W
1088	1096	1088	1096	1104	1111	1103	1111	A(K), Ar, F, P, W
1047	1054	1047	1054	1052	1080	1051	1079	Ar, L, N, P, S, W
880	884	889	8850	897	905	897	905	Ar, I, F, W
803	808	804	806	-	-	-	-	Ar, I, N, W
-	?	-	-	730	732	730	732	Ar, I, B, L
-	-	719	717	720	721	718	720	Ar, I, L
667	668	668	668	668	668	666	668	Ar, I, S
7) Wheat kept without access to sun								
3342	3342	3371	3371	3441	3441	3395	3395	Water, T
2977	2986	2975	2982	-	-	-	-	T
2930	2941	2928	2943	2954	2978	2954	2957	L, N, T
2930	2941	2896	2906	2919	2922	2918	2922	L, N, P, T
-	2846	-	2845	2850	2852	2849	2852	L, N, T
2376	2379	2374	2376	2366	2362	2374	2376	K
2328	2322	2321	2330	2328	2330	2329	2339	K
2313	2309	2317	2317	2314	2319	2317	2306	K
2197	2160	2198	2199	2164	2161	2162	2170	K
1923	1929	1928	1930	1943	1944	1938	1919	Ar
1734	1736	1734	1735	1734	1744	1734	1752	Ar, B, L, T, W
1697	1695	1696	1697	1700	1684	1695	1697	Ar, B, N
1667	1674	1672	1673	-	1674	1685	1669	B, L, W
1651	1652	1652	1652	1652	1652	1652	1652	A(N), B, N, W
1645	1645	1645	1646	1645	1646	1645	1646	B, W
1635	1635	1632	1633	1634	1635	1634	1635	A(K), B, W
1615	1616	1617	1614	1616	1616	-	1617	A(K,Q), B, W
1559	1559	1566	1568	1550	1560	1554	1575	A(E,K), Ar, B
1481	1490	1495	1497	1495	1491	1490	1497	A(K), Ar, B
-	1464	1473	1471	1473	1474	1473	1474	A, Ar, L
1455	1456	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1420	1418	1420	1420	1420	A(E)

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1383	1387	1382	1387	1374	1388	1371	1388	A(L), L, S
1328	1326	1331	1337	1317	1324	1317	1337	A(S), L, N, S
1274	1279	1275	1280	1252	1266	1249	1271	A(S), Ar, F, L
-	1204	-	1204	1202	1207	1202	1206	Ar, F, L, P
-	1160	-	1162	1159	1168	1158	1168	A(K), Ar, F, N, P, S, W
-	1128	-	1128	-	1125	-	1124	Ar, F, L, P, S, W
-	1122	-	1120	-	-	-	-	Ar, F, P, S, W
1088	1096	1089	1097	1104	1112	1103	1113	A(K), Ar, F, P, W
1047	1054	1048	1055	1051	1080	1054	1080	Ar, L, N, P, S, W
880	884	881	885	899	904	896	902	Ar, I, F, W
803	800	804	810	-	-	-	-	Ar, I, N, W
-	-	-	-	730	732	730	731	Ar, I, B, L
703	704	717	719	720	721	718	720	Ar, I, L
668	668	666	668	668	668	668	668	Ar, I, S
8) Wheat watered with coffee grounds								
3340	3340	3352	3352	3423	3423	3382	3382	Water, T
2974	2981	2974	2982	-	-	-	-	T
2927	2944	2927	2940	2954	2958	2954	2957	L, N, T
2885	2902	2888	2904	2920	2924	2918	2922	L, N, P, T
-	2846	-	2845	2850	2852	2849	2852	L, N, T
2379	2375	2373	2378	2371	2386	2374	2376	K
1217	2329	2333	2330	2324	2339	2329	2324	K
2318	2313	2314	2315	2318	2309	2317	2318	K
2197	2198	2195	2195	2160	2161	2166	2170	K
1924	1939	1925	1929	1942	1919	1918	1915	Ar
1734	1734	1734	1735	1735	1741	1734	1741	Ar, B, L, T, W
1698	1695	1695	1697	1701	1699	1701	1696	Ar, B, N
1667	1668	1664	1669	-	1674	1684	1689	B, L, W
1655	1652	1658	1653	1651	1652	1651	1652	A(N), B, N, W
1648	1648	1645	1645	1645	1648	1645	1646	B, W
-	1638	-	1633	1634	1635	1634	1635	A(K), B, W
1615	1616	1614	1614	1616	1616	-	1616	A(K,Q), B, W
1570	1575	1578	1574	1575	1575	1569	1557	A(E,K), Ar, B
-	1490	1507	1489	1508	1509	1496	1497	A(K), Ar, B
1475	1472	1478	1472	1473	1474	1473	1474	A, Ar, L
1455	1456	1455	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1420	1420	A(E)
1380	1387	1381	1387	1373	1388	1370	1383	A(L), L, S

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1330	1340	1330	1340	1320	1322	1316	1322	A(S), L, N, S
1274	1279	1274	1279	1260	1274	1257	1268	A(S), Ar, F, L
-	1203	-	1204	1201	1206	1201	1206	Ar, F, L, P
-	1161	-	-	1159	1169	1158	1168	A(K), Ar, F, N, P, S, W
-	1132	-	1128	-	1125	-	1125	Ar, F, L, P, S, W
-	-	-	1121	-	-	-	-	Ar, F, P, S, W
1089	1097	1089	1097	1104	1113	1103	1111	A(K), Ar, F, P, W
1049	1055	1049	1056	1051	1080	1051	1078	Ar, L, N, P, S, W
880	885	880	885	898	904	897	902	Ar, I, F, W
803	808	804	810	-	-	-	-	Ar, I, N, W
-	737	-	-	730	732	730	732	Ar, I, B, L
-	-	716	719	720	721	718	720	Ar, I, L
667	667	666	668	668	668	666	668	Ar, I, S
9) Wheat watered with tea extract								
3339	3339	3352	3352	3425	3425	3382	3382	Water, T
2974	2974	2974	2981	-	-	-	-	T
2927	2927	2928	2939	2955	2958	2956	2959	L, N, T
2885	2885	2886	2905	2920	2926	2918	2922	L, N, P, T
-	2846	-	2845	2850	2852	2849	2852	L, N, T
2372	2380	2375	2376	2371	2379	2374	2376	K
2329	2330	2329	2330	2338	2332	2329	2330	K
2318	2313	2317	2318	2315	2309	2317	2318	K
2200	2201	2204	2205	2165	2161	2160	2170	K
1924	1929	1925	1930	1943	1924	1918	1919	Ar
1737	1734	1734	1731	1735	1741	1735	1740	Ar, B, L, T, W
1698	1684	1685	1696	1700	1699	1701	1697	Ar, B, N
1668	1668	1665	1669	-	1684	1685	1683	B, L, W
1655	1651	1652	1653	1652	1652	1651	1652	A(N), B, N, W
1647	1648	1650	1646	1645	1646	1645	1646	B, W
1637	1634	1632	1633	1634	1635	1634	1635	A(K), B, W
1615	1616	1617	1614	1616	1616	-	1616	A(K,Q), B, W
1569	1572	1578	1573	1575	1560	1554	1557	A(E,K), Ar, B
-	1487	1508	1498	1489	1491	1496	1497	A(K), Ar, B
-	1472	1478	1471	1473	1474	1473	1474	A, Ar, L
1455	1456	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1423	1419	1418	1419	1426	1420	A(E)
1380	1387	1381	1387	1373	1388	1370	1383	A(L), L, S
1330	1336	1328	1339	1317	1341	1317	1322	A(S), L, N, S

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1274	1279	1274	1279	1256	1273	1259	1271	A(S), Ar, F, L
-	1205	1201	1202	1202	1206	1201	1206	Ar, F, L, P
-	1153	-	1159	1159	1168	1159	1168	A(K), Ar, F, N, P, S, W
-	1128	-	1128	-	1125	-	1126	Ar, F, L, P, S, W
-	-	-	1123	-	-	-	-	Ar, F, P, S, W
1089	1097	1089	1097	1104	1112	1104	1112	A(K), Ar, F, P, W
1045	1055	1048	1055	1053	1080	1052	1078	Ar, L, N, P, S, W
880	884	880	885	897	909	897	904	Ar, I, F, W
803	810	804	810	-	-	-	-	Ar, I, N, W
-	730	-	-	731	732	731	733	Ar, I, B, L
-	-	716	719	720	721	719	720	Ar, I, L
668	668	668	668	668	668	666	668	Ar, I, S
10) Wheat watered with 3% sucrose solution								
3344	3344	3346	3346	3438	3438	3361	3361	Water, T
2974	2974	2974	2981	-	-	-	-	T
2927	2927	2928	2938	2955	2959	2954	2958	L, N, T
2886	2886	2886	2904	2919	2924	2918	2923	L, N, P, T
-	2847	-	2848	2850	2852	2849	2852	L, N, T
2374	2375	2376	2374	2376	2379	2374	2376	K
2329	2320	2323	2330	2348	2340	2345	2347	K
2314	2313	2317	2318	2313	2309	2317	2318	K
2200	2194	2204	2209	2160	2161	2161	2170	K
1923	1930	1925	1929	1943	1919	1929	1921	Ar
1737	1734	1735	1731	1734	1744	1735	1740	Ar, B, L, T, W
1698	1695	1696	1696	1700	1695	1696	1697	Ar, B, N
1668	1668	1665	1669	-	1669	-	1668	B, L, W
1655	1652	1652	1650	1652	1652	1652	1652	A(N), B, N, W
1647	1648	1649	1646	1645	1646	1645	1646	B, W
1634	1634	1632	1633	1634	1635	1634	1635	A(K), B, W
1615	1616	1619	1619	1620	1616	-	1616	A(K,Q), B, W
1559	1560	1560	1555	1550	1560	1567	1557	A(E,K), Ar, B
1485	1490	1509	1503	1490	1491	1496	1497	A(K), Ar, B
1475	1473	-	1471	1473	1474	1473	1474	A, Ar, L
1455	1456	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1419	1418	1420	1420	1420	A(E)
1380	1387	1380	1387	1374	1388	1370	1380	A(L), L, S
1330	1340	1330	1339	1317	1322	1317	1323	A(S), L, N, S
1274	1280	1274	1279	1250	1266	1249	1250	A(S), Ar, F, L

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
-	1203	-	1204	1203	1207	1200	1206	Ar, F, L, P
-	1158	-	1159	1159	-	1161	1169	A(K), Ar, F, N, P, S, W
-	1127	-	1130	-	-	-	-	Ar, F, L, P, S, W
-	1123	-	-	-	-	-	-	Ar, F, P, S, W
1089	1097	1088	1092	1104	1112	1103	1112	A(K), Ar, F, P, W
1048	1055	10480	1055	1050	1080	1048	1080	Ar, L, N, P, S, W
880	885	880	885	897	905	897	904	Ar, I, F, W
803	807	804	808	-	-	-	-	Ar, I, N, W
-	731	-	-	730	732	730	732	Ar, I, B, L
-	-	723	719	720	721	719	720	Ar, I, L
668	668	665	667	668	668	666	668	Ar, I, S
11) Wheat watered with 0.5% H₂O₂ solution								
3341	3341	3339	3339	3348	3348	3352	3352	Water, T
2973	2980	2973	2980	-	-	-	-	T
2926	2936	2928	2934	2954	2958	2955	2958	L, N, T
2918	2904	2884	2905	2917	2921	2917	2921	L, N, P, T
2886	2850	-	2845	2849	2851	2849	2851	L, N, T
2372	2376	2373	2376	2369	2376	2372	2376	K
2327	2325	2332	2334	2340	2358	2348	2350	K
2317	2319	2321	2324	2323	2325	2323	2325	K
2195	2196	2198	2195	2162	2163	2162	2168	K
1923	1924	1925	1929	1980	1936	1980	1968	Ar
1738	1738	1738	1734	1734	1739	1733	1739	Ar, B, L, T, W
1698	1699	1698	1699	1698	1695	1698	1695	Ar, B, N
1668	1668	1668	1669	-	1668	1667	1668	B, L, W
1651	1652	1651	1652	1651	1652	1651	1652	A(N), B, N, W
1647	1645	1647	1648	1644	1645	1644	1648	B, W
1634	1635	1637	1638	1634	1635	1634	1635	A(K), B, W
1615	1616	1615	1616	1615	1616	1615	1616	A(K,Q), B, W
1568	1569	1569	1570	1575	1560	1574	1575	A(E,K), Ar, B
1486	1487	-	1490	1487	1491	1489	1500	A(K), Ar,B
1472	1473	-	1473	1473	1474	1472	1474	A, Ar, L
1455	1459	1455	1452	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1419	A(E)
1380	1384	1380	1387	1368	1380	1374	1375	A(L), L, S
1338	1341	1329	1340	1317	1322	1317	1320	A(S), L, N, S
1273	1279	1274	1279	1259	1266	1248	1274	A(S), Ar, F, L
-	-	-	1205	1201	1206	1201	1206	Ar, F, L, P

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1148	1162	-	1162	1158	1168	1158	1168	A(K), Ar, F, N, P, S, W
-	1134	-	1132	-	1125	-	1132	Ar, F, L, P, S, W
-	-	-	1121	-	-	-	1124	Ar, F, P, S, W
1088	1096	1088	1095	1102	1111	1102	1111	A(K), Ar, F, P, W
1046	1052	1046	1052	1047	1064	1047	1068	Ar, L, N, P, S, W
880	884	880	884	897	903	897	903	Ar, I, F, W
803	808	803	809	-	-	-	-	Ar, I, N, W
720	731	-	-	730	732	730	732	Ar, I, B, L
-	-	-	-	719	721	719	720	Ar, I, L
668	669	666	667	666	668	667	668	Ar, I, S
12) Wheat watered with 1.0% H₂O₂ solution								
3353	3382	3354	3354	3426	3426	3381	3381	Water, T
2974	2986	2974	2981	-	-	-	-	T
2928	2936	2928	2941	2956	2958	2955	2959	L, N, T
2886	2904	2890	2904	2919	2924	2920	2922	L, N, P, T
-	2847	-	2847	2850	2852	2849	2852	L, N, T
2372	2382	2373	2376	2371	2380	2374	2376	K
2329	2321	2329	2330	2322	2327	2329	2324	K
2317	2314	2317	2318	2314	2309	2317	2318	K
2193	2198	2198	2205	2160	2161	2160	2162	K
1923	1924	1925	1930	1943	1923	1929	1919	Ar
1738	1739	1734	1735	1734	1744	1735	1740	Ar, B, L, T, W
1698	1699	1695	1696	1700	1699	1701	1697	Ar, B, N
1668	1668	1665	1669	-	1674	-	1669	B, L, W
1655	1652	1657	1652	1652	1652	1652	1652	A(N), B, N, W
1647	1644	1645	1646	1645	1645	1645	1646	B, W
1634	1635	1632	1633	1635	1635	1634	1635	A(K), B, W
1615	1616	1617	1614	1616	1616	-	1616	A(K,Q), B, W
1569	1570	1566	1561	1556	1560	1554	1557	A(E,K), Ar, B
1486	1487	-	1490	1497	1491	1496	1497	A(K), Ar,B
1475	1473	1478	1470	1473	1474	1473	1474	A, Ar, L
1455	1448	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1419	1418	1419	1410	1420	A(E)
1380	1387	1380	1387	1374	1388	1370	1381	A(L), L, S
1330	1340	1330	1339	1320	1321	1317	1323	A(S), L, N, S
1274	1279	1274	1280	1250	1273	1259	1271	A(S), Ar, F, L
1196	1201	-	1202	1202	1207	1201	1206	Ar, F, L, P
-	1164	-	1160	1160	1168	1159	1168	A(K), Ar, F, N, P, S, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
-	1129	-	1128	-	1126	-	1126	Ar, F, L, P, S, W
-	1123	-	1123	-	-	-	-	Ar, F, P, S, W
1089	1097	1089	1097	1104	1112	1104	1117	A(K), Ar, F, P, W
1048	1055	1048	1055	1052	1080	1051	1080	Ar, L, N, P, S, W
880	885	880	885	898	905	897	904	Ar, I, F, W
803	807	804	806	-	-	-	-	Ar, I, N, W
-	727	-	-	730	732	730	732	Ar, I, B, L
-	-	707	719	719	721	7190	720	Ar, I, L
668	668	665	668	668	668	667	668	Ar, I, S
13) Wheat watered with 1.5% H₂O₂ solution								
3340	3340	3349	3349	3351	3351	3352	3352	Water, T
2973	2980	2973	2981	-	-	-	-	T
2927	2937	2927	2933	2954	2958	2954	2958	L, N, T
2886	2902	2883	2904	2917	2921	2917	2921	L, N, P, T
-	2849	-	2846	2849	2851	2849	2851	L, N, T
2372	2376	2372	2376	2361	2362	2372	2376	K
2324	2324	2328	2329	2323	2325	2323	2325	K
2318	2315	2314	2315	2309	2305	2315	2320	K
2195	2196	2196	2198	2162	2163	2162	2167	K
1923	1924	1923	1924	1943	1944	1918	1919	Ar
1738	1734	1733	1734	1734	1739	1732	1738	Ar, B, L, T, W
1698	1684	1683	1684	1686	1684	1698	1684	Ar, B, N
1668	1674	1668	1668	-	1662	1668	1668	B, L, W
1654	1652	1655	1652	1651	1652	1651	1652	A(N), B, N, W
1647	1645	1647	1648	1644	1645	1647	1648	B, W
1634	1635	1637	1634	1634	1635	1634	1635	A(K), B, W
1615	1616	1617	1616	1615	1616	1615	1616	A(K,Q), B, W
1568	1560	1559	1560	1558	1560	1559	1560	A(E,K), Ar, B
1486	1491	1497	1491	1489	1491	1490	1491	A(K), Ar, B
1472	1473	1471	1473	1473	1474	1472	1474	A, Ar, L
1455	1459	1455	1459	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1419	A(E)
1380	1387	1380	1387	1373	1387	1374	1388	A(L), L, S
1338	1341	1339	1340	1317	1323	1317	1320	A(S), L, N, S
1273	1278	1274	1279	1258	1274	1249	1274	A(S), Ar, F, L
1202	1201	-	1204	1201	1206	1201	1206	Ar, F, L, P
1148	1162	1177	1173	1158	1168	1159	1168	A(K), Ar, F, N, P, S, W
-	1134	-	1129	-	1125	-	1125	Ar, F, L, P, S, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
-	-	-	1121	-	-	-	-	Ar, F, P, S, W
1088	1095	1088	1095	1102	1111	1102	1111	A(K), Ar, F, P, W
1046	1052	1046	1052	1037	1055	1037	1055	Ar, L, N, P, S, W
880	884	880	884	897	903	898	904	Ar, I, F, W
803	808	803	809	-	-	-	-	Ar, I, N, W
-	-	-	-	730	732	730	732	Ar, I, B, L
741	721	-	-	719	721	719	721	Ar, I, L
668	669	664	667	667	667	667	668	Ar, I, S
14) Wheat watered with 2.0% H₂O₂ solution								
3344	3344	3339	3339	3358	3358	3352	3352	Water, T
2973	2980	2973	2980	-	-	-	-	T
2927	2936	2927	2940	2954	2958	2954	2958	L, N, T
2886	2902	2884	2904	2917	2921	2917	2921	L, N, P, T
-	2845	-	2846	2849	2851	2849	2851	L, N, T
2372	2376	2373	2376	2371	2376	2372	2381	K
2330	2332	2333	2335	2341	2349	2348	2350	K
2318	2319	2318	2320	2323	2325	2323	2325	K
2201	2196	2193	2197	2162	2163	2162	2168	K
1923	1924	1923	1924	1943	1944	1943	1944	Ar
1743	1739	1733	1734	1734	1739	1734	1739	Ar, B, L, T, W
1698	1695	1698	1695	1686	1684	1698	1695	Ar, B, N
1668	1668	1668	1668	-	1674	1668	1674	B, L, W
1651	1651	1651	1652	1651	1652	1651	1652	A(N), B, N, W
1647	1648	1648	1648	1644	1645	1644	1648	B, W
1634	1634	1634	1635	1634	1635	1634	1635	A(K), B, W
1615	1616	1615	1616	1615	1616	1615	1616	A(K,Q), B, W
1559	1551	1559	1560	1559	1560	1559	1560	A(E,K), Ar, B
1486	1490	1488	1491	1489	1491	1490	1491	A(K), Ar, B
1472	1473	1471	1473	1473	1474	1473	1474	A, Ar, L
1455	1459	1455	1459	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1419	A(E)
1380	1387	1380	13870	1368	1380	1374	1388	A(L), L, S
1339	1341	1338	1341	1318	1323	1317	1320	A(S), L, N, S
1273	1278	1274	1279	1248	1274	1260	1274	A(S), Ar, F, L
-	1201	1191	12050	1202	1206	1201	1205	Ar, F, L, P
1156	1163	-	1162	1158	1168	1158	1169	A(K), Ar, F, N, P, S, W
-	1133	-	1128	-	1126	-	-	Ar, F, L, P, S, W
-	-	-	1121	-	-	-	-	Ar, F, P, S, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1088	1095	1089	1095	1103	1101	1102	1111	A(K), Ar, F, P, W
1046	1052	1046	1053	1036	1078	1037	1064	Ar, L, N, P, S, W
880	884	880	883	897	903	897	903	Ar, I, F, W
803	809	803	807	-	-	-	-	Ar, I, N, W
-	-	-	-	730	732	730	732	Ar, I, B, L
741	721	-	-	719	721	719	721	Ar, I, L
668	669	666	667	667	668	667	668	Ar, I, S
15) Wheat watered with 2.5% H₂O₂ solution								
3340	3340	3339	3339	3354	3354	3342	3342	Water, T
2973	2980	2973	2981	-	-	-	-	T
2927	2936	2927	2940	2956	2962	2954	2958	L, N, T
2886	2902	2883	2904	2917	2921	2917	2921	L, N, P, T
-	2845	-	2841	2849	2851	2849	2851	L, N, T
2372	2376	2372	2376	2372	2376	2373	2376	K
2329	2332	2330	2335	2323	2325	2322	2325	K
2318	2318	2318	2320	2308	2302	2303	2303	K
2195	2196	2196	2198	2161	2153	2162	2168	K
1923	1924	1923	1924	1943	1944	1923	1919	Ar
1749	1734	1738	1734	1734	1744	1733	1739	Ar, B, L, T, W
1705	1695	1698	1695	1694	1695	1698	1695	Ar, B, N
1668	1662	1668	1668	1686	1684	1683	1684	B, L, W
1651	1652	1655	1652	1651	1652	1651	1652	A(N), B, N, W
1645	1645	1647	1648	1644	1645	1647	1648	B, W
1634	1634	1637	1634	1634	1635	1634	1635	A(K), B, W
1615	1616	1619	1616	1615	1616	1615	1616	A(K,Q), B, W
1569	1560	1559	1560	1558	1560	1557	1560	A(E,K), Ar, B
1486	1491	1488	1482	1490	1491	1490	1491	A(K), Ar,B
1472	1473	1472	14730	1472	1474	1473	1474	A, Ar, L
1455	1459	1455	1459	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1419	A(E)
1380	1387	1380	1387	1373	1387	1378	1388	A(L), L, S
1339	1341	1339	1341	1318	1323	1317	1322	A(S), L, N, S
1273	1278	1274	1280	1258	1275	1249	1272	A(S), Ar, F, L
-	-	1176	1174	-	1206	1200	1206	Ar, F, L, P
-	1162	1146	1148	1158	1168	1160	1169	A(K), Ar, F, N, P, S, W
-	1133	-	1128	-	1125	-	1125	Ar, F, L, P, S, W
-	-	-	1120	-	-	-	-	Ar, F, P, S, W
1088	1095	1088	1095	1102	1111	1102	1111	A(K), Ar, F, P, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
1046	1052	1048	1052	1036	1078	1037	1064	Ar, L, N, P, S, W
880	884	880	883	896	903	897	903	Ar, I, F, W
803	807	803	807	-	-	-	-	Ar, I, N, W
742	725	-	-	730	732	730	732	Ar, I, B, L
-	-	-	-	719	721	719	720	Ar, I, L
668	669	666	668	667	668	667	668	Ar, I, S
16) Wheat watered with 3.0% H₂O₂ solution								
3340	3340	3339	3339	3351	3351	3356	3356	Water, T
2973	2980	2973	2981	-	-	-	-	T
2937	2937	2928	2933	2954	2957	2955	2958	L, N, T
2884	2902	2883	2904	2917	2922	2917	2921	L, N, P, T
-	2840	-	2846	2849	2851	2849	2851	L, N, T
2372	2377	2372	2376	2372	2376	2372	2376	K
2334	2325	2324	2322	2323	2325	2322	2325	K
2318	2318	2318	2315	2314	2315	2320	2315	K
2192	2194	2196	2194	2161	2153	2162	2153	K
1923	1924	1923	1924	1943	1944	1930	1944	Ar
1734	1738	1733	1734	1734	1744	1733	1739	Ar, B, L, T, W
1694	1695	1698	1695	1698	1694	1698	1695	Ar, B, N
1668	1668	1668	1668	1682	1684	1668	1684	B, L, W
1654	1652	1655	1652	1651	1652	1651	1652	A(N), B, N, W
1647	1647	1647	1648	1644	1645	1647	1648	B, W
1634	1634	1637	1639	1634	1635	1634	1635	A(K), B, W
1615	1616	1618	1616	1615	1616	1615	1616	A(K,Q), B, W
1568	1575	1568	1575	1574	1575	1574	1575	A(E,K), Ar, B
1486	1490	1489	1491	1507	1500	1490	1491	A(K), Ar, B
1471	1477	1475	1473	1473	1474	1473	1474	A, Ar, L
1456	1459	1455	1459	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1418	1419	1418	1419	1418	1419	A(E)
1380	1387	1380	1387	1373	1380	1374	1388	A(L), L, S
1339	1341	1339	1341	1318	1323	1318	1320	A(S), L, N, S
1273	1278	1274	1280	1258	1250	1248	1256	A(S), Ar, F, L
-	1202	1200	1201	1201	1206	1202	1206	Ar, F, L, P
-	1175	1177	1180	1158	1168	1158	1169	A(K), Ar, F, N, P, S, W
-	1133	-	1133	-	1126	-	1125	Ar, F, L, P, S, W
-	1120	-	1121	-	-	-	-	Ar, F, P, S, W
1088	1096	1088	1096	1102	1111	1103	1111	A(K), Ar, F, P, W
1046	1052	1046	1052	1036	1064	1036	1064	Ar, L, N, P, S, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
880	883	880	884	896	903	897	903	Ar, I, F, W
803	807	803	808	-	-	-	-	Ar, I, N, W
741	729	-	-	730	732	730	734	Ar, I, B, L
-	-	-	-	719	721	719	721	Ar, I, L
668	669	666	667	667	668	667	668	Ar, I, S
17) Wheat watered with 1.0% NaOH solution								
3340	3340	3352	3352	3424	3424	3392	3392	Water, T
2974	2980	2974	2981	-	-	-	-	T
2927	2943	2928	2939	2955	2958	2955	2958	L, N, T
2885	2904	2886	2905	2920	2925	2918	2923	L, N, P, T
-	2846	-	2846	2850	2852	2849	2895	L, N, T
2372	2382	2376	2376	2369	2373	2374	2376	K
2329	2331	2333	2339	2331	2333	2329	2339	K
2318	2313	2317	2318	2313	2309	2317	2318	K
2206	2187	2199	2201	2160	2161	2161	2162	K
1923	1933	1925	1935	1939	1920	1933	1921	Ar
1733	1734	1735	1735	1735	1741	1734	1740	Ar, B, L, T, W
1698	1684	1685	1682	1701	1699	1685	1697	Ar, B, N
1663	1663	1665	1667	-	1668	-	1669	B, L, W
1655	1652	13652	1653	1652	1652	1652	1652	A(N), B, N, W
1647	1648	1649	1646	1645	1645	1645	1646	B, W
1637	1634	1632	1633	1634	1635	1634	1635	A(K), B, W
1618	1616	1617	1614	1616	1616	-	1617	A(K,Q), B, W
1560	1560	1560	1555	1575	1575	1564	1557	A(E,K), Ar, B
1486	1482	1508	1490	1489	1491	1496	1497	A(K), Ar,B
1475	1472	1478	1471	1473	1474	1473	1474	A, Ar, L
1455	1459	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1419	1418	1420	1419	1420	A(E)
1380	1387	1381	1387	1374	1388	1371	1383	A(L), L, S
1330	1336	1331	1339	1317	1322	1314	1323	A(S), L, N, S
1274	1279	1274	1279	1260	1266	1255	1268	A(S), Ar, F, L
-	1202	-	1203	1202	1206	1201	1206	Ar, F, L, P
-	1161	-	1159	1159	1169	1158	1169	A(K), Ar, F, N, P, S, W
-	1129	-	1128	-	1125	-	1128	Ar, F, L, P, S, W
-	-	-	1123	-	-	-	-	Ar, F, P, S, W
1089	1097	1089	1097	1104	1112	1104	1113	A(K), Ar, F, P, W
1049	1055	1048	1055	1051	1080	1049	1080	Ar, L, N, P, S, W
880	884	880	885	898	903	897	902	Ar, I, F, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
803	807	804	810	-	-	-	-	Ar, I, N, W
-	731	-	-	731	734	731	732	Ar, I, B, L
-	-	707	719	720	721	719	720	Ar, I, L
668	668	665	668	668	668	666	668	Ar, I, S
18) Wheat watered with 1.0% H₂SO₄ solution								
3344	3344	3344	3344	3423	3423	3385	3385	Water, T
2974	2981	2974	2981	-	-	-	-	T
2927	2942	2928	2941	2955	2956	2955	2960	L, N, T
2884	2903	2886	2905	2919	2924	2918	2923	L, N, P, T
-	2845	-	2848	2850	2852	2849	2852	L, N, T
2362	2365	2374	2376	2370	2386	2374	2376	K
2327	2327	2328	2330	2330	2334	2333	2339	K
2316	2317	2317	2318	23150	2309	2317	2318	K
2198	2199	2199	2199	2160	2160	2161	2171	K
1927	1930	1925	1930	1943	1943	1918	1921	Ar
1734	1734	1735	17310	1735	1752	1735	1740	Ar, B, L, T, W
1699	1700	1696	1696	1701	1699	1701	1696	Ar, B, N
1662	1663	1664	1667	-	1677	-	-	B, L, W
1652	1653	1658	1653	1652	1652	1652	1652	A(N), B, N, W
-	1646	1649	1646	1645	1646	1645	1646	B, W
1635	1635	1631	1633	1634	1635	1634	1635	A(K), B, W
1616	1617	1617	1614	1616	1619	-	1617	A(K,Q), B, W
1560	1560	1561	1568	1550	1560	1554	1558	A(E,K), Ar, B
-	1490	1508	1505	1497	1491	1496	1497	A(K), Ar, B
-	1472	1478	1471	1473	1474	1473	1474	A, Ar, L
1455	1457	1458	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1419	1418	1420	1419	1417	A(E)
1380	1387	1381	1387	1374	1381	1377	1389	A(L), L, S
1328	1340	1331	1339	1347	1323	1317	1323	A(S), L, N, S
-	1279	1274	1279	1249	1273	1255	1271	A(S), Ar, F, L
-	1208	1192	1203	1202	1206	1200	1206	Ar, F, L, P
-	1162	1180	1162	1159	1168	1158	1169	A(K), Ar, F, N, P, S, W
-	-	-	1128	-	1125	-	1126	Ar, F, L, P, S, W
-	-	-	-	-	-	-	-	Ar, F, P, S, W
1086	1097	1089	1098	1104	1112	1104	1113	A(K), Ar, F, P, W
1045	1055	1049	1055	1052	1080	1051	1080	Ar, L, N, P, S, W
880	884	881	885	898	904	897	904	Ar, I, F, W
803	809	804	810	-	-	-	-	Ar, I, N, W

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
-	734	-	-	730	732	731	733	Ar, I, B, L
-	-	709	719	720	721	719	720	Ar, I, L
667	668	665	668	667	668	666	668	Ar, I, S
19) Wheat fed with orchid fertilizer								
3340	3340	3361	3361	3429	3429	3394	3394	Water, T
2974	2982	2975	2982	-	-	-	-	T
2928	2941	2928	2939	2955	2968	2955	2967	L, N, T
2886	2932	2893	2905	2919	2924	2918	2922	L, N, P, T
-	2904	-	2848	2850	2852	2849	2852	L, N, T
2364	2365	2363	2373	2372	2377	2374	2376	K
2329	2330	2329	2330	2328	2321	2329	2324	K
2314	2318	2317	2318	2314	2309	2317	2318	K
2202	2198	2198	2200	2145	2197	2161	2162	K
1926	1929	1928	1929	1943	1945	1918	1915	Ar
1738	1733	1734	1735	1735	1738	1735	1738	Ar, B, L, T, W
1698	1700	1695	1695	1701	1699	1701	1697	Ar, B, N
1668	1669	1665	1669	-	1675	-	1669	B, L, W
1651	1652	1652	1653	1652	1652	1652	1652	A(N), B, N, W
1645	1645	1645	1646	1645	1646	1645	1646	B, W
1634	1635	1632	1632	1634	1635	1634	1635	A(K), B, W
1615	1616	1617	1614	1616	1616	-	1616	A(K,Q), B, W
1568	1560	1566	1561	1556	1560	1554	1558	A(E,K), Ar, B
1479	1490	1478	1487	1489	1491	1496	1497	A(K), Ar, B
1473	1472	1473	1471	1473	1474	1473	1474	A, Ar, L
1455	1456	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1420	1418	1420	1420	1417	A(E)
1380	1384	1381	1387	1374	1387	1377	1380	A(L), L, S
1330	1340	1329	1339	1317	1321	1318	1322	A(S), L, N, S
1274	1279	1274	1280	1260	1266	1249	1264	A(S), Ar, F, L
-	1205	-	1202	1201	1207	1201	1206	Ar, F, L, P
-	1162	-	1162	1159	1168	1158	1168	A(K), Ar, F, N, P, S, W
-	1128	-	1128	-	1125	-	1125	Ar, F, L, P, S, W
-	-	-	-	-	-	-	-	Ar, F, P, S, W
1089	1097	1089	1097	1104	1113	1103	1111	A(K), Ar, F, P, W
1048	1055	1048	1055	1052	1067	1052	1080	Ar, L, N, P, S, W
880	885	880	885	897	904	896	904	Ar, I, F, W
803	808	804	810	-	-	-	-	Ar, I, N, W
-	-	-	-	731	732	730	732	Ar, I, B, L

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
-	709	709	719	720	721	719	720	Ar, I, L
667	668	666	668	668	668	668	668	Ar, I, S
20) Wheat fed with fertilizer Polifoska 8								
3340	3340	3374	3374	3425	3425	3392	3392	Water, T
2974	2981	2976	2983	-	-	-	-	T
2927	2931	2929	2944	2955	2958	2955	2966	L, N, T
2883	2905	2899	2906	2920	2926	2918	2922	L, N, P, T
-	2849	-	2845	2850	2852	2849	2852	L, N, T
2360	2378	2374	2376	2376	2386	2374	2376	K
2332	2334	2329	2330	2341	2338	2345	2347	K
2314	2319	2317	2318	2318	2318	2317	2318	K
2199	2203	2205	2205	2160	2161	2161	2169	K
1925	1929	1923	1935	1943	1935	1918	1915	Ar
1738	1733	1734	1735	1735	1741	1735	1738	Ar, B, L, T, W
1694	1700	1700	1701	1701	1699	1701	1696	Ar, B, N
1668	1669	1664	1669	-	1675	-	1669	B, L, W
1651	1652	1652	1652	1652	1652	1652	1652	A(N), B, N, W
1645	1645	1645	1646	1645	1646	1645	1646	B, W
1631	1635	-	1633	1634	1635	1634	1635	A(K), B, W
1615	1616	-	1614	1616	1616	-	1617	A(K,Q), B, W
1563	1564	1566	1561	1575	1575	1560	1558	A(E,K), Ar, B
1489	1496	1495	1497	1489	1491	1496	1497	A(K), Ar, B
1474	1472	1480	1470	1473	1474	1473	1474	A, Ar, L
1455	1456	1456	1457	1462	1464	1462	1464	A(A), Ar, B, L
1418	1419	1419	1417	1418	1420	1420	1420	A(E)
1380	1384	1382	1387	1373	1387	1377	1380	A(L), L, S
1328	1330	1330	1329	1317	1323	1317	1323	A(S), L, N, S
1274	1279	1274	1280	1249	1250	1245	1250	A(S), Ar, F, L
-	1206	1201	1202	1201	1206	1201	1206	Ar, F, L, P
-	1158	1175	1157	1159	1169	1158	1169	A(K), Ar, F, N, P, S, W
-	1128	-	1128	-	1126	-	1125	Ar, F, L, P, S, W
-	1124	-	1121	-	-	-	-	Ar, F, P, S, W
1089	1097	1088	1096	1103	1112	1103	1114	A(K), Ar, F, P, W
1049	1055	1047	1054	1052	1080	1051	1079	Ar, L, N, P, S, W
880	885	880	884	897	904	897	904	Ar, I, F, W
803	808	803	810	-	-	-	-	Ar, I, N, W
-	729	-	-	731	732	731	732	Ar, I, B, L
-	-	717	719	720	721	718	720	Ar, I, L

Wavenumber at which the peak attains maximum value [cm ⁻¹]								Assignment to metabolic group
Extract after 14 days		Extract after 28 days		Leaves after 14 days		Leaves after 28 days		
Spectra	FDa	Spectra	FDa	Spectra	FDa	Spectra	FDa	
667	668	666	668	668	668	666	668	Ar, I, S

FDa first-order derivative of FTIR spectra; ν - stretching vibration, δ - deformation vibration, sym- symmetric, as - antisymmetric, T - fatty acids, L - lipids, N - nucleic acids and nucleotides, P - polysaccharides, B - proteins, W - hydrocarbons, A - free amino acids, Ar - aromatic, S - sulfur compounds, K - alkaloids, F - phenolic, I - terpenes. Group of amino acids and their abbreviations in brackets: S - serine, L - leucine, V - valine, A - alanine, Q- glutamine, N - asparagine, D - aspartic acid, E - glutamic acid, K - lysine, F - phenylalanine, Y - tyrosine, R - arginine.