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FT-Raman spectroscopy as a tool in evaluation the response of plants to drought stress

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Abstract: The aim of study was to evaluate the usefulness of FT-Raman spectroscopy in assessing stress-induced metabolic changes in plants. 20-d-old optimally watered plants of soybean were exposed to drought. Metabolic changes in optimally watered and drought-stressed plants were monitored using FT-Raman spectroscopy. In parallel, analyses were carried out of fatty acid composition and pigment content using analytical methods. These compounds are associated with the response of plants to environmental stress. While fatty acid assays in study were inconclusive, the pigment content analysis gave promising results. FT-Raman experiment demonstrated a decrease in carotenoid content in leaf, as a result of drought, which was confirmed by spectrophotometric analysis. In addition to the analysis of aforementioned compounds, FT-Raman spectroscopy allowed the simultaneous observation of a wider spectrum of compounds scattering the radiation in the leaves tested, and their subsequent comparative analysis. The impact of drought on metabolism of soybean was clearly visible on spectra and confirmed using cluster analysis. The technical problem of the influence of leaf water content on measurements, which appeared in studies, will be discussed. To conclude, FT-Raman spectroscopy may be a good complement to other non-invasive methods, e.g., fluorescent methods, in assessing the stress-induced damage of crops.

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1 Introduction

Abiotic and biotic environmental stresses such as drought, flooding, extreme temperatures or diseases, occurring during the growing season, affect the metabolism of plants, causing multidirectional biochemical changes in cells [1]. The purpose of these alterations is the adaptation of plants to the existing conditions and to counteract the effects of stress. The mechanisms of plant responses to various stress factors have been investigated for many years as the occurrence of environmental stress is a significant problem in agriculture. Therefore, methods are sought for rapid assessment of the post-stress damage in individual plants and entire plantations. These studies involved invasive but also non-invasive methods. The major advantage of non-invasive techniques is their non-destructive effect on plant tissue in comparison to standard analytical methods. The most popular non-invasive methods include infra-red analysis of metabolic CO_2 exchange [2], reflectance techniques [3], and fast kinetic chlorophyll *a* fluorescence [4]. Isothermal calorimetry is less known but is also applied [5,6]. These non-invasive methods allow the assessment of the status of individual leaves, whole plants or even entire crop [7], providing information on broadly defined efficiency of photosynthesis (fluorescence methods) or total metabolic activity (calorimetry). Raman spectroscopy with Fourier transformation (FT-Raman spectroscopy) is also classified as non-invasive technique where information on the chemical composition of a sample can be obtained without any need to disrupt it [8]. If characteristic key bands of individual analyte molecules are found in the spectrum, then FT-Raman spectroscopy can be successfully applied to identify various plant components [9,10]. The changes in chemical composition of the plant can be observed on the FT-Raman spectra in the intensities of visible bands originating from various plant components. There is

a simple correlation between the intensity of FT-Raman signal and the concentration of the analyte in the sample. The differences in FT-Raman band on the spectra indicate different chemical composition of the samples investigated. Generally, FT-Raman spectroscopy allows the study of analytes *in situ* in their natural environment. Primary and secondary metabolites can be analysed, such as mono- and oligosaccharides, fatty acids, amino acids, proteins, alkaloids, flavonoids, carotenoids, terpenes and polyacetylenes. In the biological sciences, FT-Raman spectroscopy has a wide range of applications, for review see [11]. Until now, FT-Raman spectroscopy has been used in plant stress physiology to study the phenomenon of allelopathy [12,13], the effects of ozone, light spectrum and pathogenesis on the metabolism of plants [6].

The aim of this study was to evaluate the usefulness of FT-Raman spectroscopy in assessing stress-induced metabolic changes in plants. Soybean was selected as the research object [*Glycine max* (L.) Merrill] (Legume), as it is one of the most important crops in the world. Initially, it was grown in China, then growth spread to other Asian countries and currently it is cultivated worldwide because of its usefulness in human and animal nourishment [14] as well as industrial and medicinal applications [15]. Despite the relatively large geographical adaptability, soybean plant is susceptible to cold stress but also, as the whole family of leguminous plants, is sensitive to drought, particularly during germination and early growth stages. In the experiments conducted in this study, soybean was exposed to water deficit. As mentioned above – many biochemical changes occur in plant cells, as a result of the action of stress factors (including drought), in the content and the chemical composition of the compounds such as fatty acids or carotenoids that are detectable by FT-Raman spectroscopy [16,17]. In parallel with the analysis of the metabolic changes of plant tissue by FT-Raman spectroscopy we have carried out biochemical analysis of fatty acids and pigments (carotenoids) in the leaves of plants watered optimally and drought-stressed plants using standard analytical methods (gas-chromatography and spectrophotometry).

2 Experimental procedure

2.1 Plant material and experimental design

The experiment was performed in pots (40 × 20 × 15 cm; 15 plants per pot). Seeds of Aldana and Augusta cultivars were soaked 24 h in water on Petri dishes. After sowing into the soil, seeds germinated in a growth chamber (darkness, 25°C) for 5 days, next the pots were moved to the greenhouse under natural light conditions (June; latitude: 50°03' North, longitude: 19°55' East) at a day/night temperature, 24/20°C. On the 20th day of vegetation, seedlings had a pair of the first foliage leaves and developed one compound leaf with three leaflets. On the 20th day of growing, the plants were watered for the last time, and then watering was ceased, which resulted in the occurrence of drought symptoms during next 5 days (gradual plant wilting was observed). At that time, only compensating watering was applied in order to avoid uneven water loss between pots.

The first foliage leaves were collected from 20-d-old watered plants and from 26-d-old drought-treated plants to measure fatty acid composition and pigment (carotenoids and chlorophylls) content. It was important that plants were in comparable stage of development due to the fact that the development during period of drought was slowed down. FT-Raman spectroscopy analysis was carried out before the drought stress (20-d-old-plants) and after drought stress (26-d-old-plants). The drought-stressed plants were subsequently watered (rehydrated) and FT-Raman spectroscopy was performed for the third time.

2.2 FT-Raman spectroscopy measurements

FT-Raman measurements were performed on fresh soybean leaves using a FT-Raman Spectrometer Nicolet NXR 9650 equipped with a Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. Spectrometer was provided with an *xy* stage, a mirror objective and a prism slide for redirecting the laser beam.

All spectra were collected in the range of 150–3700 cm^{-1}, accumulated from 128 scans, measured with the laser power of 0.6 W, with a spectral resolution of 8 cm^{-1} using an unfocused laser beam of the diameter of approx. 100 μm. FT-Raman spectra were registered by the Omnic/Thermo Scientific software. Ten spectra were collected and averaged for each object. Despite the fact that the spectra were recorded in a wide range of frequencies, only the range of 720 to 1670 cm^{-1} was analyzed. Within this region one can identify vibration modes characteristic of these groups of compounds of particular interest (namely fatty acids, carotenoids and chlorophylls).

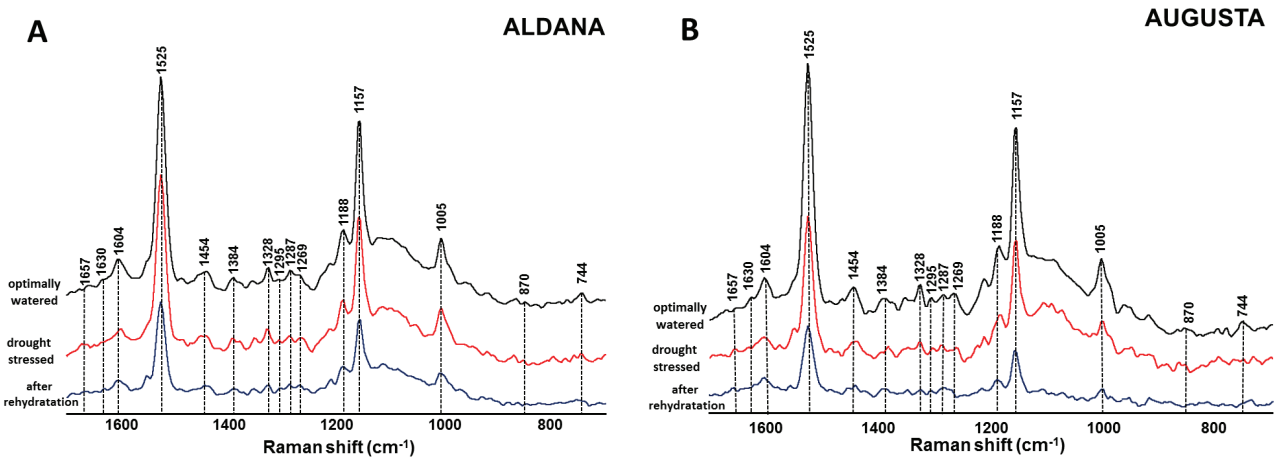

Figure 1: FT-Raman spectra of leaves of soybean (A) cv. Aldana and (B) cv. Augusta collected from optimally watered, drought-stressed and rehydrated seedlings.

2.3 Chemometrics

Similarities between FT-Raman spectra were studied using hierarchical cluster analysis in the Statistica software package 10.0 (StatSoft Inc.). The spectra were baseline corrected. The cluster analysis was performed for the whole wavenumber range using Ward's algorithm. The spectral distances were calculated with the standard algorithm.

2.4 Fatty acid analysis

Analysis of the fatty acid content was carried out according to the procedure described by Janeczko et al. (2009) [18]. Briefly, samples (0.3 g FW) were homogenized in chloroform/methanol mixture with addition of isopropanol. The separation of lipids into three classes (monogalactosyl diacylglycerols, digalactosyl diacylglycerols and phospholipids) was conducted on a silica acid column (Koch-Light Laboratories Ltd, England, type 5030h, 325 mesh activated for 18 hours at 110°C). Then the lipids were subjected to transestrification with BCl_3 catalysts in methanol yielding fatty acid methyl esters (FAME). FAMEs were spiked with methyl heptadecanoate as an internal standard and extracted with n-hexane. The analysis of FAME content was conducted with a GC (Hewlett Packard 5890, Series II), equipped with a FID detector using a GS-Alumina capillary column (30 m length, 0.542 mm in diameter) purchased from J&W Scientific. The quantitation of the FAME content was carried out using external standard calibrations corrected for internal standard. Each analysis was conducted in triplicate.

2.5 Pigment content analysis

The content of photosynthetic pigments (carotenoids and chlorophylls) in the leaves was determined spectrophotometrically according to the modified method of Lichtenthaler and Wellburn (1983) [19]. After lyophilization, leaf samples were ground, 25 mg DW was weighed and pigments were extracted with 1 cm^3 of acetone. The supernatant obtained after centrifugation was diluted and absorbance was measured at the wavelengths: 470 nm (carotenoids), 645 nm (chlorophyll *b*), 662 nm (chlorophyll *a*). Analyses were performed in five replicates (one replicate = lyophilized material from one leaf).

3 Results

3.1 FT-Raman discrimination between optimally watered, drought-stressed and rehydrated soybean seedlings

Three characteristic bands of carotenoids are visible in the FT-Raman spectra obtained for all studied objects at the following wavelengths: 1005, 1157 and 1525 cm^{-1} (Fig. 1A and 1B). Carotenoids are well known from the fact that they give a strong FT-Raman signal. They have a characteristic long central chain in the structure with a conjugated double bond system. The first, most intense C=C stretching vibration of β-carotene was observed at 1525 cm^{-1}. The second, medium in intensity at 1157 cm^{-1} was attributed to C-C stretching vibration. The third, low

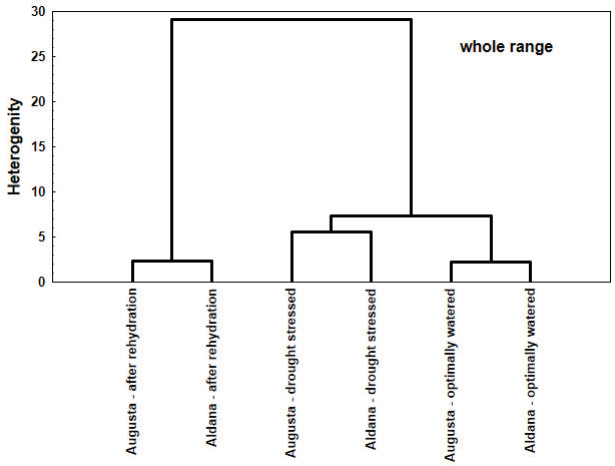

Figure 2: Cluster analysis of the FT-Raman spectra of soybean leaves (cv. Aldana and cv. Augusta) performed according to Ward's algorithm in the whole wavenumber range with standard algorithm as data preprocessing.

intensity band at 1005 cm^{-1} reflected CH_3 groups attached to the polyene chain coupled with C-C bonds. Some bands of low intensity at 1604, 1328, 1287 and 744 cm^{-1} could be assigned to chlorophyll. Bands, which appeared at 1454 and 1384 cm^{-1} could be associated with deformation vibrations of CH, CH_2 and CH_3 groups and C-C stretching vibrations of aliphatic carbohydrates, respectively. Furthermore, the spectra showed bands associated with saturated and unsaturated fatty acids at 1657 cm^{-1}, 1295 cm^{-1} and 1630 cm^{-1}, 1269 cm^{-1}. There were also bands visible on the spectra associated with disaccharides at 1188 and 870 cm^{-1}. Additionally, in the range of 1000–1140 cm^{-1} a difference in the shape and intensity of peaks was observed. They were identified as the symmetric (C-O-C, 1122 cm^{-1}) and asymmetric vibration (C-O-C, 1094 cm^{-1}) modes characteristic for polysaccharides. The observed changes are much more pronounced in cv. Augusta comparing to cv. Aldana.

3.2 Cluster analysis

Cluster analysis was applied in order to find the meaningful and systematic differences among the spectra of the leaves of cv. Aldana and cv. Augusta, which were optimally watered, drought-stressed and finally rehydrated (Fig. 2).

A distinct discrimination between these six objects was achieved throughout the wavenumber range. The first group of objects investigated consisted of well watered plants of cv. Aldana and cv. Augusta. The plants of these two cultivars were very similar in terms of their chemical

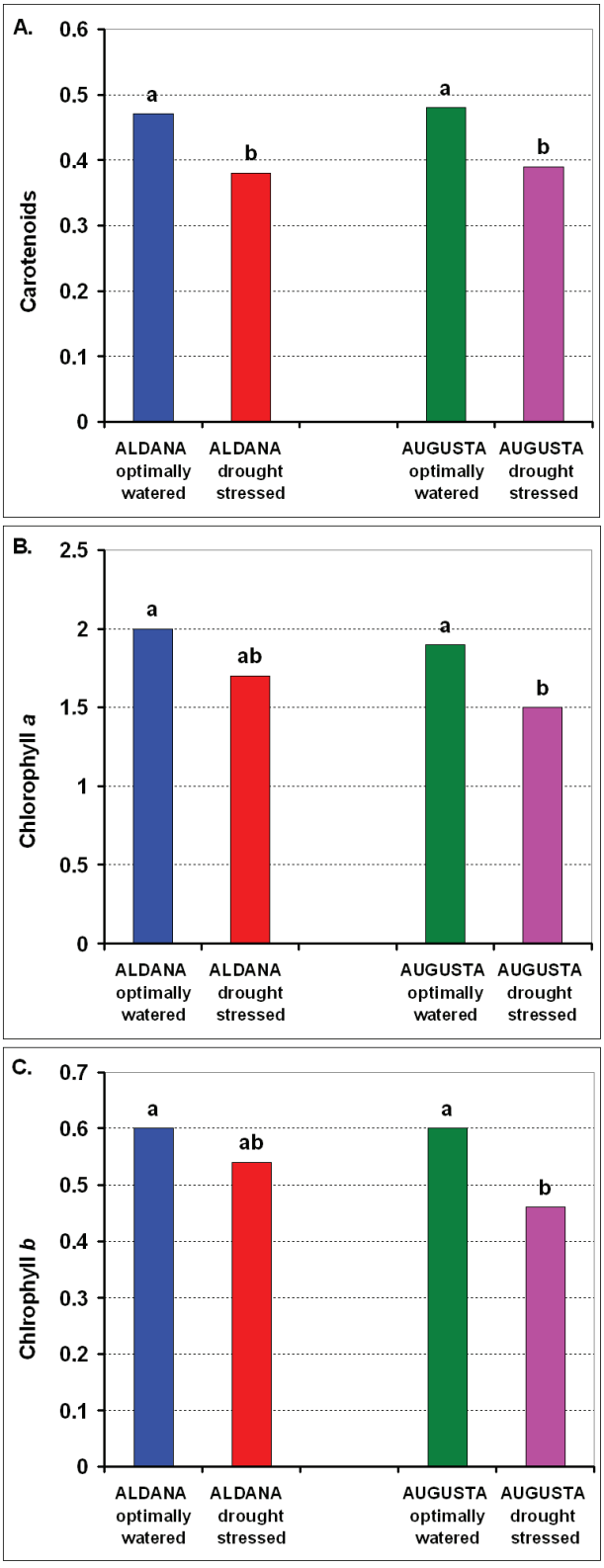

Figure 3: Content of carotenoids (A) and chlorophylls (B and C) [mg g $D.W.^{-1}$] in the leaves of optimally watered and drought-stressed soybean (cv. Aldana and cv. Augusta). Values marked with the same letters (within columns) are not significantly different according to the Duncan's multiple range test, $P < 0.05$.

Table 1: The relative amounts (% of total) of fatty acids in monogalactosyl diacylglicerol, digalactosyl diacylglycerols and phosphiolipid fractions isolated from leaves of soybean optimally watered and drought stressed. Values marked with the same letters (within columns) are not significantly different according to the multiple Duncan Test, $P < 0.05$.

Cultivar/treatment		Monogalactosyl diacylglycerols		Digalactosyl diacylglycerols		Phospho-lipids	
		Palmitic acid (16:0)					
ALDANA	optimally watered	4.0	a	12.1	b	24.3	ab
	drought stressed	3.4	a	11.0	b	25.8	a
AUGUSTA	optimally watered	4.4	a	15.4	a	22.3	b
	drought stressed	4.4	a	10.6	b	24.5	ab
		Palmitoleic acid (16:1)					
ALDANA	optimally watered	0.29	b	0.31	b	7.5	b
	drought stressed	0.27	b	0.23	b	6.8	b
AUGUSTA	optimally watered	0.77	a	6.25	a	11.2	a
	drought stressed	0.21	b	0.25	b	5.4	b
		Stearic acid (18:0)					
ALDANA	optimally watered	1.5	a	2.2	a	4.6	b
	drought stressed	1.1	a	2.2	a	4.8	ab
AUGUSTA	optimally watered	1.9	a	2.5	a	5.5	a
	drought stressed	2.0	a	2.9	a	4.6	b
		Oleic acid (18:1)					
ALDANA	optimally watered	1.0	a	0.87	a	4.4	a
	drought stressed	0.78	b	0.61	b	3.9	a
AUGUSTA	optimally watered	0.51	c	0.67	b	4.6	a
	drought stressed	0.83	b	0.70	ab	3.1	b
		Linoleic acid (18:2)					
ALDANA	optimally watered	5.2	b	4.1	a	26.2	a
	drought stressed	5.7	a	4.4	a	20.3	b
AUGUSTA	optimally watered	3.1	c	2.4	b	18.6	b
	drought stressed	4.2	c	3.7	a	14.9	c
		Linolenic acid (18:3)					
ALDANA	optimally watered	87.9	a	80.4	a	32.9	b
	drought stressed	88.8	a	81.6	a	38.5	b
AUGUSTA	optimally watered	89.3	a	72.8	b	37.9	b
	drought stressed	88.3	a	81.9	a	47.5	a

Continued **Table 1:** The relative amounts (% of total) of fatty acids in monogalactosyl diacylglicerol, digalactosyl diacylglycerols and phosphiolipid fractions isolated from leaves of soybean optimally watered and drought stressed. Values marked with the same letters (within columns) are not significantly different according to the multiple Duncan Test, $P < 0.05$.

Cultivar/treatment		Monogalactosyl diacylglycerols		Digalactosyl diacylglycerols		Phospho-lipids	
		Ratio of unsaturated to saturated fatty acids (U/S)					
ALDANA	optimally watered	17.4	a	6.0	a	2.5	a
	drought stressed	21.3	a	6.6	a	2.3	a
AUGUSTA	optimally watered	16.1	a	4.6	b	2.6	a
	drought stressed	14.8	a	6.4	a	2.4	a
		18:3/18:2					
ALDANA	optimally watered	16.8	bc	19.6	b	1.3	c
	drought stressed	15.7	c	18.4	b	1.9	bc
AUGUSTA	optimally watered	29.3	a	30.6	a	2.0	b
	drought stressed	21.0	b	22.4	b	3.2	a

composition, particularly content and composition of carotenoids, chlorophylls and fatty acids. The second group comprised drought-stressed plants of cv. Augusta and cv. Aldana. In this case, the variability between plants was somewhat larger than in objects from the first group. The third group constituted plants of cv. Aldana and cv. Augusta after rehydration and the results in this group were significantly different compared to others.

The obtained dendrogram showed that different water availability had an influence on the plant metabolic profile, especially for carotenoids, chlorophylls and fatty acids compositions in the test soybean plants.

3.3 Content of carotenoids and chlorophylls in the leaves of optimally watered and drought-stressed soybean seedlings

The content of carotenoids and chlorophylls was similar in the leaves of both studied cultivars growing under optimal watering (Fig. 3A). Under the drought, carotenoid content significantly decreased in the leaves of cv. Aldana (18%) and cv. Augusta (17%). Drought stress also reduced the amount of chlorophyll in the leaves of both varieties by 10–23%, even though the differences were statistically significant only in the case of the cv. Augusta (Fig. 3B, 3C).

3.4 Fatty acid composition in the leaves of optimally watered and drought-stressed soybean seedlings

Composition of fatty acids (FA) of phospholipids (PL) in the soybean leaves, which constitutes main components of cell membrane, differs from the composition of fatty acids of fractions of monogalactosyl diacylglycerols (MGDG) and digalactosyl diacylglycerols (DGDG), characteristic of the membranes of chloroplasts (Table 1). In the FA pool of the MGDG, DGDG fractions the largest percentage of 18:3 (linolenic acid) was found. However, in the case of MGDG and DGDG, its contribution ranges from 72.8 to 89.3%, while in PL it averages at 40%. In addition, the PL fraction had a relatively high percent (about 20%) of FA 18:2 (linoleic acid) and 16:0 (palmitic acid). The percent of these FA in the fractions of MGDG and DGDG was ranging from a few to a maximum of 15.4%. The MGDG and DGDG fractions had the lowest (typically less than 1%) relative amounts of 16:1 (palmitoleic acid) and 18:1 (oleic acid). The percent of these acids in the case of phospholipids averaged at the level of a few percent.

The soybean cv. Augusta and cv. Aldana varied in fatty acid composition in individual groups of lipids. In cv. Augusta in the MGDG fraction, there was higher percent of 16:1 than in cv. Aldana, while the percent of 18:1 and 18:2 was lower. In cv. Augusta in the DGDG fraction, there was significantly lower relative amount of 18:1, 18:2 i 18:3 when compared to Aldana, while the relative amount of 16:0

was higher. Cv. Augusta had the higher relative amount of 16:1 and 18:0 compared to Aldana, whereas there was less of the 18:2 acid in the pool.

Drought slightly modified the composition of FA lipids in the leaves of cv. Aldana, causing amongst others a decline of relative amount of 18:1 in MGDG and DGDG fraction. A decrease of relative amount of 18:2 was recorded in the PL fraction. An increase in the relative amount of 18:2 occurred in this cultivar in the MGDG fraction.

In the cv. Augusta, the drought triggered an increase in the relative amount of 18:1 (MGDG), 18:2 and 18:3 (DGDG), which was accompanied by a reduction of the percentage of 16:0 and 16:1. In the PL fraction was observed the largest increase in the proportion of unsaturated 18:3, with declines in the percentage of almost all the other FA in this fraction.

Trends in the percentage of FA (in the individual fractions of lipids) that occurred as a result of drought are synthetically expressed by the ratios: unsaturated FA/saturated FA (U/S), and the ratio 18:3/18:2. Changes resulting from the increase of FA unsaturation caused by drought are statistically significant in cv. Augusta, while in cv. Aldana only a similar trend can be noticed.

4 Discussion

4.1 Evaluation of pigment content

The lower content of carotenoids and chlorophylls in plants exposed to water deficit is a typical phenomenon [16,17]. However, there are exceptions from this rule resulting from, e.g., interspecific or intercultivar differences as well as the intensity of the stress factor [20]. In general, it is thought that the decrease in the synthesis and/or increased degradation of the pigments as a result of stress is associated with a reduction in photosynthetic efficiency. In this study, increasing water deficit during five days caused a decrease in hydration (wilting) of the leaves of soybean and resulted in a significant reduction in the content of carotenoids and chlorophylls. The content of pigments in the leaf samples collected before and after the drought was measured using one of the most popular methods, i.e., the spectrophotometric method described by Lichtenthaler and Wellburn [19], which as all classical analytical method, is time-consuming, laborious and requires the intake of reagents. Raman spectroscopy with Fourier transformation (FT-Raman) can serve as an alternative, non-invasive technique useful for the characterization and identification of the pigment content (especially carotenoids) in living tissue [21-25]. Our study has applied FT-Raman spectroscopy for the first time in the analysis of the effect of drought stress on the chemical composition of plant tissue, including the content of carotenoids and chlorophylls. The obtained spectra showed that drought stress affected the chemical composition of soybean leaves. Bands derived from carotenoids (1005, 1157, 1525 cm^{-1}) varied in height between the first two test objects, namely the leaves of optimally watered plants and the leaves withered due to water deficit in soil. The observed changes are much more pronounced in cv. Augusta, and are likely to be due to its greater susceptibility to drought stress compared to cv. Aldana. This method also allowed a quantitative estimation as FT-Raman band intensity is directly correlated with the content of a chemical compund in a sample [22]. However, data on the content of carotenoids in the stressed soybean, obtained spectrophotometrically and by FT-Raman spectroscopy, were not entirely consistent. The content of carotenoids measured spectrophotometrically decreased in both cultivars as a result of drought stress. However, data obtained using FT-Raman spectroscopy demonstrated that the content of these compounds in cv. Aldana increased during drought, while in cv. Augusta it was lower. At the same time it should be noted that the analysis of carotenoids by spectrophotometry was performed on lyophilized material and the content of these compounds was calculated based on the dry weight of the tissue. It is a commonly used procedure because it avoids the distortion of the amount of pigment that could occur when estimating the quantity of carotenoids based on fresh weight, susceptible to changes in plant tissue hydration. Particularly in the case of experiments with drought, plant leaves treated with this stress factor have a reduced water content, which may appear to increase the concentration of carotenoids and other pigments when fresh weight is used in the calculations. Our results showed that the hydration of fresh plant tissue, affecting surface structure of the leaf and its optical properties, played similarly important role in FT-Raman measurements. Influence of morphological properties of leaves on the intensity of the Raman signal was also observed in *Plantago media* L. The thin leaves, with a high water content, show a much weaker Raman scattering intensity in comparison to the less hydrated thick leaves (Skoczowski – unpublished data). The Raman bands derived from water have low intensity, because the low polarizability of water is reflected in the low intensity of scattered light. Nevertheless when comparing various objects, fluctuations in the amount of water in the examined tissue seem to affect the sensitivity of the method. Therefore, two approaches seems reasonable, i.e.,

FT-Raman analysis on the lyophilized material or rehydration of withered tissues before measurements. In our experiment, to simplify the measuring procedure, tissue was rehydrated by watering the plants. The results of the FT-Raman measurements using leaves after rehydration showed that the quantity of carotenoids significantly decreased in both cultivars compared to the content of these pigments in the leaves of the plants watered optimally. After regaining the turgor by the leaves, a decrease of the carotenoid content was observed in the FT-Raman spectroscopy and correlated with a decrease in the content of these compounds assayed by spectrophotometric method. However, the quantitative changes in the content of carotenoids in plants assayed by spectrophotometry before and after stress are low (a few percent). The results of FT-Raman spectroscopy indicated a two-, three-fold decrease in the amount of carotenoids caused by stress. In our opinion, the results of both analyses should not be directly compared in terms of quantity, because the principle of the measurement was different and the calibration was not carried out. The amount of carotenoids in the spectrophotometric method was determined based on the absorbance and molar extinction coefficient. For FT-Raman spectrometry, carotenoid content was assessed on the basis of FT-Raman intensity (FT-Raman scattering), which for these compounds is distributed in three band frequencies (the so-called carotenoid triplet). Furthermore, in addition to the chemical diagnostics, absolute quantitative changes of the selected metabolite are not necessarily needed to describe the effects of stress on the plant. Significantly more important is the so-called trend of changes. Variations in the pool of carotenoids caused by stress are analyzed on the basis of the direction of change, i.e., a decrease or increase in their content. Therefore, FT-Raman spectroscopy is ideal for the assessment of changes in the content of carotenoids in plant tissues caused by different types of stress factors.

In addition to carotenoids, this study also analyzed the content of chlorophyll using spectrophotometry and comparative method of FT-Raman spectroscopy. Spectrophotometric method allowed us to capture the changes (a decrease in chlorophyll content) in both cultivars studied. The optimally watered plants of cv. Augusta and cv. Aldana had similar levels of chlorophyll. Drought caused a decrease in the content of these pigments, while this reduction was more prominent in the leaves of more susceptible to drought (according to previous studies) cv. Augusta when compared to cv. Aldana [27]. Unlike the carotenoid analysis, FT-Raman spectroscopy did not provide unambiguous results in the analysis of chlorophylls. Bands derived from chlorophylls (1604, 1328, 1287 and 744 cm^{-1}) had very low intensity, because chlorophyll in contrast to carotenoids is a compound that demonstrates incomparably lower dispersion of the radiation.

4.2 Fatty acid assay

Plant cell membranes are one of the main targets for biotic and abiotic stresses. Fatty acids, as a structural part of membranes, participate in maintaining membrane integrity and regulating membrane fluidity. The effect of stress factors can modify the qualitative and quantitative lipid composition of the cell (including the composition of cell membranes). However, these changes very often depend on the genotype and on the type of stress. Toumi et al. (2008) [27] studied grapevine under drought stress, and observed changes in the composition of total lipids towards the increase of the proportion of 18:3. The increase in the percentage of 18:3 under the influence of drought was also reported in the study of Gigon et al. (2004) [28] in certain lipid fractions (DGDG and phosphatidylcholine fraction) in *Arabidopsis thaliana* L. What is more, these authors found an overall decrease in the amount of lipids reaching even 75% as a result of drought. In turn, Zhong et al. (2011) [29] recorded a decline in polyunsaturated fatty acids 18:2 and 18:3, and an increase in saturated fatty acids 16:0 and 18:0 in bermudagrass exposed to drought.

The FA changes in soybean have previously been studied mainly in relation to the seeds. It was found that the FA composition of the soybean seed is influenced by such factors as environmental conditions, fertilization and genotype [30,32]. Moreover, the content of fatty acids in the seeds involves mainly storage lipids (so-called neutral lipids), while in leaves polar lipids are mainly found. We have evaluated the effects of water stress on lipid composition in the leaves of soybean. The results obtained by gas chromatography indicated that the composition of the FA polar lipids under drought in the leaves of cv. Aldana did not change significantly. There was an increase in the percentage of 18:3 in cv. Augusta in digalactosyl diacylglycerol and phospholipid fractions. This translated into an increase of the U/S ratio. FA were also visible in the FT-Raman spectra. The bands present were derived from saturated (1657 and 1295 cm^{-1}) and unsaturated fatty acids (1630 and 1269 cm^{-1}). The low intensity of the bands resulted from the low content of the compounds analyzed in the leaf tissue as well as high intensity of carotenoid bands, which influenced the visibility of the bands derived from

the fatty acid spectrum. Since the bands derived from FA in the spectra had low intensity, the U/S ratio could not be determined with sufficient accuracy. This parameter (U/S) is sometimes calculated in order to determine the level of plant stress [18,32]. Therefore, the results obtained in our study showed, that the method of FT-Raman spectroscopy was less suitable for the analysis of FA in leaves (due to the small amounts of these compounds located mainly in biological membranes), nevertheless it was also very well suited for seeds high in fats [33,34]. Previous studies on cotyledons of white mustard and rapeseed showed that the lipid content determination by gas chromatography was considerably less accurate as compared to an in situ assay using FT-Raman spectroscopy [34]. The differences were observed in the spectra in the content of saturated and unsaturated fatty acids caused by allelopathic substances present in plant extracts, in which seedlings were grown, while the chromatographic technique did not demonstrate significant statistical differences [34].

4.3 General metabolic changes

For the purpose of this study, metabolites were investigated, the quantitative changes of which were expected in drought conditions. However, FT-Raman spectroscopy allowed the simultaneous observation of a wider spectrum of compounds scattering the radiation, which were present in the objects tested, and a subsequent comparative analysis between them. Cluster analysis showed that the tested objects are grouped into three clusters. The first one was optimally watered plants. The second constituted drought-stressed leaves, and third one was composed of the leaves after rehydration. The impact of drought on metabolism was already visible in the measurements performed on the withered leaves, but the significantly clearer effect of metabolic changes that have occurred during the drought was demonstrated in plant leaves exposed to drought after rehydration. These results constituted entirely separate branch of the dendrogram.

5 Conclusions

FT-Raman spectroscopy allows a detailed analysis of the chemical composition of tissues and identifies changes in the qualitative content of various chemical compounds scattering the radiation. Hence it seems to be a convenient tool for monitoring the effect of environmental factors on plants. The analysis can be preferably be carried out based on the changes of carotenoids levels, even though FT-Raman spectroscopy allows the simultaneous observation of a wider spectrum of compounds scattering the radiation present in the objects tested, and a subsequent comparative analysis. This method can complement other non-invasive methods, e.g., fast kinetic of chlorophyll *a* fluorescence, in assessing the stress-induced damage of crops. However, in the measurements conducted for the stress causing the loss of the leaf water content, the problem of proper hydration of the tissue should be bourne in mind and resolved by full rehydration of leaves before analysis.

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