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STUDY OF THE LOW INTENSITY SPECTRAL BANDS WITHIN THE INFRARED SPECTRA OF KERNELS OF HIGH-YIELDING MAIZE HYBRIDS

ABSTRACT: The nature, role, and significance of low intensity spectral bands contained within the infrared spectra of kernels of high-yielding maize hybrids: ZP 341, ZP 434, and ZP 505 were observed in this study. The observations were performed to identify organic molecules and their structural properties. The occurrence of unstable state of organic compounds and their functional groups are conditioned by such a process. The set hypothesis holds that there is a necessity to study the existence of many and low intensity spectral bands, not observed so far, occurring in different patterns (low intensity bands, single or grouped). They should be observed and the dynamics of their formation, caused by their different movements, including the possibility of their cancellation or amplification, should be explained. Such spectral bands most often appear in the wave number range of 400–950 cm^{-1} . They occur in several wave numbers up to 3,000 cm^{-1} and are caused by different types of vibration movements (valence and deformation vibrations) of organic compounds and their functional groups: primary, secondary, and tertiary amides, proteins, free amino acids, alkanes, alkenes, aldehydes, ketones, aromatic compounds, cellulose, carbohydrates, carboxylic acids, ethers, and alcohols. An unbiased analysis of low intensity spectral bands of maize hybrid kernels reveals that their occurrence is similar. Small differences, for some cases of the occurrence of low intensity spectral bands, can barely be ascertained. In this way, it is possible to establish not only the chemical composition of organic compounds of kernels of observed maize hybrids, but also it is possible to indicate their unstable, conformational, and functional properties.

KEYWORDS: *Zea mays* L, hybrid, kernel, infrared spectra, spectral bands

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INTRODUCTION

Nowadays, the fundamental improvement in the diagnosis of the state of organs and vital functions of the whole plant at the molecular level is achieved with modern methods of spectroscopy. The vibrational spectroscopy (infrared and Raman) is an unavoidable method in the analysis of infrared spectra of organic molecules resulting from molecular vibrations, thus it is possible to obtain many other results related to the structure of the studied systems. (Васильев и др., 2007; Свердлов и др., 1970; Тарасевич, 2012; Krim and Bandekar, 1986; Ribnikar, 1985).

In our previous papers (Radenović et al., 1994; Radenović et al., 1994a; Radenović et al., 1995; Radenović et al., 1998), studies are presented on the structural changes of carotenoid molecules in kernels of various maize inbred lines and hybrids. It was also shown that the structure of these molecules could be used as molecular markers in the evaluation of agronomic values of the maize inbred lines and hybrids. Our more recent papers (Radenović et al., 2015; Radenović et al., 2015a; Radenovich et al., 2016) discuss studies on the formation of infrared spectra, and in particular of 5–6 spectral bands that were most pronounced. No other, numerous and less developed spectral bands were analysed in these studies.

The method of infrared spectroscopy was applied in this study with the aim to analyse weak and insufficiently differentiated spectral bands, and thus, to identify the unstable state of the excited system and life functions of kernels of observed maize hybrids. It was shown that even by the use of infrared spectroscopy it was possible to determine the structure of organic compounds in kernels of studied maize hybrids (Macura and Radenović, 2016).

The objective of this study was to develop the methodology for analyses of numerous low intensity spectral bands of different shapes and kinetics, in order to identify organic compounds and their functional groups and to determine their stability and structure in kernels of analysed maize hybrids.

MATERIAL AND METHODS

Kernels of three highly productive maize hybrids, ZP 341, ZP 434, and ZP 505, developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia were used as a plant material in this study. Morphological and agronomic traits of observed maize hybrids, including those relevant to breeding and seed production, have been thoroughly described in our previously published papers (Radenović et al., 2015a; Radenovich et al., 2016).

The method of infrared spectroscopy applied to the grain of maize hybrids includes spectrophotometers used in the infrared spectrum region. They do not differ from spectrophotometers in the ultraviolet-visible spectrum regarding the sequence of components. Specificities, however, occur in the very principle of the work of the spectrophotometer. The fundamental differences

relate to the source of radiation, nature of samples, the principle of absorption of radiation, as well as to the use of various detectors (thermal and photo-detectors) (Васильев и др., 2007; Ribnikar, 1985).

Today, a special type of spectrophotometers is used, based on the principles of interferometry. Interferometers do not produce the spectrum itself, but an interferogram, which is then processed by a computer into a common spectral shape. This is co-called the Fourier transformation, and hence the name the Fourier Transform Spectroscopy (FTS). These devices are especially suitable for use in the far-infrared region and are characterised by good resolution (Radenović et al., 2015a; Radenovich et al., 2016).

In order to register the infrared spectrum of the observed maize hybrids, kernels were homogenised and compressed into tablets with the addition of potassium bromide (KBr). The spectrum was recorded with the Fourier Transform Infrared Spectrometer, Shimadzu IR-Prestige 21 (Instruction Manual User System Guide IRPrestige-21), within the spectral range of 400–4,000 cm^{-1} . This method has been described in details in our previous paper (Radenovich et al., 2016), including the modus operandi and the optical scheme of the device for registering infrared spectra.

Thirty kernels of each studied hybrid were randomly selected. Samples were prepared in the following way: kernels were ground and homogenised in a mortar and then mixed with potassium bromide (KBr) and rolled into samples – tablets with the component ratio of 1:100.

The primary processing of infrared spectra was done by the OriginPro software package, 2017 (OriginLab Corporation, USA).

The calculations were made by Microsoft Excel 2013 software package (Microsoft Corporation, USA).

The largest part of the statistical calculations was done using the program package Statistica, version 10 (StatSoft, Inc, USA).

RESULTS AND DISCUSSION

Kernels of the observed high yielding maize hybrids (ZP 341, ZP 434, and ZP 505) were homogenised and compressed into tablets and thus prepared for registering the infrared spectrum (Figure 1 a, b, c). Generally, the analysed infrared spectra for three maize hybrids were characterised by numerous spectral bands. The number of bands was up to 40 within the range of the wave number from 400 to 4,000 cm^{-1} . The registered spectral bands were of the unequal intensity, different shapes and kinetics.

Distinctively pronounced spectral bands (4–6) were analysed and presented in our recently published papers (Radenović et al., 2015a; Radenovich et al., 2016). However, in addition to these bands in the infrared spectrum of kernels of maize hybrids, a series of very different spectral bands appeared – high or low intensity bands, clearly separated or grouped, and spectral bands of a complex structure. Such spectral bands cannot be found in the literature and have not been analysed yet. Our study was actually focused on stated low

intensity bands with specific kinetic parameters. The careful consideration and analysis of kernel spectra of three studied maize hybrids (Figure 1 a, b, c) show a great number of spectral bands within the wave number range of 400–2,040 cm^{-1} for all three maize hybrids. According to their kinetic parameters, they differed considerably from one another. There were at least three classes. Similar spectral bands were formed within the range of the wave number of 400–950 cm^{-1} . Somewhat different spectral bands were formed within the range of the wave number of 1,200–1,600 cm^{-1} . Finally, there were spectral bands that were formed within the ranges of the wave numbers of 1,680–2,400 cm^{-1} and 1,680–2,800 cm^{-1} (Figure 1 a, b, c).

According to our hypothesis, the low intensity spectral bands with different kinetic parameters suggest an unstable (excited) state of the biological system (Macura and Radenović, 2016; Кољс и др., 1993; Radenović, 1998; Radenović et al., 2001). Furthermore, the excited state is expressed in certain functional groups that are presented in Table 1. It should not be forgotten that the unstable states of the biological system (tissue, cells, membranes) are a consequence of the excited state of molecules, radicals, atoms or ions and that they are inevitably caused by the occurrence of kinetic energy and their different movement modes (oscillation, vibration, rotations, and translations). This can cause the cancellation or the enhancement of the movement processes, which condition the different occurrence of low intensity spectral bands. The similar events happen in the process of the ion oscillatory transport through the excited membrane (Кољс и др., 1993; Radenović, 1998; Radenović et al., 2001). Moreover, today, beyond any doubt, great attention is paid to the contemporary study of the biological systems, seeking for information on genomes and proteomes and primarily their metabolomes, i.e. the concentration of all metabolites and their interactions. This is enabled by the application of infrared spectroscopy in studying of the structure and properties of organic compounds of kernels of maize hybrids (Radenović et al., 2015; Radenović et al., 2015a; Radenovich et al., 2016; Macura i Radenović, 2016).

Based on everything stated above, at least two questions may be posed. First, how to obtain reliable information on the existence of different biogenic organic molecules (substances), whose specificities regarding oscillations and deformations cause the occurrence of different spectral bands (Figure 1 a, b, c)? Second, are there any differences in kernels of the observed maize hybrids (ZP 341, ZP 434, and ZP 505) in relation to the integrity of the formation of spectral bands? If such differences exist, then it can be concluded that there are various structural properties of organic compounds in kernels of the studied maize hybrids.

The answer to the posed questions can be largely found out in our gained results presented in Table 1 and Figure 1 a, b, c. The careful analysis of literature data – the intensity, shape, kinetic values, as well as the range of the wave number of spectral bands (Volhardt and Schore, 1996; White and Johnson, 2003; Amir et al., 2013; Jackson and Mantsch, 2006; Skoog et al., 2007) – provides the identification of functional groups of organic compounds (Table 1). When the same parameters presented in Table 1 and Figure 1 a, b, c are compared,

it can be concluded that structural properties of organic compounds of all three maize hybrids are similar. Smaller differences occurred in the hybrid ZP 505.

Finally, according to the gained results the following can be added: the importance of intensity, shape, and kinetics of low intensity spectral bands that express unstable processes and states in biological systems and bioactive organic molecules contained in kernels of three studied maize hybrids, have been studied and emphasised for the first time.

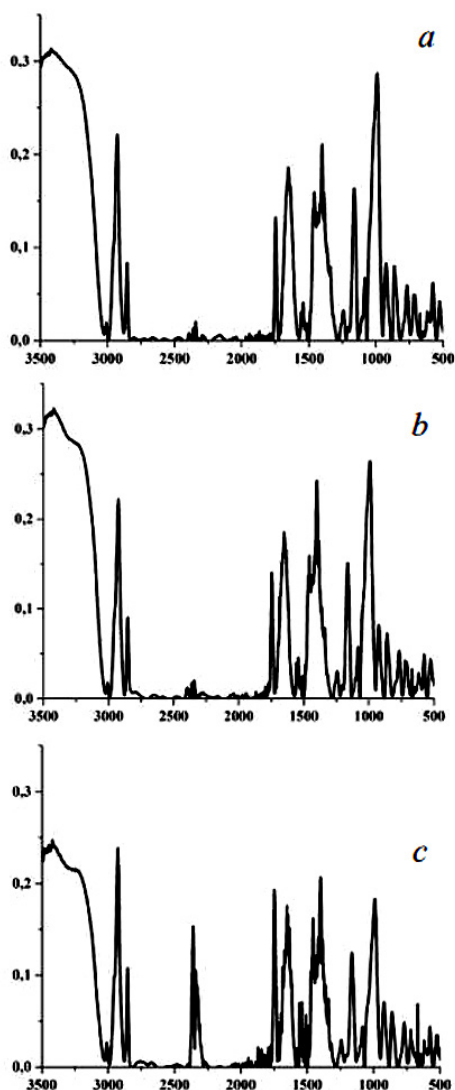


Figure 1 a, b, c. Infrared spectra of kernels of three maize hybrids – low intensity spectral bands were observed: a – ZP 341, b – ZP 434, c – ZP 505.

Abscissa: Wave number, cm⁻¹. *Ordinate:* Intensity, relative units

Table 1. Absorption bands of infrared spectra in kernels of maize hybrids: ZP 341, ZP 434, and ZP 505

Maize hybrids			Assignment of the IR absorption bands to functional groups in organic molecules
ZP 341	ZP 434	ZP 505	
Wave number of spectral band, cm ⁻¹	Wave number of spectral band, cm ⁻¹	Wave number of spectral band, cm ⁻¹	
3,360 h	3,360 h	3,360 h	Alcohols, amides. ^{a, f, g} Valence vibrations of a free and bound OH group; valence vibrations of intramolecular and intermolecular H-bonds in dimers and polymers; valence vibrations of N-H bonds (primary and secondary amides); valence vibrations of free OH groups (water, carbohydrates, amino acids); valence vibrations of NH-groups (proteins, amino acids and their derivatives). ^{b, c, d, f, g}
3,010 l	3,010 l	3,010 l	
2,920 h	2,920 h	2,920 h	Valence vibrations of -CH ₂ - bonds in alkanes (2,940–2,915 cm ⁻¹). ^{c, f}
2,855 l	2,855 l	2,855 m	
2,350 m	2,350 m	2,350 m	Valence vibrations of O=C=O bonds in atmospheric carbon dioxide CO ₂ , used for graduation (2,349.3 cm ⁻¹). ^c CO ₂ is not contained in maize grains.
2,340 l	2,340 l	2,340 m	CO ₂ bend. ^h
2,000–1,750 l	2,000–1,750 l	2,000–1,750 l	
1,720 l	1,720 l	1,720 l	
1,700 l	1,700 l	1,700 l	
1,680 m	1,680 m	1,680 m	Alkenes, aldehydes, ketones. ^{a, f}
1,750 m	1,750 m	1,750 m	Ethers, aldehydes, ketones, carboxylic acids (1,750–1,690 cm ⁻¹). ^{a, f}
1,650 m	1,650 m	1,650 m	Valence vibrations of C=O bonds in primary, secondary and tertiary amides; stretching vibrations of N-H and C-N bonds in secondary amides, proteins, and free amino acids; vibrations of OH groups in crystal water of cellulose. ^{b, c, f}
1,645 m	1,645 m	1,645 m	
1,640 m	1,640 m	1,640 m	
1,610 l	1,610 l	1,610 l	
1,585 l	1,585 l	1,585 l	
1,565 l	1,565 l	1,565 l	
1,550 m	1,550 m	1,550 m	Amide II (protein N-H bending vibrations and C-N stretching vibrations). ^{e, f} 1,420 cm ⁻¹ , glucan ⁱ
1,545 m	1,545 m	1,545 m	
1,520 m	1,520 m	1,520 m	Vibration of C=C bonds in aromatic rings, vibration of NO ₂ in nitro compounds. ^{e, f}

1,395 h	1,395 h	1,395 h	Valence vibrations of $-\text{C}(\text{CH}_3)_3$ bonds in alkanes, (1,395–1,385 cm^{-1}) and (1,365 cm^{-1}), Two spectral bands with absorption intensities of about 1: 2. ^{d, f}
1,380 m	1,380 m	1,380 m	
1,355 m	1,355 m	1,355 m	
1,310 l	1,310 l	1,310 l	
1,235 l	1,235 l	1,235 l	
1,150 m	1,150 m	1,150 m	Alcohols, ethers 1,150 cm^{-1} (1,000–1,260 cm^{-1}). ^a Valence vibrations of $=\text{C}-\text{O}-\text{C}$ -bonds in simple ethers. ^{d, f}
1,100 l	1,100 l	1,100 l	
1,050 h	1,050 h	1,050 h	Stretching plane vibrations of C-H bonds in aromatic compounds. ^{b, c, d, f}
925 l	925 l	925 l	
850 l	850 l	850 l	
765 l	765 l	765 l	
710 l	710 l	710 l	
665 l	665 l	665 l	
615 l	615 l	615 l	
575 l	575 l	575 l	
525 l	525 l	525 l	

Intensity of spectral bands – Abbreviations:

l – low intensity (<0.1 rel. units), m – medium intensity (0.1–0.2 rel. units),

h – high intensity (>0.2 rel. units).

Source: ^a (Radenovich et al., 2016), ^b (Васильев и др., 2007), ^c (Свердлов и др., 1970), ^d (Тарасевич, 2012), ^e (Amir et al., 2013), ^f (Yu et al., 2004), ^g (Jackson and Mantsch, 2006), ^h (Chalmers, 2002), ⁱ (Yu et al., 2004).

CONCLUSION

The infrared spectroscopy was applied for the first time to register and study low intensity spectral bands contained in the infrared spectra in kernels of three high yielding maize hybrids: ZP 341, ZP 434, and ZP 505. According to the obtained results, the following can be concluded:

- Infrared spectra of kernels of the observed maize hybrids are characterised by up to 40 spectral bands within the range of wave number from 400 to 4,000 cm^{-1} ;
- Spectral bands can be differently pronounced, can be of unequal intensity, different shapes, and complex kinetics;
- Low intensity spectral bands (approximately 33 in the infrared spectrum of kernels of the observed maize hybrids) were studied. Besides being of low intensity, they can be single or grouped, can have different shapes and a complex kinetic structure.
- Although they are low intensity spectral bands they provide the identification of organic compounds and their functional groups: primary, secondary and tertiary amides, proteins, free amino acids, alkanes, alkenes,

aldehydes, ketones, aromatic compounds, cellulose, carbohydrates, carboxylic acids, ethers, and alcohols.

- An attempt has been made to clarify the nature, role, and significance of low intensity spectral bands that indicate an unstable (excited) state of the biological system (kernel) in which various movement modes occur, and thereby the possibility of the cancellation or the enhancement of the movement processes, which condition their different formation.

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ПРОУЧАВАЊЕ СЛАБО РАЗВИЈЕНИХ СПЕКТРАЛНИХ ТРАКА
САДРЖАНИХ У ИНФРАЦРВЕНИМ СПЕКТРИМА ЗРНА
ВИСОКОПРИНОСНИХ ХИБРИДА КУКУРУЗА

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РЕЗИМЕ: У овом раду чини се покушај изучавања природе, улоге и значаја слабо развијених спектралних трака, садржаних у инфрацрвеним спектрима зрна високоприносних хибрида кукуруза: ZP 341, ZP 434 и ZP 505. Ова проучавања вршена су ради идентификације органских молекула и утврђивања њихових структурних карактеристика. Оваквим процесом условљава се појава нестабилног стања органских једињења и њихових функционалних група. Износи се хипотеза да се постојање бројних и слабо развијених спектралних трака, које до сада нису изучаване, а које се појављују у различитој форми (слабог интензитета), појављују појединачно или груписано. Њих је неопходно посебно изучавати и објашњавати динамику њиховог настајања условљавану различитим карактером њиховог кретања, што укључује и могућност њиховог потирања или увећавања. Овакве спектралне траке најчешће се појављују у опсегу таласног броја од 400 до 950 cm^{-1} . Оне се успостављају, ту и тамо, на више места таласног броја све до 3.000 cm^{-1} , а настају различитим карактером вибрационог кретања (валенционо осциловање и деформационо вибрирање) органских једињења и функционалних група и то: примарни, секундарни и терцијарни амиди, протеин, слободне аминокиселине, алкани, алкени, алдехиди, кетони, ароматична једињења, целулоза, угљоводоници, карбоксилне киселине, етри и алкохоли. Непристрасном анализом слабо развијених спектралних трака зрна три проучавана хибрида кукуруза стиче се сазнање да је њихово појављивање слично. Мање разлике, за неке случајеве појављивања слабих спектралних трака, једва да се могу констатовати. На овај начин, могуће је утврдити не само структуру органских једињења у зрну проучаваних хибрида кукуруза, него и указати на њихова нестабилна, конформациона и функционална стања.

КЉУЧНЕ РЕЧИ: *Zea mays* L, хубрид, зрно, инфрацрвени спектри, спектралне траке