Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 129, 35—44, 2015

UDC 633.15:631.527.5
DOI: 10.2298/ZMSPN1529035R

Čedomir N. RADENOVIĆ[1,2*], *Georgij V. MAKSIMOV*[3], *Evgenij V. TYUTYAEV*[4], *Ilja V. SYUSIN*[4], *Vitalina V. SHUTOVA*[4], *Mile D. SEČANSKI*[1], *Jelena Ž. SRDIĆ*[1], *Živorad V. VIDENOVIĆ*[1], *Aleksandar S. POPOVIĆ*[1]

[1] Maize Research Institute,
11185 Zemun Polje, Belgrade, Serbia
[2] University of Belgrade, Faculty of Physical Chemistry,
Studentski trg 12–16, 11000 Belgrade, Serbia
[3] M. V. Lomonosov Moscow State University, Faculty of Biology,
1–12 Leninskie Gory, 119991 Moscow, Russia
[4] Mordovia N. P. Ogarev State University, Faculty of Biology,
Bolshevistskaya 68, 430005 Saransk, Russia

STRUCTURAL PROPERTIES OF MAIZE HYBRIDS ESTABLISHED BY INFRARED SPECTRA

ABSTRACT: This paper discusses the application of the infrared (IR) spectroscopy method for determination of structural properties of maize hybrid grains. The IR spectrum of maize grain has been registered in the following hybrids: ZP 341, ZP 434 and ZP 505. The existence of spectral bands varying in both number and intensity, as well as their shape, frequency and kinetics have been determined. They have been determined by valence oscillations and deformation oscillations of the following organic compounds: alkanes, alkenes, alkynes, amides, alcohols, ethers, carboxylic acids, esters and aldehydes and ketones, characteristic for biogenic compounds such as carbohydrates, proteins and lipids. In this way, possible changes in the grain structure of observed maize hybrids could be detected.

KEYWORDS: Maize hybrid, grain, structural properties of molecules, infrared spectra, spectral bands

INTRODUCTION

At present, contemporary methods of spectroscopy and biotechnology provide essential progress in diagnostics of a state of organs and vital functions of the whole plant at the molecular level. Vibrational spectroscopy (infrared and Raman) is an unavoidable method in the analysis of spectra originating

* Corresponding author e-mail: radenovic@sbb.rs

from molecular vibrations, which provide numerous data on structures of observed systems [Krimm and Bandekar 1986; Vasilev 2007; Tarasevich 2012; Sverdlov 1970].

Our previous scientific papers [Radenović *et al.*, 1994a; 1994b; 1995; 1998) described changes in the molecular structure of carotenoids in grain of various maize hybrids and inbred lines and showed that these molecules can be used as molecular markers in evaluation of agronomic values of maize inbred lines and hybrids.

The IR spectroscopy method was applied in the present study to diagnose the state of grain of observed hybrids. It is well known that IR spectroscopy provides the analysis of molecular composition and structure by registering the intensity of oscillations and deformations of molecular bonds [Vasilev 2007; Tarasevich 2012].

The aim of this study was to develop methods for registration of the IR spectrum of grain of observed maize hybrids and to identify structural differences in its biogenic compounds.

MATERIAL AND METHODS

Plant material − The following three hybrids of high quality and with high yields were studied: ZP 341, ZP 434 and ZP 505. These hybrids were developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia. The stated maize hybrids have been released not only in Serbia, but also in Russia and another three European countries. These hybrids are annually sown on more than one million hectares.

Methods − Overall studies of high yielding and high quality maize hybrids encompass several sets of experiments in which new and standard methods and procedures were applied.

1. Infrared spectroscopy of maize hybrid grain

Measurements of infrared spectrum were done by the IR Furie spectrometer (Shimadzu IR – Prestige 21) in the range of 400–4,000 cm^{-1}. Spectrophotometers used in infrared spectral region, in principle, do not differ from those used in the visible and ultraviolet spectral region. The specifics of the behaviour of IR radiation, particularly with regard to the middle and far spectral region, still impose some differences, first of all, the principles of vibrational spectroscopy, the nature of the materials, sources of IR radiation, the application of thermal detectors, etc.

Shimadzu IR –Prestige 21 is based on the principle of interferometers. Namely, it does not give the spectrum itself, but an interferogram, which is additionally processed by computers and transformed into a common shape of a spectrum – it is called Fourier transformation and therefore this type of spectroscopy is called Fourier transform spectroscopy (FTS). These devices are particularly suitable for use in the far IR regions and are characterised by high power of breakdown. In order to register the IR spectrum of observed maize hybrids, grain was homogenised and packed as a tablet with the addition of potassium bromide (KBr).

2. Chemical composition of grain of maize hybrids

Methods used to determine the chemical composition of grain of observed maize hybrids are generally accepted and standardised and described in detail in papers written by Radosavljević *et al.*, 2000; White and Johnson 2003; Radenović *et al.*, 2009, 2010.

3. Functional dependence of maize hybrid yields in different locations in Serbia

Studies of functional dependence of high yielding and high quality maize hybrids (ZP 341, ZP 434 and ZP 505) were performed in many locations in Serbia with the application of the standard cropping practices [Videnović *et al.*, 2011].

4. A broad overview of breeding, seed production and technological traits of maize hybrids

Since the hybrids are high yielding and recently developed a broader overview of relevant breeding, seed production and technological traits, properties and parameters obtained by the application of standard ranking methods are presented.

RESULTS

1. Infrared spectroscopy of maize hybrid grain

Grains of the observed maize hybrids (ZP 341, ZP 434 and ZP 505) were homogenised and pressed into the tablet form with the addition of KBr and thus prepared for the measurement of the IR spectrum, Figures 1–3.

The observed IR spectrum was characterised by spectral bands. There was about 20–23 bands in the wavenumber range of 400 to 4,000 cm^{-1}. Spectral bands were differently pronounced, of uneven intensity, with special kinetics and various width in their base. There were 3–5 distinctly pronounced bands, 2–3 bands were moderate, and 4–5 were very low intensity bands. There were several spectral bands that could not be separated, or that indicated the unstable state of the system.

1.1. Infrared spectroscopy of the maize hybrid ZP 341

Figure 1 shows the IR spectrum of the maize hybrid ZP 341 grain. There are three very prominent spectral bands at 3,400, 1,000 and 2,900 cm^{-1}. Moreover, spectral bands at 1,650, 1,175, 2,850 and 1,145 cm^{-1} are also distinctively observable. A detailed survey shows weakly pronounced spectral bands at 3,780, 2,300, 1,550, 1,145, 1,100, 925, 825, 775, 700 and 600 cm^{-1}. There is an indication of an unstable state of the system in the wavenumber range of 400 to 4,000 cm^{-1}: at 3,000, 1,700 and 700 cm^{-1}.

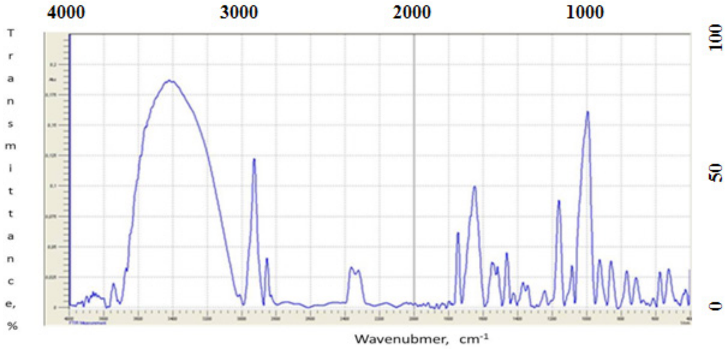

Figure 1. *Infrared spectrum of the maize hybrid ZP 341 grain*

1.2. Infrared spectroscopy of the maize hybrid ZP 434

Figure 2 shows the IR spectrum of the maize hybrid ZP 434 grain. There are four very outstanding spectral bands at 3,400, 1,000, 1,700 and 2,900 cm^{-1}. Furthermore, spectral bands at 2,825, 1,775 and 1,185 cm^{-1} are also particularly observable. Weakly pronounced spectral bands are observable at 3,750, 1,500, 1,225, 1,100, 975, 900, 800, 700 and 600 cm^{-1}. There is also an indication of an unstable state of the system at 3,700, 2,300, 1,800, 1,400 and 550 cm^{-1}.

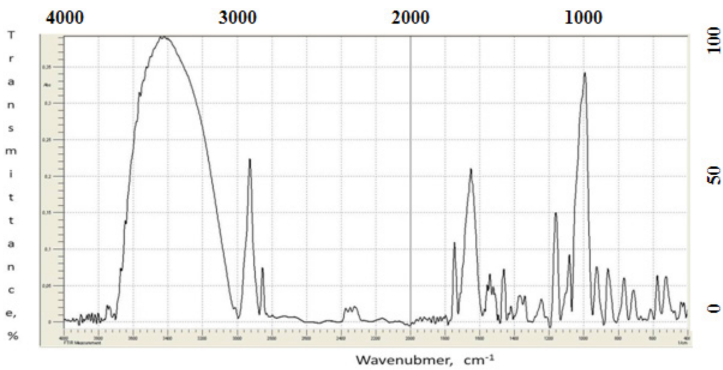

Figure 2. *Infrared spectrum of the maize hybrid ZP 434 grain*

1.3. Infrared spectroscopy of the maize hybrid ZP 505

Figure 3 shows the IR spectrum of the maize hybrid ZP 505 grain. There are eight very significantly expressed spectral bands at 3,400, 2,900, 1,750, 1,000, 2,850, 1,700, 1,450 and 1,150 cm^{-1}. Besides, spectral bands at 3,750, 3,025, 2,350, 1,550, 1,300, 1,100, 900, 775, 700, 575 and 500 cm^{-1} are weakly pronounced. There is an indication of instability of the system at 3,750, 1,900, 1,800, 1,460 and 1,430 cm^{-1}.

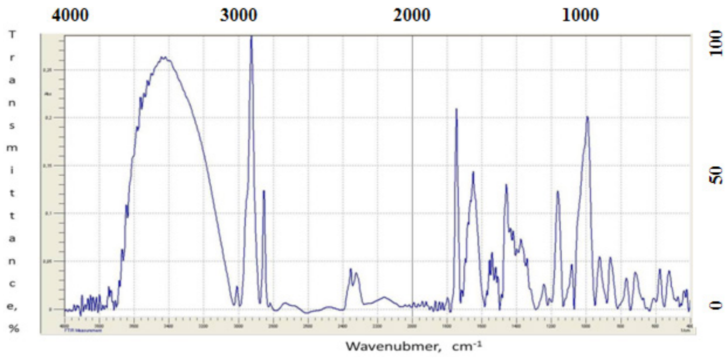

Figure 3. *Infrared spectrum of the maize hybrid ZP 505 grain*

2. Chemical composition of grains of studied maize hybrids

Results of overall studies of the chemical composition of the studied maize hybrids are presented in Table 1.

Table 1. *Results of the analyses of the chemical composition of maize hybrid grains*

Chemical composition of grains of maize hybrids	Range of the chemical composition in the literature*	Average chemical composition in the literature*	Average chemical composition of maize hybrids		
			ZP 341	ZP 434	ZP 505
Moisture (%)	7-23	16	11.96	11.56	11.14
Starch (%)	61-78	71.7	70.40	72.04	73.38
Proteins (%)	6-12	9.5	9.75	10.15	9.88
Lipids (oil) (%)	1-5.7	4.3	6.28	6.02	6.38
Ash (%)	1.1-3.9	1.4	1.34	1.40	1.31

* *Source*: [White and Johnson 2003]

3. Functional dependence of yields of observed maize hybrids in various locations in Serbia

High yielding and high quality maize hybrids: ZP 341, ZP 434 and ZP 505 are primarily intended for cultivation in European maize growing regions. Results of the yields of the stated maize hybrids are presented in Table 2.

Table 2. *Maize hybrid yields (t ha^{-1}) in several different locations in Serbia in the period 2008–2011*

Hybrid		Year				Average	
		2008	2009	2010	2011	t ha^{-1}	%
ZP 341		7.299	9.318	8.389	7.626	8.158	100.0
ZP 434		7.432	9.522	8.393	7.788	8.284	101.6
ZP 505		7.580	9.706	8.752	7.918	8.489	104.1
Mean	t ha^{-1}	7.437	9.515	8.511	7.777	8.310	-
	%	100.0	127.9	114.4	104.6	111.7	-

Genetic potential of the yield of maize hybrids ZP 341, ZP 434 and ZP 505 was observed in 38, 35, 41 and 37 locations in Serbia in 2008, 2009, 2010 and 2011, respectively. Common cropping practices required for maize hybrid cultivation were applied in trials. Irrigation was not applied. Results of the yields (Table 2) show that there are differences in yields among hybrids, but they are not significant. If the average yield of the hybrid ZP 341 is considered 100%, then the yields of hybrids ZP 434 and ZP 505 are higher by 1.6% (0.126 t/ha^{-1}) and 4.1% (0.331 t/ha^{-1}), respectively. These data point out to the fact that genetic potential of these hybrids is very similar and that only the hybrid ZP 505 has a certain advantages that is actually a result of its somewhat longer growing season. However, yields obtained over years differ much more. Hence, if the lowest yield recorded in 2008 is considered 100%, then yields were much higher in remaining years: 4.6% or 0,340 t/ha^{-1} in 2011, 14.4% or 1.074 t/ha^{-1} in 2010 and 27.9% or 2.078 t/ha^{-1} in 2009. These data unambiguously show to which extent climatic characteristics over years affected the maize yield.

4. A broad overview of breeding, seed production and technological traits of studied maize hybrids

Results of these analyses are presented in Tables 3a and 3b.

Table 3a. *Agronomic traits of studied maize hybrids*

Agronomic trait	ZP 341	ZP 434	ZP 505
Type of hybrid	TC	SC	SC
FAO maturity group	300	400	500
Plant height (cm)	210	220	230
Ear height (cm)	100	105	110
1000-kernel weight (g)	350	350	400
Type of kernel	dent	dent	dent
Sowing density (000 plants ha^{-1})	70	70	60-65
Leaf position	erect	erect	erect
Resistance to drought	good	good	good
Resistance to diseases	good	good	good
Appearance of leaves at harvest	stay green	stay green	stay green
Growing region (altitude, m)	up to 600	up to 600	up to 500
Silage yield (t/ha^{-1})	50	50	60

Table 3b. *Morphological traits of ears of studied maize hybrids*

Morphological trait	ZP 341	ZP 434	ZP 505
Grain moisture	11.96	11.56	11.14
Ear length (cm)	21.53	21.53	23.05
Ear weight (g)	281.43	296.62	309.13
Number of kernel rows per ear	14.6	14.7	15.7
Number of kernels per row	604.1	599.5	706.2
Embryo weight (g)	41.31	43.07	45.18
Kernel weight (g)	240.33	253.56	263.95

DISCUSSION

As already stated, different spectral bands varying in the number, intensity, shape, frequency and kinetics were established in IR spectra of maize hybrids (Figures 1–3). These bands occurred in the wavenumber range of 400 to 4,000 cm^{-1}. The intensity of spectral bands was designated as a transmittance (%) and it ranged from 0 to 100. Spectral bands were determined by valence oscillations and deformation oscillations of numerous functional groups within biogenic organic molecules, starch, proteins and lipids (Table 1). Essentially, this procedure resulted in possible changes in grain structure of studied maize hybrids.

Based on previously stated, the following two questions arise. First, how to acquire information on the individual biogenic organic molecules by valence oscillations and deformations of functional groups that result in appearance of the spectral bands?

And second, are there any differences in totality of IR spectra of grains of observed maize hybrids ZP 341, ZP 434 and ZP 505? If such differences exist, than it can be concluded that various structural properties of grains of studied maize hybrids exist.

The answers to these questions can be, to a great extent, found in Table 4.

The overall observation of the columns in the Table 4, especially those related to intensities of spectral bands, wavenumber values at which the bands occurred, wavenumber range taken over from the references [Vollhardt and Schore 1996], then the observation of the biogenic organic molecules with valence vibrations of functional groups, the information on structural properties of molecules in grains of studied maize hybrids were gathered. Organic molecules from maize hybrids grain were compared with molecules of organic compounds [Vollhardt and Schore 1996], which provided their identification.

When the same parameters for the studied maize hybrids are compared (Table 4), it can be concluded that the structural properties of the hybrids ZP 341 and ZP 434 were similar, while those in the hybrid ZP 505 differed to a greater extent.

Table 4. *Properties of IR spectra caused by valence vibrations of biogenic organic molecules of grains of studied maize hybrids*

Maize hybrid	Intensity of five most pronounced spectral bands, %	Wavenumber, cm^{-1}	Biogenic organic molecule*
ZP 341	87.5	3,400 3,200–3,650* 3,250–3,500*	alcohols amides
	70.5	1,000 1,000–1,260*	alcohols ethers
	56.0	2,950 2,840–3,000* 2,500–3,300*	alkanes carboxylic acid
	44.0	1,650 1,620–1,680* 1,690–1,750*	alkenes aldehydes ketones
	38.0	1,150 1,000–1,260`	alcohols ethers
ZP 434	100	3,410 3,200–3,650* 3,250–3,500*	alcohols amides
	88.5	1,000 1,000–1,260*	alcohols ethers
	60.5	2,925 2,840–3,000* 2,500–3,300*	alkanes carboxylic acid
	50.0	1,625 1,620–1,680* 1690–1750*	alkenes, aldehydes, ketones
	39.0	1,175 1,000–1,260*	alcohols ethers
ZP 505	93.0	3,410 3,200–3,650* 3,250–3,500*	alcohols amides
	100	2,975 2,840–3,000* 2,500–3,300*	alkanes carboxylic acid
	73.0	1,775 1,735–1,750* 1,690–1,750* 1,710–1,760*	esters aldehydes, ketones carboxylic acid
	67.0	1,000 1,000–1,260*	alcohols ethers
	49.5	1,650 1,690–1,750* 1,620–1,680*	aldehydes, ketones alkenes

**Source*: [Vollhardt and Schore 1996]

CONCLUSION

The IR spectrum was for the first time registered in grain of the maize hybrids ZP 341, ZP 434 and ZP 505 by the application of the IR spectroscopy method. According to obtained results the following can be concluded:

- IR spectrum of maize hybrid grain is characterised by 20–23 spectral bands that can occur in the wavenumber range of 400–4,000 cm^{-1} ;
- Spectral bands can be differently pronounced, can be of uneven intensity (transmittance, %), particular kinetics and various width at the base;
- Five spectral bands for each studied maize hybrid were analysed and the following data were gathered: intensity (%), value of the experimentally established wavenumber wavenumber range; literature data were also analysed; possibly present organic molecules;
- The following biogenic organic molecules: alcohols, amines, ethers, alkanes, carboxylic acids, alkenes, aldehydes, ketones and esters were registered (by their functional groups) in the IR spectrum of grain of maize hybrids;
- It can be concluded that the structural properties of the hybrids ZP 341 and ZP 434 are similar, while those in the hybrid ZP 505 differed to a greater extent.

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СТРУКТУРНЕ КАРАКТЕРИСТИКЕ ХИБРИДА КУКУРУЗА ПОКАЗАНЕ ИНФРАЦРВЕНИМ СПЕКТРИМА

Чедомир Н. РАДЕНОВИЋ[1,2], Георгиј В. МАКСИМОВ[3], Евгениј В. ТЈУТЈАЕВ[4], ИЉА В. СЈУСИН[4], Виталина В. ШУТОВА[4], Миле Д. СЕЧАНСКИ[1], Јелена Ж. СРДИЋ[1], Живорад В. ВИДЕНОВИЋ[1], Александар С. ПОПОВИЋ[1]

[1] Институт за кукуруз „Земун Поље“, 11185 Земун Поље, Београд, Србија
[2] Универзитет у Београду, Факултет за физичку хемију, Студентски трг 12–16, 11000 Београд, Србија
[3] „М. В. Ломоносов”, Московски државни универзитет, Биолошки факултет, Ленински гори 1–12, 119991 Москва, Русија
[4] Мордовиа Н. П. Огарев државни универзитет, Биолошки факултет, Болшевицкаја 68, 430005 Саранск, Русија

РЕЗИМЕ: У овом раду разматра се примена методе инфрацрвене спектроскопије на зрну хибрида кукуруза ради утврђивања његових могућих структурних карактеристика. Извршено је регистровање инфрацрвеног спектра зрна хибрида кукуруза: ЗП 341, ЗП 434 и ЗП 505. Показано је постојање различитих спектралних трака, како по броју и интензитету, тако и по њиховом облику, фреквенцији и кинетици. До њих се долази валентним осцилацијама и деформацијама функционалних група: алкана, алкена, алкина, амида, алкохола, етра, карбоксилне киселине, естра, алдехида и кетона, које су карактеристичне за биогена једињења угљоводоника, протеина и липида. На овај начин се долази до могућих разлика у структури зрна испитиваних хибрида кукуруза.

КЉУЧНЕ РЕЧИ: хибрид кукуруза, зрно, структурне карактеристике молекула, инфрацрвени спектри, спектралне траке