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# IDENTIFICATION OF ORGANIC MOLECULES IN KERNELS OF MAIZE INBRED LINES DISPLAYED WITH INFRARED SPECTRA

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The application of the infrared (IR) spectroscopy method to grain of maize inbred lines with high-quality traits to determine possible conformational and functional properties of their organic compounds is discussed. The IR spectrum of maize grain has been registered in the following inbred lines: ZPPL 186, ZPPL 225 and M1-3-3 Sdms. The existence of spectral bands varying in number, intensity, shape, frequency and kinetics has been determined. These bands have been determined by valence oscillations and deformation oscillations of the following functional groups: alkanes, alkenes, alkynes, amides, alcohols, ethers, carboxylic acids, esters, aldehydes and ketones that are characteristic for biogenic compounds of carbohydrates, vitamins, pigments and dietary fibres. In such a way, possible conformational and functional changes in the grain components of observed maize inbred lines could be detected. This was shown by total parameters of infrared spectra of grain.

*Key words: Zea mays* L., maize inbred lines, chemical composition of grain, conformational properties of organic molecules, infrared spectrum, spectral band.

At present, contemporary methods of spectroscopy and biotechnology provide crucial progress in diagnostics of a state of organs and vital functions of the whole plant at the molecular level. Vibratory spectroscopy (infrared and Raman) is an essential method in the analysis of spectra originating from molecular vibrations and deformations of functional bonds, which provide numerous data on properties of observed systems [1, 2, 3, 4, 12].

Our previous scientific papers [7, 8, 9, 10] described changes in the molecular structure of carotenoids in grain of various maize hybrids and inbred lines and showed that the structure of these molecules can be used as a molecular marker in evaluation of agronomic values of maize inbred lines and hybrids.

The IR spectroscopy method was applied in the present study to diagnose, at the molecular level, the state of biogenic compounds and life functions of grain of observed maize inbred lines. It is acknowledged that the IR spectroscopy provides the analysis of molecular composition and structure by registering the intensity of oscillations and deformations of molecular bonds [1, 3].

The aim of this study was to develop methods of the registration of the IR spectrum of grain of observed maize inbred lines and to identify conformational and functional changes in molecular bonds in grain biogenic compounds by the analysis of these changes.

## Methods

The following three maize inbred lines with high-quality traits were studied: ZPPL 186, ZPPL 225 and M1-3-3 Sdms. These inbreds are owned by the Maize Research Institute, Zemun Polje, Belgrade, Serbia. Since these inbred lines have high-quality traits their properties will be described in detail. These inbreds were used to develop more than 20 maize hybrids among which the most important are: ZP 341, ZP 360, ZP 434, ZP 544, ZP 633, ZP 735 and ZP 737. They are sown on approximately two million hectares per year. These hybrids have been released not only in Serbia, but also in Russia and another five European countries.

Overall studies of maize inbred lines with high-quality traits encompass several sets of experiments in which new and standard methods and procedures were applied.

*1. Infrared spectroscopy of grain of maize inbred lines.* Spectrophotometers used in the 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 however impose some differences [1, 12] first of all, the principles of vibration spectroscopy, the nature of the materials, sources of IR radiation, the application of thermal detectors, etc.

Today, spectrophotometers are more often based on the principle of interferometers. These devices do 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 so 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 inbred lines (ZPPL 186, ZPPL 225 and M1-3-3 Sdms) maize grain was homogenised and packed as a tablet with the addition of potassium bromide (KBr) while the registration of the IR spectrum was done by the IR spectroscope Fourier (Shimadzu IR-Prestige 21) in the range of 400—4000 $cm^{-1}$ (Fig. 1—3) values.

*2. Chemical composition of grain of maize inbred lines.* Methods used to determine the chemical composition of grain of observed maize inbred lines are generally accepted and standardised and described in detail in papers [5, 11, 14].

*3. Leaf angle and leaf area of maize inbred lines.* The third set of experiments referred to studies of erect position of top leaves in maize inbred lines. A specially designed protractor was used to measure the angle between lines of the position of the above-ear leaf and the position of the plant stalk of observed maize inbred lines. The leaf area was measured by using the portable area meter (model LI-3000, LI-COR Biosciences, USA). Measures of the angle between the above-ear leaf and the stalk and the leaf areas were carried out on 120 plants for each inbred line. These methodical procedures were described in previously published papers [5, 6].

*4. Overview of breeding and seed production traits of maize inbred lines.* Since the maize inbred lines have high-quality traits and efficient photosynthesis, a broader overview of relevant breeding and seed production traits, properties and parameters obtained by the application of standard ranking methods are presented.

## Results and discussion

*1. Infrared spectra of grain of maize inbred lines.* The observed IR spectrum was characterised by spectral bands. There was about 22—24 bands in the wave number range of 400 to 4000 cm$^{-1}$. Spectral bands were differently pronounced, of uneven intensity, special kinetics and various widths in their base. There were 5—6 distinctly pronounced bands, while 8—10, i.e. 4—5 bands had moderate, i.e. very low intensity, respectively. There were several spectral bands that could not be separated, or that indicated the unstable state of the system.

Figure 1 shows the IR spectrum of grain of the maize inbred line ZPPL 186. There are six very prominent spectral bands at 3400, 1000, 2900, 1700, 1175 and 2375 cm$^{-1}$. Moreover, spectral bands at 2850, 1775, 1450, 1550, 900, 850, 750, 700, 650 and 550 cm$^{-1}$ are also distinctively observable. A detailed survey shows weakly pronounced spectral bands at 3750, 1350, 1225, 1075 and 3085 cm$^{-1}$. There is an indication of an unstable state of the system in the wave number range of 400 to 4000 cm$^{-1}$: at 3900, 1875 and 650 cm$^{-1}$.

Figure 2 shows the IR spectrum of grain of the maize inbred line ZPPL 225. There are six very outstanding spectral bands at 3400, 1000, 1675, 2925, 2375 and 1175 cm$^{-1}$. Furthermore spectral bands at 1475, 1100, 1750, 2850, 1500, 1100, 925, 875, 775, 700 and 575 cm$^{-1}$ are also particularly observable. Weakly pronounced spectral bands are observable at 3250, 1275, 2850, 700 and 1225 cm$^{-1}$. There is also an indication of an unstable state of the system at 3850, 850, 1375 and 675 cm$^{-1}$.

Figure 3 shows the IR spectrum of grain of the maize inbred line M1-3-3 Sdms. There are five very significantly expressed spectral bands at 3400, 1000, 1650, 2950 and 1175 cm$^{-1}$. Besides, spectral bands at 1100, 1750, 1450, 950, 850, 775, 700 and 2850 cm$^{-1}$ are weakly pronounced. There is an indication of instability of the system at 3900, 2350 and 1900 cm$^{-1}$.

*2. Chemical composition of grain of maize inbred lines with high-quality traits.* Results of overall studies of the chemical composition of the studied

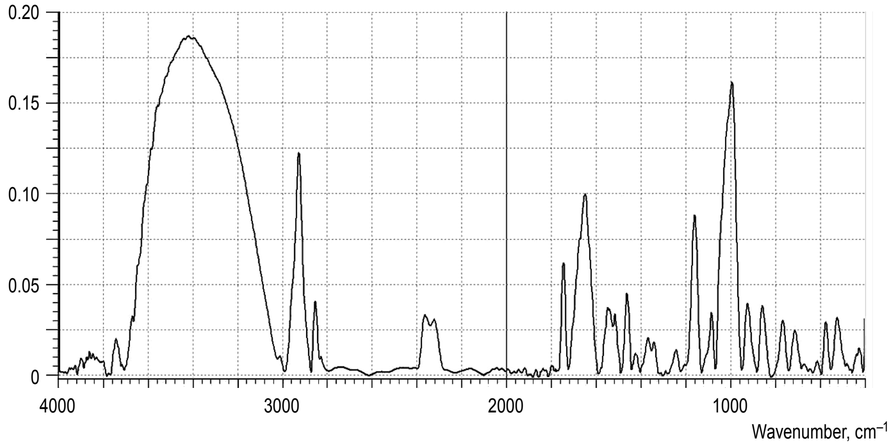


Fig. 1. Infrared spectrum of grain of the maize inbred line ZPPL 186

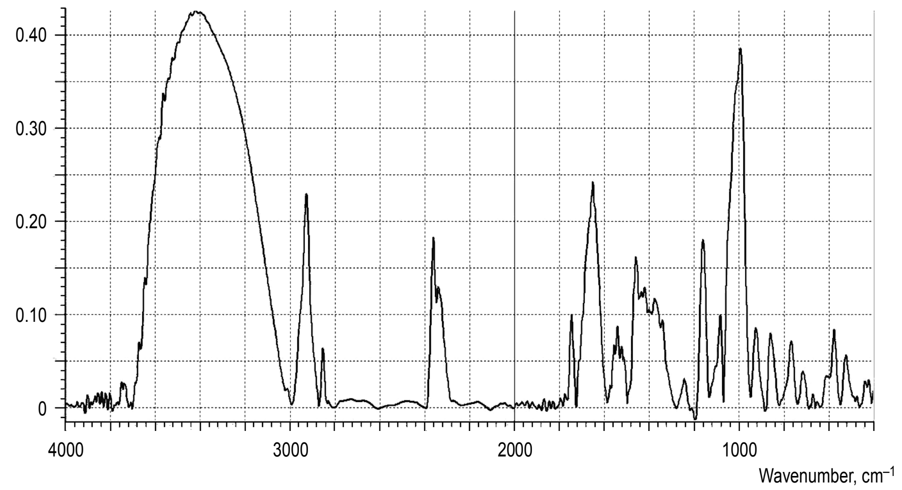


Fig. 2. Infrared spectrum of grain of the maize inbred line ZPPL 225

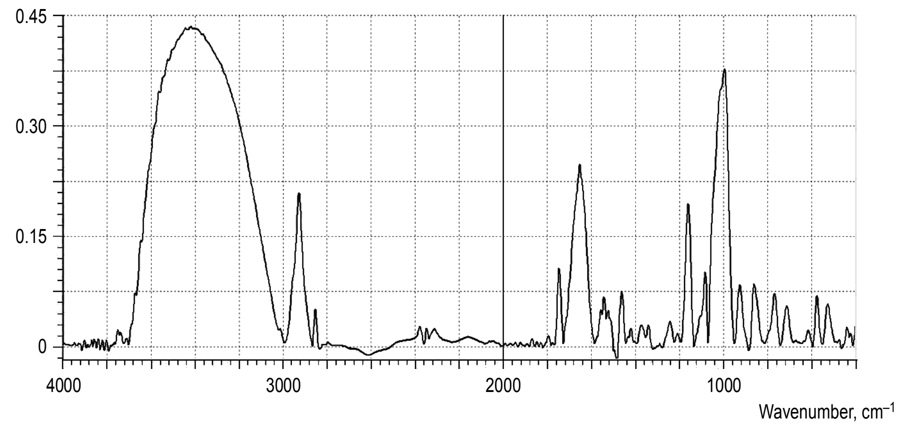


Fig. 3. Infrared spectrum of grain of the maize inbred line M1-3-3 Sdms

maize inbred lines are presented in Tab. 1. The obtained results refer to the grain chemical composition of the observed maize inbred lines and refer to important chemical constituents.

The study of the chemical composition of the grain showed that inbred lines contain more lipids then the average for maize based on literature data.

*3. Leaf angle and leaf area of the first leaf above ear shoot of maize inbred lines.* Tab. 2 presents the angle between the first leaf above ear shoot and the stalk, as well as average leaf areas. According to obtained results, the inbred lines have high-quality traits and belong to the recently developed inbred lines with erect top leaves, and are good photosynthetic models.

*4. Overview of breeding and seed production traits of maize inbred lines with high-quality traits and efficient photosynthetic functions.* The observed inbred lines ZPPL 186, ZPPL 225 and M1-3-3 Sdms have been used in the breeding process for the last five years. Therefore their selected traits, properties and parameters are shown in Tab. 3. As can be seen, inbred lines are very different in relation to the technological properties.

As already revealed different spectral bands varying in the number, intensity, shape, frequency and kinetics were established in IR spectra of maize

*TABLE 1. The chemical composition of grain of maize inbred lines*

| Chemical composition of grain of maize inbred lines | Range of the chemical composition in the literature [14] | Average chemical composition in the literature [14] | Average chemical composition of maize inbred lines | | |
|---|---|---|---|---|---|
| | | | ZPPL 186 | ZPPL 285 | M1-3-3 Sdms |
| Moisture (%) | 7—23 | 16 | 10.24 | 8.64 | 8.78 |
| Starch (%) | 61—78 | 71.7 | 67.80 | 64.22 | 65.52 |
| Proteins (%) | 6—12 | 9.5 | 10.22 | 11.33 | 12.44 |
| Lipids (oil) (%) | 1—5.7 | 4.3 | 7.53 | 6.96 | 7.14 |
| Ash (%) | 1.1—3.9 | 1.4 | 1.48 | 1.47 | 1.43 |
| Cellulose (%) | — | 3.0 | 2.26 | 2.31 | 1.99 |

*TABLE 2. Angle between the above-ear leaf and the stalk and leaf area of maize inbred lines with efficient photosynthesis*

| Maize inbred line* | FAO maturity group | Heterotic origin of inbred line* | Angle of the above-ear leaf, degree | | Leaf area of the above-ear leaf | |
|---|---|---|---|---|---|---|
| | | | $\bar{X}$ | σ | $\bar{X}$ | Σ |
| ZPPL 186 | 650—700 | BSSS, Zemun Polje | 21.50° | 1.13 | 3822 | 358 |
| ZPPL 225 | 350—400 | BSSS, Zemun Polje | 23.50° | 1.18 | 3686 | 334 |
| M1-3-3 Sdms | 530—560 | BSSS, Zemun Polje | 21.20° | 1.14 | 3159 | 310 |

*Observed maize inbred lines represent good heterotic pairs, have good combining abilities for grain yield, their propagation is well and they are highly yielding inbreds.

*TABLE 3. Relevant traits of maize inbred lines with efficient photosynthetic functions*

| Breeding and seed production traits | Brief description of breeding and seed production traits | | |
|---|---|---|---|
| | ZPPL 186 | ZPPL 225 | M1-3-3 Sdms |
| Grain yield $ha^{-1}$ in kg at 14% moisture | 2100 kg | 2100 kg | 2320 kg |
| % moisture in grain at harvest | 26.3 % | 19.62 % | 19.8 % |
| Number of plants $ha^{-1}$ at harvest<br>a) dry land farming<br>b) irrigation | <br><br>55000<br>67500 | <br><br>59000<br>75000 | <br><br>59000<br>67500 |
| Description and essential grain properties | Semi-flint, increased carotenoids content | Dent | Dent, increased carotenoids content alongside the kernel margins |
| Description and essential stalk properties | Moderately tall and elastic stalk | Low, slender and elastic stalk | Moderately tall, firm and elastic stalk |
| Data on emergence | Good emergence | Good emergence | Exceptionally good emergence, good early growth |
| Data on initial growth | Good initial growth | Good initial growth | Inbred suitable for early sowing |

*Continued on the next page*

*TABLE 3 (continuation)*

| Breeding and seed production traits | Brief description of breeding and seed production traits | | |
|---|---|---|---|
| | ZPPL 186 | ZPPL 225 | M1-3-3 Sdms |
| Inbred tendency towards greater densities with adequate nutrition | Stay green trait exceptionally pronounced | Stay green trait less pronounced | Inbred suitable for the development of hybrids for moderate densities |
| Does the leaf remain green for a long time? | Inbred remains green for a long time, leaves remain green until physiological maturity of grain | Leaves above the ear remain green until waxy ripeness | Stay green trait less pronounced |
| Does the inbred require any specific growing conditions? Estimation of resistance, tolerance and adaptability of the inbred | The inbred does not require any specific growing conditions; it is tolerant to all major disease-producing agents | The inbred does not require any specific growing conditions; it is tolerant to all major disease-producing agents | The inbred is tolerant to lower positive temperatures, and is moderately resistant to drought |
| Is harvest of the inbred easy and is mechanised harvest suitable? | Harvest is easy | Harvest is easy | Inbred is suitable for both hand and mechanised harvest |
| Is inbred zonation necessary? | Inbred is very adaptable and zonation is not necessary | Inbred is very adaptable and zonation is not necessary | Inbred is adaptable and zonation is not necessary |
| Is grain suitable for nutrition of ruminants and could it be recommended for nutrition of pets? | Inbred has grain of high quality and hybrids developed from it have grain of quality | Inbred has grain of high quality and hybrids developed from it have grain of quality suitable for nutrition of ruminants and nonruminants | Inbred is suitable for nutrition of ruminants, nonruminants and domestic animals |
| Is inbred resistant, tolerant or adaptable under drought conditions? | Inbred endures drought, increased and high temperatures | Inbred endures drought, increased and high temperatures | Inbred is moderately tolerant to drought |
| Suitability for grain and silage | Inbred is exceptionally suitable for the development of silage hybrids | Inbred is primarily intended for the development of grain hybrids | Inbred is suitable for the development of grain hybrids |
| Is the kernel large, of good quality, reddish and has it some other traits? | The medium large kernel, semi-flint type | The medium large kernel belongs to the dent type and has plenty of anthocyanins on its flanks | The kernel belongs to the dent type and has carotene on its flanks |

inbred lines (Fig. 1—3). These bands occurred in the wavenumber range of 400 to 4000 cm$^{-1}$. Spectral bands were determined by valence oscillations and deformations of numerous functional groups within biogenic organic molecules. Basically, this procedure resulted in possible changes in conformational and functional traits of the substances included in the grain of studied maize inbred lines.

Based on previously stated the following two questions can be asked: 1) 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 7 and 20? 2) Are there any differences in totality of IR spectra of grains of observed maize inbred lines ZPPL 186, ZPPL 225 and M1-3-3 Sdms? If such differences exist, then it can be concluded that various functional properties of grains of studied maize inbred lines exist.

*TABLE 4. Properties of IR spectra caused by valence vibrations of biogenic organic molecules of grain in studied maize inbred lines*

| Maize inbred lines | Intensity of the most pronounced spectral bands, % | Wavenumber, $cm^{-1}$ | Biogenic organic molecule* |
|---|---|---|---|
| ZPPL 186 | 81.5 | 3400<br>3200—3650*<br>3250—3500* | Alcohols,<br>amines,<br>alkynes, |
| | 73.0 | 1000<br>1000—1260* | Alcohols,<br>ethers |
| | 58.0 | 2900<br>2840—3000*<br>2500—3300* | Alkenes,<br>carboxylic acids |
| | 50.0 | 1700<br>1690—1750* | Aldehydes,<br>ketones |
| | 38.0 | 1175<br>1000—1260` | Alcohols,<br>ethers |
| | 36.0 | 1350<br>1000—1260* | Alcohols,<br>ethers |
| ZPPL 225 | 100 | 3410<br>3200—3650*<br>3250—3500*<br>3260—3330* | Alcohols,<br>amines,<br>alkynes |
| | 94.5 | 1000<br>1000—1260* | Alcohols,<br>ethers |
| | 53.0 | 1675<br>1620—1680*<br>1690—1750* | Alkenes,<br>aldehydes,<br>ketones |
| | 51.0 | 2925<br>2500—3300*<br>2840—3000* | Carboxyl,<br>oxygen,<br>alkenes |
| | 48.0 | 2375<br>2100—2260*<br>2220—2260* | Alkynes,<br>nitriles |
| | 39.5 | 1175<br>1000—1260* | Alcohols,<br>ethers |
| M1-3-3 Sdms | 76.5 | 3400<br>3200—3650*<br>3250—3500* | Alcohols,<br>amines |
| | 66.5 | 1000<br>1000—1260* | Alcohols,<br>ethers |
| | 48.5 | 1650<br>1620—1680* | Alkenes |
| | 36.5 | 2950<br>2500—3300*<br>2840—3000* | Carboxylic acids,<br>alkenes |
| | 34.5 | 1175<br>1000—1260* | Alcohols, ethers |

*Characteristic IR regions of valence vibrations of organic molecules according to which our obtained results were compared in relation to the compound class and the functional group. Source: [13].

The answers to these questions can be, to a great extent, found in Tab. 4. The intensity of spectral bands was designated as a transmittance (%) and it ranged from 0 to 100. The overall observation of the columns in the Table 4, especially those related to intensities of spectral bands, wave number values at which the bands occurred, wave number range taken over from the references [13] then the observation of the biogenic organic molecules with valence vibrations of functional groups, the information on structural and functional properties of molecules in grain of studied maize inbred lines (ZPPL 186, ZPPL 225 and M1-3-3 Sdms) is gathered. Organic molecules from grain of maize inbred lines were compared with molecules of organic compounds [13] which provided their identification.

When the same parameters for the studied maize inbred lines are compared (Tab. 4) it can be concluded that there were no differences for spectral bands at 3400, 2950, 2925, 2900, 1175 and 1000 $cm^{-1}$. However, maize inbred lines differed at 2375, 1700, 1675, 1650 and 1350 $cm^{-1}$. It turns out that we deal with: 1) aldehydes and ketons (ZPPL 186 at 1700 $cm^{-1}$), and then alcohols and esters (ZPPL 186 at 1350 $cm^{-1}$); 2) alkenes and nitriles (ZPPL 225 at 2375 $cm^{-1}$), and then alkanes, aldehydes and ketones (ZPPL 255 at 1675 $cm^{-1}$); 3) alkenes (M1-3-3 Sdms at 1650 $cm^{-1}$).

The IR spectra were for the first time registered in grain of the maize inbred lines ZPPL 186, ZPPL 225 and M1-3-3 Sdms by the application of the IR spectroscopy method. According to obtained results the following can be concluded.

IR spectrum of grain of maize inbred lines is characterised by 22—24 spectral bands that can occur in the wave number range of 400—4000 $cm^{-1}$.

Spectral bands can be differently pronounced, they can be of uneven intensity, particular kinetics and of various widths at the base.

Five or six spectral bands for each studied maize inbred line was analysed and the following data were gathered: intensity (%), value of the experimentally established wave number, wave number range, and at the end, the classes of organic molecules with their functional groups.

The following biogenic organic molecules with their functional groups: alcohols, amines, ethers, alkanes, carboxylic acids, alkenes, aldehydes, ketones, esters and nitriles were registered in the IR spectrum of grain of maize inbred lines.

It can be concluded that the conformational and functional properties of the organic molecules for all three studied inbred lines were mainly similar.

Despite the fact that inbred lines of maize differ in their morphological and physiological characteristics they retain the close chemical composition in grain of high quality.


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## ИДЕНТИФИКАЦИЯ ОРГАНИЧЕСКИХ МОЛЕКУЛ В ЗЕРНЕ ИНБРЕДНЫХ ЛИНИЙ КУКУРУЗЫ ПРИ ПОМОЩИ ИНФРАКРАСНЫХ СПЕКТРОВ

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Обсуждается применение метода инфракрасной (ИК) спектроскопии для определения возможных конформационных и функциональных свойств веществ, входящих в состав зерна инбредных линий кукурузы с признаками высокого качества. ИК спектры зерна кукурузы измерены у инбредных линий ZPPL 186, ZPPL 225 и M1-3-3 Sdms. Выявлены спектральные полосы, различающиеся по количеству, интенсивности, форме, частоте и кинетике. Они определены по колебаниям связей и смещению атомов таких функциональных групп: алканов, алкенов, алкинов, амидов, спиртов, эфиров, карбоксильных групп, сложных эфиров, альдегидов и кетонов, характерных для биологических соединений углеводов, витаминов, пигментов и клетчатки. Таким образом, можно определить возможные конформационные и функциональные изменения веществ зерна исследованных инбредных линий. Это было показано обобщенными параметрами ИК спектров зерна.

# ІДЕНТИФІКАЦІЯ ОРГАНІЧНИХ МОЛЕКУЛ У ЗЕРНІ ІНБРЕДНИХ ЛІНІЙ КУКУРУДЗИ ЗА ДОПОМОГОЮ ІНФРАЧЕРВОНИХ СПЕКТРІВ

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Обговорюється застосування методу інфрачервоної (ІЧ) спектроскопії для визначення можливих конформаційних і функціональних властивостей речовин, що входять до складу зерна інбредних ліній кукурудзи з ознаками високої якості. ІЧ спектри зерна кукурудзи виміряні в інбредних ліній ZPPL 186, ZPPL 225 і M1-3-3 Sdms. Виявлено спектральні смуги, що різняться за кількістю, інтенсивністю, формою, частотою і кінетикою. Вони визначені за коливаннями зв'язків і зміщенням атомів таких функціональних груп: алканів, алкенів, алкінів, амідів, спиртів, ефірів, карбоксильних груп, складних ефірів, альдегідів і кетонів, які характерні для біологічних сполук вуглеводів, вітамінів, пігментів і клітковини. Отже, можна визначити можливі конформаційні і функціональні зміни речовин зерна досліджених інбредних ліній. Це було показано узагальненими параметрами ІЧ спектрів зерна.