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Original scientific papers

High-yielding and chemically enriched maize hybrids bred in Serbia: The best basis for super quality feed and food

Высокоурожайные и химически обогащенные гибриды кукурузы сербской селекции – лучшая основа для высококачественных продуктов питания и кормов для домашних животных

Високоприносни и хемијски обогаћени хибриди кукурузасрпске селекције као најбоља основа за најквалитетнију храну

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Abstract:

Introduction/purpose: This paper presents the results of several different research studies. The inbred lines ZPPL 146 and ZPPL 159 and the maize hybrids ZP 633, ZP 735, and ZP 737 are primarily intended for human and livestock nutrition. Their selection took about four decades.
Methods: Spectral bands were registered using the method of resonant Raman spectroscopy of the leaves of inbred maize lines. These spectral bands indicate the conformational characteristics of not only carotenoid molecules but also other compounds (phosphate, gluten, and amide III) in the leaf.
Results: A systematic examination of the inbred lines ZPPL 146 and ZPPL 159 and their maize hybrids ZP 633, ZP 735, and ZP 737 was performed in this paper. It was stated that the new inbred lines of corn, i.e. ZPPL 146 and ZPPL 159, are rich in carotenoids and yellow pigments. These lines also have significant quantities of other valuable bioactive compounds and good physical characteristics. The lines have an upright position of the top leaves and belong to the group of maize lines with significant characteristics of the photosynthetic model. They are resistant to high temperatures and are drought tolerant.
Conclusion: This paper presents the relevant properties, characteristics and parameters of the new studied inbred maize lines that can be used in selection processes in the future. High-yielding and high-quality maize hybrids, i.e. ZP 633, ZP 735, and ZP 737, have been created from the mentioned inbred maize lines. They are recognizable by their qualities. The hybrid ZP633 is especially noteworthy for human consumption (children and the elderly). Further, from the agronomic-veterinary point of view, it is confirmed that the hybrids ZP 735 and ZP 737 are the most suitable for livestock feeding with the programmed use of corn silage. The relevant agronomic, morphological and nutritional properties of the maize hybrids ZP 633, ZP 735, and ZP 737 are also presented in this paper. The results regarding the grain structure and yield height for grain and silage for the hybrids ZP 677 and ZP 684, produced in Serbia and the countries of Southeastern Europe, are also given.

Keywords: Delayed chlorophyll fluorescence, Raman spectroscopy of leaves, photosynthetic model, Zea mays L, inbred lines, hybrids, thylakoid membrane, pigment properties, nutritive values, good quality food and feed.

Резюме:

Введение/цель: В данной статье представлены результаты ряда различных исследований. Селекция инбредных линий ZPPL 146 и ZPPL 159, произведенная на основе гибридов кукурузы, предназначенных в первую очередь для производства продуктов питания и кормов для домашних животных, заняла около четырех десятилетий: ZP 633, ZP 735 и ZP 737.
Методы: В исследовании применялся метод резонансной рамановской спектроскопии листьев инбредных линий кукурузы, с помощью которой были обнаружены спектральные полосы, которые указывают на конформационные характеристики молекул каротиноидов, а также других соединений (фосфата, глютена и амида III).
Результаты: На основании проведенного систематического исследования инбредных линий: ZPPL 146 и ZPPL 159 и гибридов кукурузы: ZP 633, ZP 735 и ZP 737 выявлено, что новые инбредные линии кукурузы: ZPPL 146 и ZPPL 159 богаты каротиноидами и желтыми пигментами. Эти линии в большом объеме обладают и другими важными биологически активными соединениями и благоприятными физическими характеристиками. Изучаемые инбредные линии кукурузы отличаются прямостоячим положением верхних листьев и относятся к группе линий кукурузы со значительными характеристиками модели фотосинтеза. Это жаростойкие и засухоустойчивые растения.
Выводы: В данной статье представлены релевантные свойства, характеристики и параметры изученных новых инбредных линий кукурузы, которые в перспективе могут быть использованы в процессе селекции. С помощью вышеуказанных инбредных линиий кукурузы созданы высокоурожайные и высококачественные гибриды кукурузы: ZP 633, ZP 735 и ZP 737, отличающиеся по своим качествам. Особенно узнаваем гибрид ZP 633, предназначенный для производства продуктов питания (для детей и пожилых людей).

Ключевые слова: замедленная флуоресценция хлорофилла, рамановская спектроскопия, модель фотосинтеза, Zea mays L, инбредная линия, гибрид, тилакоидная мембрана, пигментные свойства, пищевая ценность, продукты питания и корма для домашних животных.

Abstract:

Увод/циљ: У овом раду излажу се резултати више различитих истраживања. Констатује се да је око четити деценије трајало селекционисање инбред-линија ZPPL 146 и ZPPL 159 и са њима створених хибрида кукуруза: ZP 633, ZP 735 и ZP 737, првенствено намењених за исхрану људи и домаћих животиња.
Методе: Применом методе резонантне Ранамове спектроскопије листа инбред-линија кукуруза регистроване су спектралне траке које указују на конформационе карактеристике молекула каротеноида, али и других једињења (фосфата, глутена и амида III) у листу.
Резултати: У раду је извршено систематско испитивање инбред-линија: ZPPL 146 и ZPPL 159 и хибрида кукуруза: ZP 633, ZP 735 и ZP 737. Констатовано је да су нове инбред-линије кукуруза: ЗППЛ 146 и ЗППЛ 159 богате каротеноидима и жутим пигментима. Те линије имају, у знатним количинама и вредностима, и друга релевантна биоактивна једнињења и добре физичке карактеристике. Проучаване инбред-линије кукуруза имају усправан положај вршних листова и спадају у групу линија кукуруза са значајним карактеристикама фотосинтетичког модела. Оне су отпорне на високу температуру и толерантне су на сушу.
Закључак: У раду су изложена релевантна својства, карактеристике и параметри проучаваних нових инбред-линија кукуруза која се могу користити у будућности у селекционом процесу. Са наведеним инбред-линијама кукуруза створени су високоприносни и квалитетни хибриди кукуруза: ZP 633, ZP 735 и ZP 737, који су препознатљиви по својим квалитетима,а нарочито хибрид ZP 633 за исхрану људи (деце и старијих лица). Исто тако, са агрономско-ветеринарског становишта је потврђено да су хибриди ZP 735 и ZP 737 најпогоднији за исхрану домаћих животиња уз програмирано коришћење кукурузне силаже. Такође, изложена су релевантна агрономска, морфолошка и нутритивна својства хибрида кукуруза ZP 633, ZP 735 и ZP 737. Презентовани су и резултати о структури зрна и висини приноса за зрно и силажу и код хибрида ZP 677 и ZP 684, који се производе у Србији и земљама југоисточне Европе.

Keywords: закаснела флуоресценција хлорофила, Раманова спектроскопија листа, фотосинтетички модел, Zea mays L, инбред-линија, хибрид, тилакоидна мембрана, пигментна својства, нутритивна вредност, храна за људе и животиње.

Introduction

The period from 1978 to the present day (2020) has become historically significant because a tremendous success in maize breeding and high quality hybrid seed production was achieved. Because of such activities, over 1500 grain and silage hybrids have been derived (Duvick, 1984), (Sprague, 1984), (Trifunović, 1986), (Dumanović, 1986), (Hallauer, 1988) and (Ivanović et al, 1995).

Modern technical and technological prerequisites were provided for carrying out the process of breeding, efficient production of hybrid maize seeds and significant amounts of seeds of commercial and silage hybrids (Kojić & Ivanović, 1986), (Petrović et al, 1992), (Pejić, 1994), (Jovanović, 1996, 1998), (Bekrić, 1997, 1999), (Dumanović & Pajić, 1998), (Jovanović et al, 2000), (Radenović & Somborac, 2000), (Antov et al, 2004), (Dinić & Đorđević, 2005).

In the context of the stated dynamics of the development, interdependent studies of many scientific disciplines (physiology, biochemistry, biophysics, biotechnology, breeding, photosynthesis, Raman spectroscopy, infrared spectra of grain, processing technology of cereals, silage production practices, silage utilisation and food science) have been linked with the aim of modernising and efficient implementation of contemporary programmes on maize breeding and seed production (Radenović, 1994), (Pajić et al, 1995), (Radenović et al, 2004, 2008, 2009), (Radenović & Somborac, 2000), (Konstantinov et al, 2010). In addition to the outstanding results achieved in the selection of standard grain and maize silage hybrids, there was a pressing need to develop new inbred lines and better quality maize hybrids with an improved chemical composition of the grain, especially in essential bioactive compounds. Moreover, the intensive work has been carried on the improvement and development of new methods for the preservation of plants, especially maize hybrid plants in the form of silage of the whole plant and grain. Silage is biologically fermented or chemically preserved feed of a plant origin. Silage maize growing practices differ, to a certain extent, from the practices used in the commercial maize cultivation. Namely, it is very important to produce the maximum quantity of silage per area unit and it is necessary that the produced silage be of high quality. Therefore, in order to succeed in this, it is necessary to select a plot with good soil properties, use high quality seed, apply the necessary amount of mineral fertilisers, perform proper and timely sowing and apply tested herbicides that have no residual effects and toxic effects on silage mass. All this has to provide silage that contains approximately 50% grain, as it is a prime quality part. Furthermore, it is important that the crop be disease free, so that there are no adverse effects in feeding ruminants and no negative effects on their health, quality of meat and milk, and dairy products. Nowadays, silage is the basis of cost-effective and contemporary animal husbandry and the closest substitute for green forage (Bekrić, 1997, 1999), (Dumanović & Pajić, 1998), (Pejić, 1994), (Jovanović, 1996, 1998), (Jovanović et al, 2000), (Antov et al, 2004), (Dinić & Đorđević, 2005), (Liu, 2007), (Strati et al, 2012), (Radenović, 2002, 2013), (Bacchetti et al, 2013), (Đorđević & Dinić, 2003), (Kurlich & Juvik, 1999), (Luo & Wang, 2012).

To meet many demands, justifiable and increased needs for functional and quality nutrition of people (mainly children and the elderly) and livestock (primarily cows and sheep), it was necessary to select new maize inbred lines with significantly richer pigment-complex properties and the exceptional nutritional value. This aim was achieved with the increased content of carotenoids and other bioactive compounds. With such inbred lines, it was possible to develop high-quality maize hybrids which would meet requirements of medicine, veterinary medicine and agronomy and be necessary for healthy food and feed, which was the objective of the present study.

Materials and methods

Plant material – The genetic and breeding studies of the new maize inbred lines have been performed for over 40 years. These inbreds, future parental forms, primarily ZPPL 146 and ZPPL 159, have increased chemical compositions, whereas the hybrids of high yields and extra quality derived from these inbreds are used as follows: ZP 633 - standard in nutrition of children, the elderly and athletes; products: flour, semolina; ZP 737 - standard in nutrition of fattening chickens up to 1.5-3kg and laying hens; products: ground maize, coarse meal, flour and grain silage; and ZP 735 - standards in nutrition of calves, fattening heifers, dairy cows, sheep, goats and pigs; products: coarse meal, ground maize, grain silage, ear silage and the whole plant silage. In recent times, the hybrids ZP 677 and ZP 684 have also been used for silage. Figure 1 shows the actual appearance of the elite maize inbred lines with erect top leaves, i.e. ZPPL 146, ZPPL 159, and high-quality maize hybrids ZP 633, ZP 735, and 737 with their erect top leaves.

The observed maize inbred lines and hybrids have the increased content of carotenoids and other bioactive compounds. They have been developed and owned by the Maize Research Institute, Zemun Polje, Belgrade, Serbia.

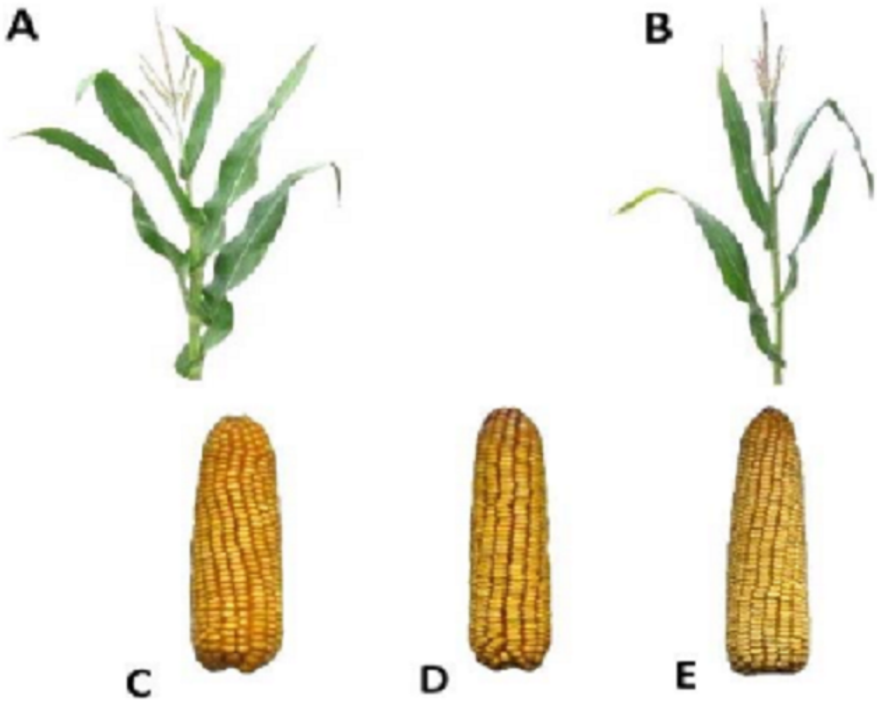

FIGURE 1
Actual appearance of the elite maize inbred lines with erect top leaves ZPPL 146 (A) and ZPPL 159 (B) and the high-quality maize hybrids ZP 633 (C), ZP 735 (D) and ZP 737 (E) with their erect top leaves

Methods – Overall studies of the stated new inbred lines and hybrids with erect top leaves developed from these inbreds encompassed several series of experiments in which new and standard methods and procedures were applied.

1. Chemical compositions of the new inbred lines and high-quality maize hybrids

The methods applied to determine the grain chemical composition of the maize inbred lines and hybrids are generally accepted and standardised and already described in detail in previous papers (Radosavljević et al, 2000), (Bekrić, 1997), (White & Jonson, 2003), (AACC, 2013), (Strati et al, 2012), (Radenović et al, 2010).

2. Resonance Raman spectroscopy method applied to the maize inbred lines

The measurements of resonance Raman spectroscopy of the maize inbred line leaves were done in accordance with the procedure and the method was described in our previously published papers (Radenović et al, 1994, 1998).

3. The measurement of the angle and the leaf area of the maize inbred lines

This series of experiments was related to studying the erect position of top leaves in the maize inbred lines. A specially designed protractor was used to measure the angle between the position of the above-ear leaf and the position of the plant stalk on the maize inbred lines. The leaf area was measured by the LI-3000 Portable Leaf Area Meter (LI-COR Biosciences, USA). The measurements of the angle between the above-ear leaf and the stalk and the leaf areas were carried out on 122 plants for each maize inbred line during the three-year period. These methodical procedures were described in the previously published papers (Radenović et al, 2009, 2010).

4. Photosynthetic fluorescence measurements

This series of the experiments was related to photosynthetic-fluorescence studies, including thermal processes of delayed chlorophyll fluorescence, critical temperatures (phase transitions), and activation energies. The test maize inbreds were grown in the experimental field of the Maize Research Institute, Zemun Polje. The plants were brought from the experimental field to the laboratory between 7 a.m. and 8 a.m. These plants sampled in the field were transversally cut in the ground internode. In the laboratory, the plants were placed in water along the length of one internode. Prior to the fluorescence experiment, all plants were kept under the black ball glass for two hours. A segment of intact above ear leaves was taken from such plants and placed into a chamber of the phosphoroscope. The intact leaf segments were kept in the chamber (in the dark) for at least 15 minutes, and then the thermal processes of delayed chlorophyll fluorescence were measured. These tests were performed on 111 plants of each maize inbred line. An improved, noninvasive photosynthetic-fluorescence method was applied for these measurements. This method was developed at the Maize Research Institute and was described in the previously published papers (Radenović et al, 2002, 2004, 2007, 2008, 2010, 2013).

5. Survey of the breeding and seed production properties of the new maize inbred lines

Since these maize inbred lines with efficient photosynthesis, rich in pigments and with exceptional nutritive qualities are promising, a broad survey of their relevant breeding and seed production properties, traits and parameters obtained by standard methods of ranking (Radenović et al, 2007, 2008, 2009, 2013) are presented in this article.

6. Functional dependence of the yield of the studied maize grain and silage hybrids

Numerous and long-term studies on the yields (t ha-1) of the three high-yielding and high-quality grain and maize silage hybrids (ZP 633, ZP 735, ZP 737) were performed in many locations in Serbia and other countries of Southeastern Europe. Standard methods for maize production, tinning and processing were applied in these studies (Pejić, 1994), (Bekrić, 1997, 1999), (Jovanović, 1996, 1998), (Jovanović et al, 2000), (Antov et al, 2004), (Đorđević & Dinić, 2003), (Dinić & Đorđević, 2005), (Videnović et al, 2011), (Radenović, 2013).

7. Medical, veterinary, agronomic, and nutrition estimation of the need for human and animal nutrition with products based on maize hybrids bred for a specific purpose

7.1. Human nutrition with products based on maize hybrids enriched with pigments and other nutrition ingredients

Empirical efforts to acquire knowledge about the need for maize diet in human nutrition were initiated a long time ago, perhaps 300-400 years ago. Much later, in the 1950s, the scientific literature related to this topic emerged, primarily in medical institutions. However, the authors of this study became interested in this topic in the early 1990s (Radenović, 1991).

7.2. Animal nutrition with products based on maize hybrids bred for silage

It is believed that 1150 years have passed since the first procedures of preserving green crops. Modern and improved technology of preserving crops by ensiling flourished as late as the early second half of the 20th century (1955-1965) (Bekrić, 1997, 1999), (Pejić, 1994), (Jovanović, 1998), (Jovanović et al, 2000), (Đorđević & Dinić, 2003), (Dinić & Đorđević, 2005). Modern, intensive and cost-effective production in cattle husbandry can no longer be imagined without silage. Furthermore, the advantages of such feed have been growing in goat and sheep breeding and to a lesser extent in pig breeding (Pejić, 1994), (Jovanović, 1996, 1998), (Jovanović et al, 2000), (Đorđević & Dinić, 2003), (Dinić & Đorđević, 2005), (Radenović, 2013). The authors of this paper became interested in this topic at the beginning of the 21st century (in 2002). At that time, a great number of high-quality maize hybrids intended for production of high-quality silage were developed with the aim to regulate metabolic processes in domestic animals thus improving their growth and quality of meat and milk (Radenović, 2002).

Results and discussion

1. Chemical composition and physical traits of grain of maize inbred lines and high-quality maize hybrids

The results of the studies of the chemical composition and physical traits of the grains of the observed maize inbred lines and hybrids are presented in Table 1. The obtained results relate to important chemical and physical constituents.

TABLE 1
Results obtained in the analyses of the chemical composition and physical traits of the grain of maize inbred lines and hybrids (Zea mays L.) (three-year average, trial field of the Maize Research Institute, Zemun Polje, Belgrade, Serbia)

Chemical composition and physical traits of the maize (*Zea mays* L.) grain	Published data*		Average data of the observed grain for storage in silos of the elite maize (*Zea mays* L.) inbred lines and hybrids				
			Inbred lines		Hybrids		
	Range	Average	**ZPPL 146**	**ZPPL 159**	**ZP 633**	**ZP 735**	**ZP 737**
Moisture (%, wet basis[a])	7-23	16.0	10.24	10.12	9.90	9.84	10.15
Starch (%)	61-78	71.7	67.80	66.26	68.23	64.39	67.86
Protein (%)	6-12	9.5	10.22	12.57	11.11	12.27	11.57
Fat (oil) (%)	1.0-5.7	4.3	7.53	5.38	6.11	5.82	7.16
Ash (%)	1.1-3.9	1.4	1.48	1.45	1.51	1.54	1.47
Cellulose (%)	-	3.0	2.26	2.33	2.37	2.43	2.00
Pentosans (as xylose), %	5.8-6.6	6.2	-	-	-	-	-
Fibres, %	8.3-11.9	9.5	-	-	-	-	-
Cellulose + lignin, %	3.3-4.3	3.3	-	-	-	-	-
Sugars, total (as glucose), %	1.0-3.0	2.6	-	-	-	-	-
Yellow pigment, (µg βCE/g d.m.)**	-	-	19.00	18.10	27.30	21.90	21.60
Total carotenoids (mg/kg)	12-36	26.0	33.20	31.80	32.40	28.30	27.80
1000-kernel weight (g)	217-438	343.70	277.45	283.03	333.82	295.81	296.95
Chemical composition and physical traits of the maize (*Zea mays* L.) grain	Published data*		Average data of the observed grain for storage in silos of the elite maize (*Zea mays* L.) inbred lines and hybrids				
			Inbred lines		Hybrids		
	Range	Average	**ZPPL 146**	**ZPPL 159**	**ZP 633**	**ZP 735**	**ZP 737**
Test weight (kg/m^3)	693-843	791.00	829.84	844.96	809.03	808.27	817.07
Density (g/cm^3)	1.21-1.38	1.26	1.27	1.29	1.27	1.28	1.28
Flotation index (%)	0-68	27.10	10.56	10.68	25.12	13.36	7.91
Grinding resistance (%)	7.0-25.8	15.90	8.77	14.33	15.80	13.27	11.07
Hard endosperm fractions (%)	54.3-71.3	59.20	11.00	10.67	9.67	11.33	9.67
Soft endosperm fractions (%)	45.7-28,7	40.80	23.33	18.66	23.33	21.67	23.33
Water absorption index	0.180 - 0.284	0.245	0.245	0.237	0.215	0.237	0.227

* Source: P.J. White & L.A. Jonson (White & Jonson, 2003). ** Done by the AACC Methods 14-50.01 (AACC, 2013).

2. Conformational changes in carotenoid molecules in the leaf of txe maize inbred lines

The Raman spectra are very suitable for studying photosynthetic pigments in terms of conformational changes of carotenoid molecules. The authors of the present study have been dealing with this topic for a long period of time (Radenović et al 1994, 1995, 1998).

However, this study will highlight some conformational changes in molecules of carotenoids in the leaf of the observed maize inbred lines. Thus, the following six characteristic resonance Raman spectral bands were established within the 900 cm^{-1}-1800 cm^{-1} interval of Raman frequencies: 962, 1026, 1160, 1187, 1206, and 1520 cm^{-1}, Figure 2.

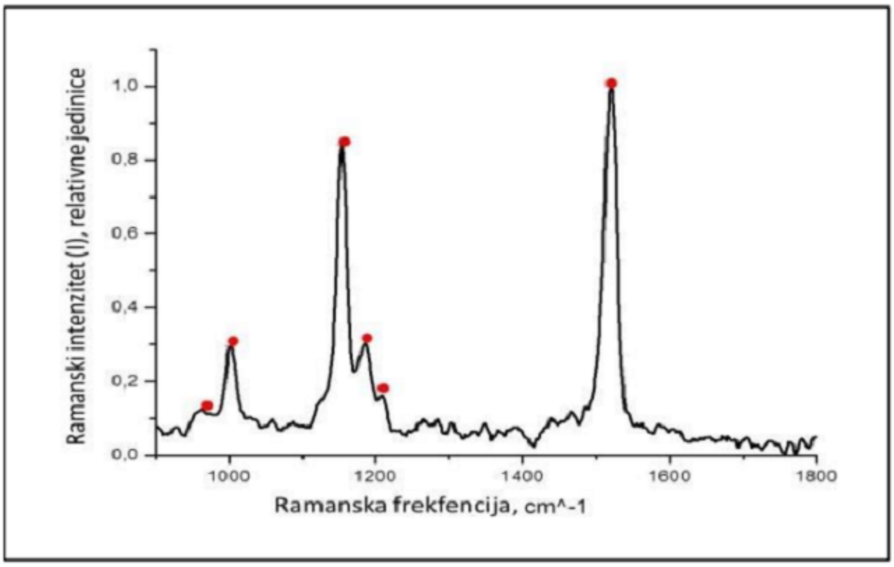

FIGURE 2
Resonance Raman spectrum of the leaf of the maize inbred lines ZPPL 146 and ZPPL 159

Four spectral bands with lower intensities (I_{926}, I_{1026}, I_{1187}, I_{1206}) were caused by conformational changes of phosphates, glycogens, and amides III. The remaining two spectral bands with significantly higher intensities (I_{1160}, I_{1520}) have been regularly analysed in relation to the conformational changes in the carotenoid molecule. It is common to analyse the differences in the intensities of spectral bands (I_{1520} and I_{1160}) and even more often the differences in their ratio (I_{1520}/I_{1160}). The resonance Raman spectrum of the leaf of the observed inbred lines with dominant spectral bands (I_{1520} and I_{1160}) is presented. This spectrum conditions the carotenoid molecules placed in the non-polar phase of the thylakoid membrane of the leaf of the inbred lines. In this paper, the effort was made to emphasize the application of resonance Raman spectroscopy in studying important vital functions of leaves of maize inbred lines, especially under agroecological conditions atypical for the maize growing region. Carotenoid molecules (β caroten, $C_{40}H_{56}$, with the activity of vitamin A, but also two xanthophylls: cryptoxanthin $C_{40}H_{56}O$ and zeaxanthin $C_{40}H_{56}O_2$), since localised in the non-polar phase of the thylakoid membrane of maize inbred leaves, showed to be a very suitable natural probe, capable of contributing to registering not only higher and more significant, but also smaller and finer conformational changes. These changes in the molecular structure of carotenoids may be expressed in the form of bending, stretching, compressing, and physical disruption of chemical bonds, which is caused by intensive actions of environmental factors, unfavourable critical temperatures in the first place. In the end, each conformational change in the carotenoid molecule unconditionally changes the function not only of the carotenoid molecule but also of the thylakoid membrane in leaves of maize inbred lines. Conformational changes in chemical bonds – C = C – are reflected in the spectral band at 1520cm^{-1}. In addition, conformational changes in chemical bonds = C – C = are reflected in the spectral band at 1160cm^{-1} (Karnauhov, 1988).

3. The measure of the angle and the area of the above-ear leaf of maize inbred lines

The results of the measurements of the angles between the above-ear leaf and the stalk are presented in Table 2 as well as the average leaf areas. Based on the obtained angle measurement results, it can be stated that the observed maize inbred lines belong to the group of contemporary inbred lines with erect top leaves and the status of the photosynthetic model.

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

Maize inbred line*	FAO maturity group	Heterotic origin of the inbred line*	Angle of the above-ear leaf, (°)		Leaf area of the above-ear leaf (cm^2)	
			Import Image	σ	Import Image	σ
ZPPL 146	650-700	BSSS, USA Zemun Polje	20.8°	1.2	3762.7	238
ZPPL 159	550-600	Landrace from Argentina (S13) crossed to the in-bred PE 25-10-1, Zemun Polje	21.3°	1.2	2378.1	241

* The observed maize inbred lines represent good heterotic pairs which have good combining abilities for grain yield and silage as well as good propagation and high yield These inbreds are rich in pigments and have extraordinary nutritive qualities

4. Empirical procedure for photosynthetic and fluorescence studies on the above-ear leaf of maize inbred lines bred for the production of healthy food

The thermal processes of delayed chlorophyll fluorescence of the observed maize inbred lines intentionally selected for developing maize hybrids to be used in the production of various feed and food products were studied in detail.

The thermal curve is a curve that shows the changes in the delayed chlorophyll fluorescence level intensity depending on temperature. The trend of its establishment is most often analogous to changes in the duration in seconds for the segments designated with a, b, c, d, e, f, and g, Figure 3, which was determined by the empirical procedure (Radenović et al, 2008, 2009, 2010).

Monitoring the course of the thermal curve and the analysis of the duration of certain segments provided data on the existence of a greater number of critical temperatures (phase transition temperatures) at which greater or smaller structural and functional changes occurred in the thylakoid membrane of the observed maize inbred lines.

In accordance with such information, it is possible to draw a conclusion on their different responses to the phenomena of resistance, drought and high temperature as well as on the phenomenon of their adaptation.

5. The exact temperature dependence of the delayed chlorophyll fluorescence intensity for the thylakoid membrane of new maize inbred lines bred for the production of healthy food

The changes in the intensity of the stationary level of delayed chlorophyll fluorescence (I_{DF}) depending on temperature which ranges from 25 °C to 60 °C were measured. The dynamics of the changes of the temperature dependence for the observed maize inbred lines is presented in Figures 4a and 4b.

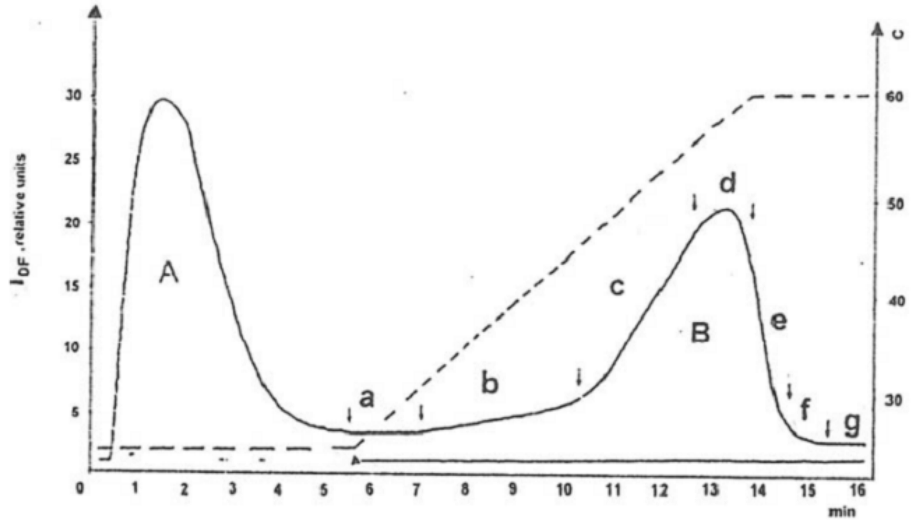

FIGURE 3
Schematic presentation of the empirical procedure of typical changes of delayed chlorophyll fluorescence intensities (I_{DF}) on the intact above-ear leaf of the observed maize inbred lines with significant breeding properties (solid line) and changes of temperatures (dashed line): the curve A indicates induction processes of delayed chlorophyll fluorescence, while the curve B encompasses photosynthetic fluorescence thermal processes of delayed chlorophyll fluorescence. Typical temporal segments (a, b, c, d, e, f, and g) on the thermal curve B correspond to the dynamics of IDF changes at the time of delayed chlorophyll fluorescence formation. Conformational and functional changes in the thylakoid membrane of the observed maize inbred lines with erect top leaves occur at the interception points of typical temporal segments.

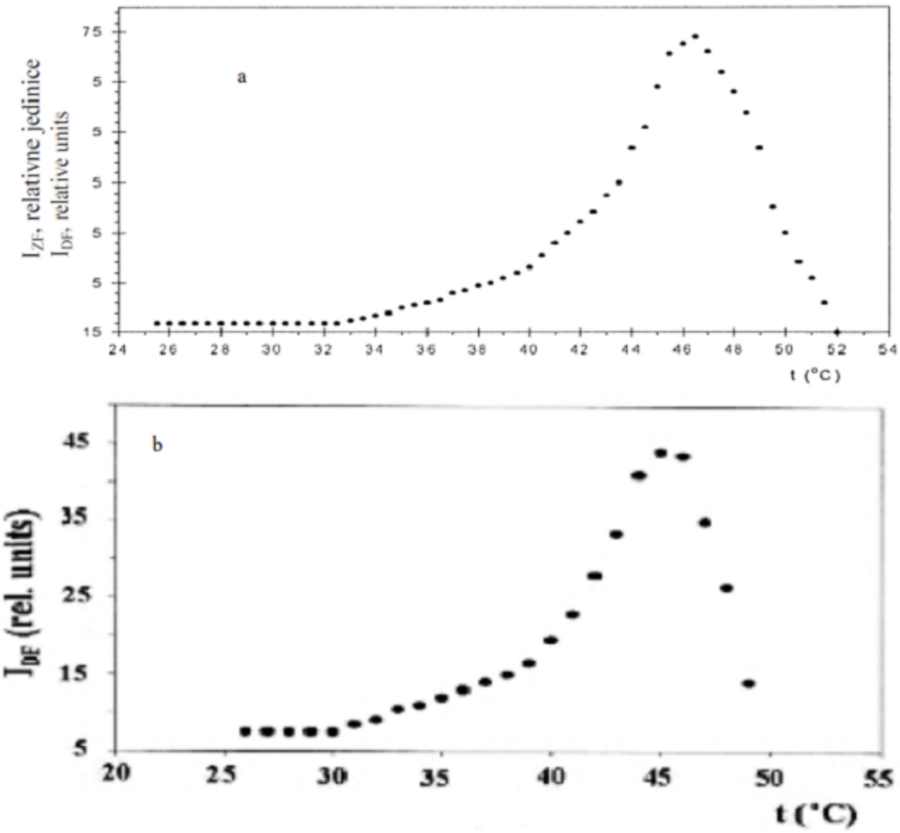

FIGURE 4
Changes in the intensity of the delayed chlorophyll fluorescence (I_{DF}) of the thermal processes depending on temperature in the thylakoid membrane and chloroplasts of the intact above-ear leaf of the new air dried maize inbred lines ZPPL 146 (a) and ZPPL 159 (b).

5.1. The Arrhenius plot for the determination of critical temperatures and conformational changes in chloroplasts and thylakoid membranes of the new maize inbred lines bred for the production of healthy food

The Arrhenius plot is based on the linearisation of the delayed chlorophyll fluorescence exact temperature dependence of the observed maize inbreds. Critical temperatures (phase transition temperatures) at which conformational changes occur in chloroplasts and the thylakoid membrane are determined by the application of the Arrhenius plot. Figures 5a and 5b present the results of the Arrhenius plot application to the observed maize inbred lines.

Such dependence (Figures 5a and 5b) is known as the Arrhenius plot for the determination of critical temperatures that cause conformational and functional changes, of chemical nature, in chloroplasts and the thylakoid membrane. Using the Arrhenius plot and linearisation of the exact temperature dependence of DF chlorophyll, all critical temperatures (phase transition temperatures) at which even the smallest conformational change occurred in the thylakoid membrane of the new air dried maize inbred lines were determined.

The values of critical temperatures in °C, their frequency and inter-distance characterise the observed new inbred lines with erect top leaves in relation to their tolerance, resistance and adaptability to increased and high temperatures as well as to drought (Radenović et al, 2013). The Arrhenius plot is based on the existence of straight lines. Each Arrhenius's straight line represents activation energy (Ea). The intercept of two straight lines is denoted by the critical temperature.

The results of Ea values in the ascending and descending parts of the thermal curve are explained by the fact that with the temperature increase, smaller or larger conformational and functional changes occur in pigment molecules (chlorophyll, carotenoids) in the thylakoid membrane and chloroplasts. Due to the changes, these molecules became more reactive thus acquiring additional energy which was used in the recombination process of DF chlorophyll formation (Radenović et al, 2013).

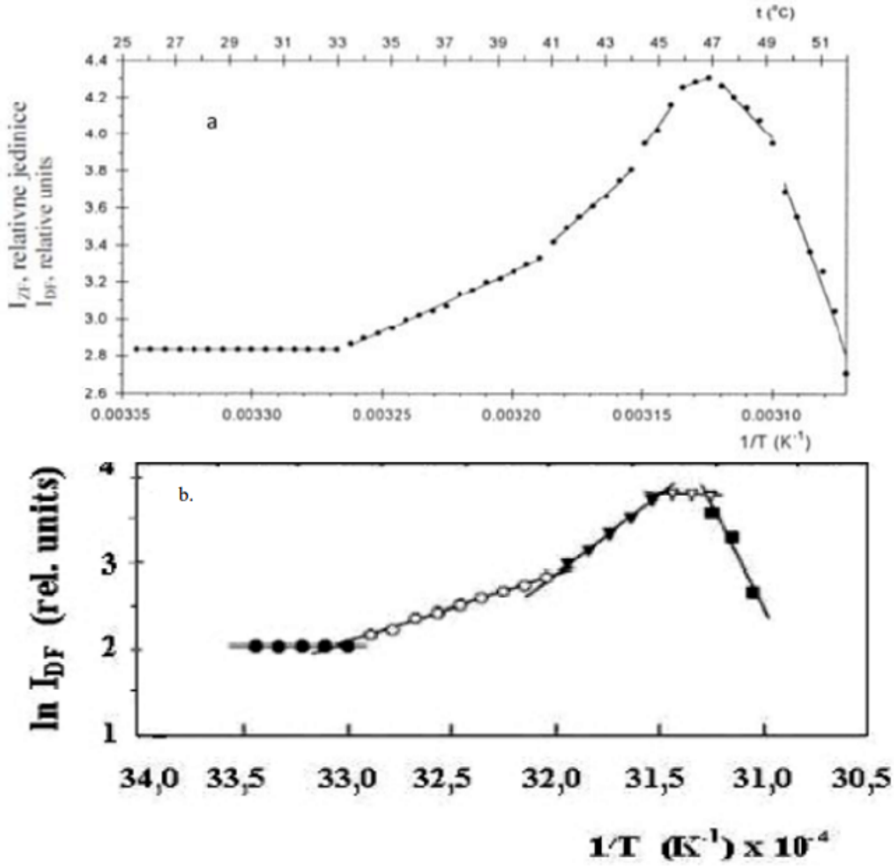

FIGURE 5

The change of the logarithm of the delayed chlorophyll fluorescence intensity (ln I_{DF}) of the thermal processes as a function of the reciprocal temperature value in the thylakoid membrane and chloroplasts of the intact above-ear leaf of the observed air dried maize inbred lines ZPPL 146 (a) and ZPPL 159 (b).

5.2. Activation energy and critical temperatures in the thylakoid membrane of the observed maize inbred lines bred for the production of healthy food

Detailed studies of the thermal processes of delayed chlorophyll fluorescence, and especially the analysis of the experimental thermal curve, encompassed not only the temperature dependence and the Arrhenius plot, but also the estimation of the values of activation energies (Ea) for the critical temperatures (phase transition temperatures) in chloroplasts and the thylakoid membranes of the observed maize inbreds bred for the production of healthy food, i.e. ZPPL 146 (a) and ZPPL 159 (b). The obtained results are shown in Table 3.

TABLE 3
Changes in activation energies (Ea) and critical temperatures (t °C) during the thermal processes in chloroplasts and the thylakoid membrane of the intact above-ear leaf of air dried maize inbred lines

ZPPL 146		ZPPL 159	
Ea, kJ/mol	t °C	Ea, kJ/mol	t °C
/	33.5	/	25
54.5	40.0	32.0	30
105.0	44.0	100.3	38
174.0	46.0	176.7	42
41.0	47.0	259.9	47
128.5	49.0	/	50
326.0	/		

6. Brief survey of the breeding and seed production traits of the new maize inbred lines with efficient photosynthesis

The observed new maize inbred lines ZPPL 146 and ZPPL 159 are very promising in the process of maize breeding. For these reasons, some relevant observations of their selected traits, performances, and parameters are presented in Table 4a b.

TABLE 4A
Relevant breeding and seed production traits of the new maize inbred lines with photosynthetic efficiency

Ordinal numeral	Name and definition of a trait	Brief description of the breeding and seed production traits of the maize inbred lines	
		ZPPL 146	ZPPL 159
1.	Heterotic origin	BSSS, USA, Zemun Polje	Landrace from Argentina (S13), crossed to the inbred PE 25-10-1, Zemun Polje
2.	FAO maturity group	650-700	550-600
3.	Grain yield ha^{-1} in kg at 14% moisture a) dry land farming b) irrigation	3500 5000	2000 3000
4.	Number of plants ha^{-1} at harvest a) dry land farming b) irrigation	50000 60000	50000 60000
5.	Stalk properties	Stalk is moderately high with a prolific trait. Tassel has an elongated central branch with fewer side branches	Stalk is short. Tassel has closed side branches that shed for a long time
6.	Stalk resistance to lodging	Inbred is resistant to lodging	Inbred is resistant to lodging
7.	Erect position of above ear leaves	first leaf < 20.8° second leaf < 17.9° third leaf < 15.3°	first leaf < 21.3° second leaf < 18.1° third leaf < 15.4°

TABLE 4B
Relevant breeding and seed production traits of the new maize inbred lines with photosynthetic efficiency

Ordinal numeral	Name and definition of a trait	Brief description of the breeding and seed production traits of the maize inbred lines	
		ZPPL 146	ZPPL 159
8.	Stay green	Leaf remained green until harvest	Leaf remained moderately green until harvest
9.	Tolerance of the inbred to stress factors (drought and high temperatures, etc.)	Inbred is tolerant to drought and high temperatures	Inbred is tolerant to drought and high temperatures
10.	Kernel traits and cob colour	Semi-dent type, orange kernels, white cob	Semi-flint, orange kernels, red cob
11.	% grain moisture at harvest	20-25	20-25
12.	Dry down rate in the stage of grain maturing	Dry down rate is not fast, but hybrids are suited for silage	Dry down rate is not fast, but hybrids are suited for silage
13.	Harvest of inbreds	Harvest is easy	Harvest is easy
14.	Emergence of inbreds	Inbred emerges well	Inbred emerges well
15.	Early growth of inbreds	Early growth is moderate	Early growth is moderate
16.	Suitability of the hybrid grain for nutrition of ruminants and nonruminants	Grain of the hybrid developed from this inbred is suitable for nutrition of ruminants, nonruminants, human nutrition and for industrial processing	Grain of the hybrid developed from this inbred is suitable for nutrition of ruminants, nonruminants, human nutrition and for industrial processing
17.	Carotene content in the inbred grain	33.2 (mg/kg)	a) 31.8 (mg/kg)
18.	Suitability of the inbred for the development of silage hybrids	Inbred is very suitable for developing silage hybrids	Inbred is very suitable for developing silage hybrids
19.	Digestibility of hybrids	Hybrids developed from this inbred have good digestibility of the whole plant and of the grain	Hybrids developed from this inbred have good digestibility of the whole plant and of the grain

7. Functional dependence of the yield of the observed grain and maize silage hybrids

The high-yielding and high-quality maize hybrids ZP 735, ZP 737, ZP 677, and ZP 684 are mainly intended for grain and silage production of grain, ear and the whole plant, under the agroecological conditions of

Southeastern Europe. According to our studies and good agricultural practice, the hybrid ZP 633 is very suitable for human diet (Radenović, 1991). However, the hybrids ZP 735, ZP 737, ZP 677, and ZP 684 are significantly better for nutrition of domestic animals through high-quality grain silage and even more often the whole plant silage. The important agronomic and morphological traits of these hybrids are presented in Tables 5, 6, 7, 8 and 9.

TABLE 5
Agronomic traits of the observed maize hybrids

Agronomic traits	Hybrid		
	ZP 633	ZP 735	ZP 737
Hybrid type	SC	SC	SC
FAO maturity group	550-650	750-850	750-850
Plant height (cm)	250	280	290
Ear height (cm)	120	130	135
1000-kernel weight (g)	380	370	370
Kernel type	semi-dent	dent	dent
Sowing density of silage hybrid ($x10^3$ plants ha^{-1})	60-70	70-75	70-75
Leaf position on the plant	semi-erect to erect	semi-erect to erect	semi-erect to erect
Tolerance to drought	good	good	good
Tolerance to diseases	good	good	good
Leaf appearance at harvest	stay green	stay green	stay green
Hybrid growing regions (altitude, m)	300-400	250-400	250-400
Hybrid biomass yield (t ha^{-1})	60-65	75-80	75-80
Hybrid grain yield (t ha^{-1})	7.819*	8.108**	12.732**

* Hybrid yield achieved in 30 locations in Serbia in the 2008-2011 period
** Hybrid yield achieved in 6 locations in Greece in the 2006-2009 period

TABLE 6
Ear morphological traits of the observed maize hybrids with a grain structure

Traits	Hybrid		
	ZP 633	ZP 735	ZP 737
Grain moisture (%)	18	19	20
Ear length (cm)	22	25	25
Ear weight (g)	252.3	286.4	226.7
Rows per ear	16	18	18
Kernel row number	700	800	850
Kernel weight on ear (g)	228.4	248.4	200.4
% kernel pericarp on ear	5.3	6.5	4.6
% kernel embryo on ear	11.3	12.1	10.7
% kernel endosperm on ear	83.4	81.4	84.7

TABLE 7
Whole plant silage yield depending on the maize hybrid sowing density

Hybrid	Sowing density (000/ha)				Grain moisture in silage period (%)
	55	60	65	70	
	Whole plant silage yield (t/ha)	Whole plant silage yield (t/ha)	Whole plant silage yield (t/ha)	Whole plant silage yield (t/ha)	
ZP 677	66.60	71.80	76.20	78.60	28.5
ZP 684	56.10	61.80	66.09	72.40	29.3
ZP 735	62.42	67.62	72.82	77.28	31.4
ZP 737	64.50	69.50	74.70	78.90	32.1

The results of the silage yields of the whole plant as a function of the sowing density of the observed maize hybrids (Table 7) should be taken conditionally. They indicate a possible trend of silage yield increase of the whole plant depending on the crop densities. It should be noted that the silage yield of the whole plant depended, to a large extent, on the type of soil, supply of nutrients, crop protection products, water and other measures within contemporary crop growing practices (Jovanović, 1996, 1998), (Jovanović et al, 2000), (Antov et al, 2004), (Dinić & Đorđević, 2005), (Radenović, 2013), (Đorđević & Dinić, 2003).

TABLE 8
Yield of fresh matter, dry matter and digestible dry matter of the observed maize hybrids sown at the common sowing density under arid conditions

Hybrid	Fresh matter yield (t•ha^{-1})	Dry matter yield			
		Whole plant yield (t•ha^{-1})	Plant without ear yield (t•ha^{-1})	Ear yield (t•ha^{-1})	Digestible dry matter yield (t•ha^{-1})
ZP 677	76,2	29,7	11,8	17,9	19,9
ZP 684	66,0	29,0	11,1	17,9	19,1
ZP 735	64,2	23,7	10,8	12,9	12,2
ZP 737	66,1	25,1	13,8	11,3	15,9

TABLE 9
Content of dry matter lignocellulosic fibres and dry matter digestibility of the observed maize hybrids*

Hybrid	Whole plant dry matter content (%)	Content of lignocellulosic fibres (%)					Dry matter digestibility (%)
		NDF*	ADF*	ADL*	Hemicellulose*	Cellulose*	
ZP 677	38.96	41.09	19.51	1.68	21.58	17.83	68.29
ZP 684	44.02	39.46	18.40	1.50	21.06	16.90	65.85
ZP 735	35.58	60.10	32.07	3.06	28.03	29.01	51.30
ZP 737	38.00	42.90	22.01	3.54	18.58	18.58	63.51

* Source of data, abbreviations and explanations: The analyses of contents of NDF, ADF and ADL were performed according to the method of Van Soest P. J. (Van Soest, 1963); dry matter content was established according to the Rulebook on Sampling Methods and Methods of Physical, Chemical and Microbiological Analyses of Animal Feed (Službeni list SFRJ, 15/87) and dry matter digestibility was obtained by the INRA method, whereas the content of hemicellulose and cellulose was computed (Hemicellulose = NDF – ADF, and Cellulose = ADF - ADL). NDF – neutral detergent fibres; ADF – acid detergent fibres; ADL - acid detergent lignin (72% sulphuric acid)

According to the data presented in Tables 5-9, it is noticeable that the observed hybrids belong to long-season hybrids with a modern architecture, leaves that remain green and are rich in lignocellulosic fibres. Moreover, these hybrids have more than 50% of grain dry matter in dry matter of the whole plant, which is very important for silage quality. In addition, the embryo content in grain amounts to above 10%, which is especially important for the quality of nutritive values of hybrids in nutrition of people (especially children and the elderly) (Radenović, 1991) but also in nutrition of livestock (particularly cows and sheep, chicks and laying hens) (Jovanović, 1996, 1998), (Jovanović et al, 2000). The results on silage yields of grain, ear and the whole plant and grain moisture in the silage period are important for dry matter yield, digestible matter yield and the content of dry matter and lignocellulosic fibres (Tables 7, 8, 9).

After 1950, from the initial procedures of plant ensiling, the technology of fodder tinning by ensiling flourished only in the period from 1955 to 1965. Modern, intensive and economical production in cattle breeding can no longer be imagined without ensiled fodder. Moreover, the importance of such feed has been increasing in sheep and goat breeding, and to a lesser extent in pig breeding (Pejić, 1994), (Jovanović, 1996, 1998), (Jovanović et al, 2000), (Dinić & Đorđević, 2005), (Radenović et al, 2013), (Đorđević & Dinić, 2003). The authors of this study became interested in this topic in the beginning of 2002. Since then, a large number of quality maize hybrids have been developed for the production of high-quality silage in order to regulate the metabolic processes of domestic animals and thus improve their gain in weight and the quality of meat and milk (Radenović et al, 2002), (Radenović, 2002).

It is well known that maize is one of the most suitable field crops for the production of silage for ruminants. This is important for several reasons. First, very high yields of green mass are recorded in maize. It is also important that more than 50% of grain dry matter participate in the dry matter of the whole plant, which is an excellent prerequisite for the production of high quality silage. Ruminants need lignocellulosic fibres for the activity of the rumen microflora and these fibres are mainly found in maize stalks, leaves, husk, and cobs (lignocellulosic parts of the plant) (Table 9). On the other hand, starch, proteins and oils are predominantly found in the maize grain (Table 1). With the addition of some other micronutrients, maize silage prepared in this way presents a modern way of ruminants feeding. It is particularly important that such a way of animal feeding is very economical, because the process of silage preparation is completely mechanised, while the way of storing and its taking during the use is also simple.

According to the previous studies (Jovanović, 1996, 1998), (Jovanović et al, 2000), the observed hybrids (ZP 677, ZP 684, ZP 735 and ZP 737) have significantly better digestibility than some short-season hybrids, and therefore they belong to the group of the highest quality silage hybrids. It is this fact that indicates the higher nutritional value of these hybrids, which directly affects the productive performances of ruminants. Based on our studies (Radenović, 2002, 2013), the hybrids ZP 677, ZP 684, ZP 735, and ZP 737 had satisfactory yields of green mass silage under the conditions of Leskovac with the application of standard cropping practices and without irrigation. According to the obtained results, the highest yield of green mass was recorded in the maize hybrid ZP 677. However, to draw the final conclusion on which hybrid is more suitable for silage, grain yield as well as silage digestibility should be taken into account. Since these are long-season hybrids (FAO maturity group 750-850), they have a very developed leaf mass and the intensive photosynthetic activity. This, among other things, classifies them into a group of hybrids most suitable for the silage production under the climate conditions of Serbia and Southeastern Europe (Greece, Bulgaria, and Turkey).

As already mentioned, a huge success has been achieved in maize breeding and the production of high quality foundation seed and hybrid seed in the last 42 years. Furthermore, a great success was achieved in modern technologies for the commercial maize production. Since 1978, the number of maize plants per area unit has been significantly increased by the application of the new maize breeding programme. This programme, known as a "plant density" breeding programme, directly affected the increase of yield of both foundation and hybrid seeds as well as the yield of commercial maize (Radenović et al, 2004, 1978). A few years later, the breeding programme for the development of maize inbred lines with erect top leaves - inbreds with more efficient photosynthesis - was implemented (Radenović et al 2004, 2008, 2009, 2007). Some of these inbred lines with the erect top leaves were thought to be the closest to the assumed photosynthetic maize model. At the same time, the breeding programme for maize inbreds rich in pigments and other chemical properties and excellent nutritional values was initiated (Kojić & Ivanović, 1986), (Petrović et al, 1992), (Pejić, 1994), (Jovanović, 1996, 1998), (Dumanović & Pajić, 1998), (Jovanović et al, 2000), (Dinić & Đorđević, 2005), (Pajić et al, 1995), (Liu, 2007), (Strati et al, 2012, (Tyutyaev et al, 2015), (Đorđević & Dinić, 2003), (Kurlich & Juvik, 1999), (Granado et al, 2003), (Luo & Wang, 2012).

This study was an attempt to answer the following question by using different interdependent tests and analyses: „Is there a reliable and dominant trait (one or more) of the observed maize inbred lines rich in the pigment complex that would be the basis for the development of new extra-quality maize hybrids that would be suitable for human diet and nutrition of domestic animals?“ The analysis of the presented overall results, obtained in the series of experiments, can easily give the positive answer to this question. Consequently, the new maize inbred lines (ZPPL 146 and ZPPL 159) and the hybrids developed from them (ZP 633, ZP 735 and ZP 737) are the best confirmation of the stated. The bred inbred lines and hybrids developed from them are rich in pigments, have significant nutritive values, especially of carotenoids that give the colour (Abdel-Aal et al, 2006) to cereal kernels used in the nutrition of poultry. Carotenoids have many biological functions in both people and animals (Strati et al, 2012), (Kurlich & Juvik, 1999), (Granado et al, 2003), (Bacchetti et al, 2013). This aspect of the observed maize inbred lines and hybrids will get priority within the healthy extra-quality maize-based diet for people and nutrition for animals.

Conclusions

Based on the presented numerous and diverse results of the studies of the new inbred lines (ZPPL 146 and ZPPL 159) and the maize hybrids developed from these inbreds (ZP 633, ZP 735 and ZP 737) that have high nutritive values, are rich in pigments and, in accordance with their chemical composition, have efficient photosynthesis and other relevant parameters characteristic for the best standard maize hybrids (ZP 677 and ZP 684) for silage of grain, ear and the whole plant, the following can be concluded:

- Selected new, unique maize inbred lines (ZPPL 146 and ZPPL 159), rich in carotenoids, yellow pigments, also have significant amounts of other relevant bioactive compounds and good physical traits.

- Observed inbred lines have erect top leaves and are classified into a group of maize inbreds with significant properties of the photosynthetic model - they are high yielding and tolerant to high temperatures and drought.

- Spectral bands pointing to the conformational characteristics of molecules of carotenoids but also other compounds (phosphates, glutens, and amides III) were established by the resonance Raman spectroscopy method applied to the leaf of the maize inbred lines.

- Relevant traits, properties and parameters of the observed new maize inbred lines that can be successfully used in the breeding process are presented.

- These maize inbred lines were used to develop high yielding and extra quality maize hybrids (ZP 633, ZP 735 and ZP 737) that are recognisable for their quality. The hybrid ZP 633 is particularly recognisable in human nutrition (children and the elderly). Furthermore, in relation to veterinary and agronomic estimations, the hybrids ZP 735 and ZP 737 are the most suitable for feeding domestic animals with a programmed use of maize silage, ground maize and coarse meal.

- Relevant agronomic, morphological and nutritive properties of the maize hybrids ZP 677, ZP 684, ZP 735 and ZP 737 are presented. Moreover, the results regarding the grain structure and grain and silage yields obtained in the regions of Serbia and Southeastern Europe (Greece, Bulgaria, and Turkey) are also displayed.
- All studied maize hybrids (ZP 633, ZP 735, ZP 737, ZP 677, and ZP 684) are intended for large-scale production of flour, semolina, ground maize, silage of grain, ear and the whole plant, which provides healthy and extra quality food and feed.

Special acknowledgement

The authors express deep gratitude and appreciation to Dr. Obrad Stojnić (1960 -1997) for his remarkable contribution to the development of Serbian maize breeding and selection. Had his life not been prematurely and forcibly interrupted, his scientific results in maize breeding would have been even more successful. Even so, he will be always remembered for his deeds - the Serbian maize breeding has a leading place in Europe.

Acknowledgments

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