Zsanett Simon[1], Csaba Lovász[1]

Received: 2015. November – Accepted: 2016. February

Genetic purity testing of maize hybrids using a MALDI-TOF mass spectrometric method

Modern "test of origin" of seeds

1. Summary

Mass spectrometry is one of the most versatile techniques of modern instrumental methodologies. Starting from the most sensitive analytical instruments, through application in space research, to the intelligent scalpel (iknife), there is almost no scientific area where the advantages of the method cannot be exploited. In this paper the application of mass spectrometry in a new area is presented.

From an economic point of view, maize is one of our most important crops 5 to 8 million tons of which is produced annually in Hungary. For this industrial scale production, high quality seeds are essential, and this requires a systematic, strict control of the seeds. In this spirit, identification and elimination of seeds of dubious origin or of poor quality are important tasks of growers and seed producers. Genetic purity of seeds the varietal identity is one of the most important qualification parameters.

Our research goal was to develop a state-of-the-art, effective analytical method to test the genetic purity and varietal identity of maize seeds, and to prove the applicability of our method by measurements.

The procedure is based on the application of MALDI-TOF-MS (Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry), with the help of which the reserve proteins of maize grains can be investigated. Following the extraction of maize grains, proteins characteristic of the given parents can be selected from among the different mass number peaks (proteins) appearing in the mass spectra of the extracts, which can then be followed as genetic markers during the testing of hybrid seeds. To check the MALDI-TOF-MS method, our analytical results were compared to results obtained by the recommended reference method (isoelectric focusing – IEF). Results of the tests performed so far are promising, there is a very good agreement with the results obtained by the reference method. Exact results were also provided by our method when testing fungus-infected maize seed batches, while such lots can only be analyzed by the reference method in a limited way. Running on the gel using isoelectric focusing is affected by fungal infection, which makes the evaluation of the results harder, in some cases even impossible.

Our MALDI-TOF mass spectrometric method developed for the genetic purity testing of maize seeds proved to be satisfactory not only because of its speed, sensitivity and comparable results, but it also made the analysis of fungus-infected hybrids possible.

[1] WESSLING Hungary Kft.

2. Introduction

The population of Earth is growing rapidly, therefore, it is important to ensure foodstuffs of adequate quality and quantity. Plant breeding contributes to the realization of this goal, because it can increase crop yields and improve the genetic characteristics of the species. The primary task of plant breeding is to produce new, better quality genotypes with higher yields.

For crop production to be effective, high quality seeds are essential. Hungary is among the most important seed producers in Europe, it has played an important role in seed production since the beginning of the 20th century. Seed certification conditions and the parameters required are prescribed by regulations. The analysis of these qualification parameters is important, because serious losses to producers are caused by seeds of dubious origin or poor quality. Because of yield planning and the resistance of the given varieties to plant diseases it is not indifferent what the genetic origin of the seeds that the producers supply is.

Corn as a crop plays a very important role in the food supply of the Earth's population, in feeding animals, and in other, newer industrial application areas, such as the production of bioethanol. The new, mass spectrometric method for the genetic purity testing of hybrid maize seeds is described below. The method used is MALDI-TOF (matrix-assisted laser desorption ionization) mass spectrometry, with the help of which the protein profiles of maize seeds were analyzed. Using the mass spectrometric method developed by us, it was possible to recognize accidental self-pollination or foreign fertilization, and the genetic purity of fungus-infected maize seeds could also be determined.

3. Literature overview

3.1. Composition and constituents of the corn kernel

The main constituents of the corn kernel are the tip cap, the bran, the germ and the so-called *endosperm* **[1]**. The germ is basically an inactive plant embryo, which starts to develop when conditions (temperature, humidity) are adequate. Roughly 11% of the weight of the kernel is made up by the germ, and it contains mainly oil, proteins and sugars. The additional 83% of the weight of the kernel is made up by the *endosperm*, 88% of the weight of which consists of starch grains and proteins necessary for the development of the germ.

5.3% of the weight of the kernel is made up by the bran, consisting of two parts. The outer part is the so-called *pericarp*, with a thickness of 25 - 140 µm, composed of dead cells, and whose material is mainly lignocellulose **[2]**, and the inner part is the so-called *aleurone*, directly connected to the *pericarp*, and whose thickness is monolayer. In addition to lignocellulose, the bran contains some protein and oil.

Reserved or stored proteins are in the *endosperms* of grain seeds, and they make up roughly one half of the total protein found in the seed. Seed proteins represent a valuable biological substance not only for the food and feed industry, but also for basic research, because their genes only operate during a well-defined stage of the plant lifecycle **[3]**. The reserve protein of maize, named zein by Osborne **[4]**, is rich in glutamic acid, proline, leucine and albumin, but it contains only small amounts of the essential amino acids lysine and tryptophan. Zein is synthesized in the *endosperm* by the ribosomes of the rough endoplasmic reticulum (RER), starting from approximately the 15th day after fertilization. Synthesis is started by a signal peptide of 1–2 kD molecular weight. Synthesized molecules are transported through the membrane by the peptide, which is then excised, and a protein body bound to the RER is formed by the molecules inside the membrane.

3.2. Seed certification

In Hungary, in accordance with European Union legislation, commercial distribution of plant varieties is regulated by law LII of 2003 about state recognition of plant varieties and about the production and marketing of reproductive materials, and its implementation is regulated by decree 48/2004. (IV. 21.) of the Ministry of Agriculture and Rural development. In Hungary, only certified seeds of plant varieties that appear on the National List of Varieties and the EU list of varieties can be produced for commercial purposes, distributed and used for production purposes. Seeds are classified according to requirements set forth in a separate regulation, and a certificate about the results has to be issued.

Therefore, seed certification is a prerequisite of seed distribution, and its subprocesses are as follows:

- Certification of origin;
- Field inspection;
- Sampling;
- Laboratory testing;
- Metal sealing;
- Growing of variety identification specimen;
- Issuing quality certificate.

Through the processes listed above, the quality of the seed produced can be traced, and it can be verified whether the most important international standards have been observed during seed production. One of the main goals of laboratory testing is to determine the genetic purity, genetic homogeneity and varietal identity of the seed batch.

In plant breeding, to determine self- and foreign fertilization, to verify varietal purity, for variety identifica-

tion and variety protection, isoenzyme analyses are used. However, today isoenzyme analyses are mainly used in variety recognition and patenting processes. Currently, the internationally accepted method for the varietal purity testing of maize hybrids is gel electrophoresis with isoelectric focusing. When using this method, reserve proteins (zein) found in corn are extracted by kernel, and then separated on an ultrathin polyacrylamide gel (Ultra Thin Layer Isoelectric Focusing - UTL-IEF method). The use of ultrathin gels is more economical and it requires higher voltages than usual, the analysis takes a shorter time and the dyeing process is faster than in the case of conventional gels **[5]**.

During isoenzyme-based variety identification, the protein pattern of the variety tested is compared to the protein pattern of the authentic variety and, by contrast, when determining the genetic purity of a hybrid, the protein pattern of the parents is compared to the protein pattern of the hybrid. The protein band pattern of the gel chromatograms is characteristic of the given variety or the inbred line. Those bands – or marker proteins – always have to be marked in the parents which are inherited by the hybrid. Usually, the genetic purity of the hybrid produced is evaluated with the help of one or more marker bands or marker proteins that can be found in the father, but missing in the mother. These bands can be used during the variety identification of the hybrids as well. The genetic line of the hybrid is indicated by the presence or absence of marker bands, and the ratio of self-pollinated and foreign pollinated kernels can be determined with the help of these.

3.3. Polymorphism and heterogeneity of the zein pattern

Polymorphism in the zein pattern was first detected by Turner et al. **[17]** in 1965. This polymorphism may be due to the different compositions of the prolamin fractions obtained from the seeds of homozygous specimens, and on the other hand, it can originate from the fact that the number of separable components depends on the method applied and the hybrid. Prolamin components can differ by hybrid depending on the parent genotype, due to their different electrophoretic properties **[3]**. Since the zein pattern is not influenced by environmental factors, genetic identity or diversity of maize lines and hybrids inbred can be demonstrated by zein polymorphism. However, complete genetic dissimilarity, based on which agronomic performance (e.g., heterosis) could be accurately predicted, cannot be revealed using this method either.

The following methods were considered to be suitable for zein polymorphism analysis by Wilson **[6]**:

- SDS-PAGE (sodium dodecyl sulfate - polyacrylamide gel electrophoresis) – separation according to molecular weight in the presence of urea;
- UTL-IEF (ultra thin layer gel electrophoresis with isoelectric focusing) – separation based on the isoelectric characteristics of the proteins – according to the locations of protein bands;
- RP-HPLC (reverse phase high performance liquid chromatography);
- CE (capillary electrophoresis);
- Analysis according to amino acid composition;
- Analysis of regulator genes affecting zein (e.g., O2 gene);
- Mapping of zein structural genes;
- Cloning of zein cDNS.

The method of Bietz **[7]**, RP-HPLC separated the components of proteins – including zein – based on their surface hydrophobic groups. By this technique, heterogeneous zein can be separated into a higher number of fractions than using any other chromatographic or electrophoretic method. Today, the latter two methods are combined by fractionating zein using RP-HPLC first, and then the fractions are separated by UTL-IEF based on their isoelectric properties.

SDS-PAGE and HPLC methods are routinely used in breeding programs to separate high and low molecular weight proteins **[8]**. Separation by the SDS-PAGE method is based on electrophoretic mobility, however, this method is not exact enough and identification is hard in certain cases **[9]**. To some extent, RP-HPLC can present a solution to these problems, but the identification of certain subunits is not successful using this method either **[10]**.

The road to the mass spectrometric analysis of proteins was opened by the discovery and development of MALDI-TOF mass spectrometry **[11]**. Since zein polymorphism can be traced back to the analysis of zein protein composition or profile analysis, the possibility arises to inspect the composition of the reserve protein using MALDI-TOF mass spectrometry.

Gluten proteins of wheat can be identified quickly and extremely successfully by the MALDI-TOF method **[8]**. Compared to earlier methods, the MALDI-TOF method is much more accurate and sensitive, and last, but not least, it is a time-saving method, since the measurement requires only a few minutes per sample **[8]**. Glutenin and gliadin proteins of wheat were analyzed using a MALDI-TOF mass spectrometric method by Ghirardo **[12]**. When testing corn kernels, the extract preparation method used by Ghirardo was applied, with some modifications.

3.4. MALDI-TOF mass spectrometry

Mass spectroscopy is a powerful method of structure research, during which the molecules to be analyzed enter the gas phase, they are ionized and then accelerated using a high-voltage electric field. The ac-

celerated ions are separated by a magnetic, electrostatic or radio frequency field according to their mass and charge, and the charge of the ions is determined. The size, or molecular weight of the molecule analyzed is one of the most important structural information that can be provided by mass spectrometry, therefore, ionization is the key issue during the mass spectrometric analysis of macromolecules. For the analysis of biologically active molecules – proteins, enzymes – one of the solutions can be the application of the MALDI ionization technique.

The acronym MALDI-TOF refers to a specific mass spectrometer, in which the MALDI (*Matrix-Assisted Laser Desorption Ionization*) is the ion source and the TOF (*Time of Flight*) is the mass number analyzer, which serves for the separation of the ions produced during ionization according to their mass/charge ratio. The schematic of the instrument is shown in **Figure 1**.

The procedure patented by Tanaka **[11]** in 1985 (SLD – soft laser desorption, with a matrix of glycerol and metal powder) was rarely used for the analysis of proteins, instead, the much more sensitive solution of the German research duo of M. Karas and F. Hillenkamp **[16]** has been used to this day. In this procedure, the matrix is a small organic molecule, which can absorb the energy of the laser used for ionization, and ionize the target compounds. Most often, aromatic compounds containing hydroxyl or carboxyl groups are used as the matrix, the structural formulas and names of the most commonly used ones being shown in **Figure 2**.

One of the matrices most often used in protein analysis is *alpha*-cyano-4-hydroxycinnamic acid (α-HCCA, HCCA), with a UV absorption maximum at 332 nm, therefore, it can utilize the energy of the UV laser used in the MALDI-TOF mass spectrometer very efficiently (according to the manufacturer's specification, the radiation maximum of the UV laser is at 337.1 nm). The energy of the emitted photon pulses can be varied freely, it can even be changed pulse by pulse by the instrument control software, according to the ionization efficiency.

Most often, the matrix is applied to the sample plate and the sample as a solution. Together with the proteins found in the samples, it forms a solid composite crystal, in which macromolecules are embedded. During ionization, the energy of the laser serves to transfer proteins into the gas phase on the one hand, and on the other hand, it helps their ionization. This way, from the proteins analyzed, gas-phase, protonated, mostly singly charged positive ions, mainly molecular ions are obtained ($[M+H]^+$). Molecular adduct formation is matrix dependent, but multiply protonated ions ($[M+2H]^{2+}$ or $[M+3H]^{3+}$) also appear in the mass spectrum, with smaller intensities.

Ions that form in the specially designed ion source are accelerated using a high-voltage field, they are focused, and forwarded into the detector through a flight tube. The application of the time-of-flight mass spectrometer allows for a solution for the separation of the accelerated ions according to their mass **[13]**. The MALDI-TOF instrument provides information about the macromolecules, the protein profile of the sample analyzed simply and efficiently. The mass spectrum recorded during the analysis can be used in several ways to draw further conclusions regarding the macromolecules.

The spectrum obtained by the MALDI-TOF technique during the analysis of the reserve proteins of maize is not the mass spectrum of a specific compound, but the total of different molecular weight ions that form in the ion source of the instrument, i.e., of protonated molecular ions of proteins found in the sample analyzed. Therefore, each and every peak in the spectrum corresponds to a single protein ***(Figure 3)***. When analyzing the genetic purity of corn hybrids, the evaluation is based on the reserve proteins, or on marker proteins selected from among them. During our research, we could not find any publications, in which the genetic purity of seeds had been tested by a MALDI-TOF mass spectrometric method, therefore, our goal was to develop a new mass spectrometric method that can replace the reference method (UTL-IEF), one that is more efficient and can provide adequate results in critical cases (fungus-infected kernels) as well.

3.5. Materials and methods

3.5.1. Samples and sample preparation

The maize seed samples tested came from hybrid maize seed production performed in a large area. Seeds were obtained from the Seed testing laboratory of the National Food Chain Safety Office (NÉBIH), in broken or whole kernel forms. Among the samples, there were healthy, fungus-infected and treated seeds as well.

As an international standard reference method, the method described in Chapter8 of the *International Seed Testing Association* (ISTA) regulation was used **[5]**. This is gel electrophoresis performed on ultrathin polyacrylamide gel using isoelectric focusing (UTL-IEF, or IEF in short). This is the method that was used by the Seed testing laboratory of NÉBIH as well, so our measurement results obtained by the MALDI-TOF method were compared to the analytical results provided by them, as reference values.

Since, for the MALDI-TOF analyses, proteins had to be extracted from the corn kernels in the first step, extraction experiments were performed before the genetic purity testing of maize seeds, to select marker proteins on the one hand, and to be able to choose the extraction agent most suitable for the analyses on the other hand. Analyses were performed on composite samples consisting of 25-50 maize kernels (ul-

tracentrifugal grinding mill, particle size below 1 mm) to avoid errors due to potential genetic inhomogeneity.

3.5.2. Extraction of marker proteins, sample application, recording of mass spectra

For the extraction, several extraction agents were used: aqueous sodium chloride buffers, and more apolar, alcoholic extraction buffers (containing 1-propanol, 2-propanol or 2-chloroethanol). Our experience showed that the solubility of the proteins analyzed was helped by the presence of reducing agents (for example, dl-dithiothreitol – DTT) that breaks down disulfide bridges, therefore, DTT was also added to the extraction agent mixtures (***Table 1***).

Since our analyses showed that the quality and quantity of extracted proteins, and so the number and intensity of the protein peaks in the mass spectrum, are significantly influenced by the choice of extraction agent, several extraction agents were tried during our preliminary experiments. While sodium chloride buffers proved to be the best for certain hybrid maizes, for other hybrids more useful results were obtained in the case of one or the other of the alcoholic buffers. It was decided after the evaluation of the protein spectra, which extraction mixture was going to be used during the genetic purity testing.

In ***Figure 4***, mass spectra of the preliminary extraction experiments of a hybrid maize are shown. Marker proteins characteristic of the hybrid are highlighted by a green frame. It can be seen that the signal-to-noise ratio of the marker proteins was best in *extraction buffer 3 (***Table 1***)*, so for the genetic purity testing of this hybrid, this mixture was selected.

For genetic purity testing, ground samples of adequate particle size were prepared from 95-100 individual kernels of the maize hybrid seeds, and then these samples were treated with the extraction agents that had been selected according to the results of the preliminary experiments. **Figure 5** summarizes the work process of genetic purity testing.

Extracts were applied to the target of the MALDI-TOF instrument in a diluted state, in duplicate. Sodium chloride buffers were diluted with acetonitrile, while alcoholic extracts were diluted with a water-acetonitrile mixture. Acetonitrile helps drying of the droplets applied to the sample plate (target), and also the application of alcoholic solutions. The matrix solution was spotted on the plate after the drying of the extracts (***Figure 6***).

Standards necessary for mass number calibration were applied to selected positions of the sample plates. In the case of maize seed analyses, the reference standard is identical to the reference material used for the identification of microorganisms (*Escherichia coli* DH5).

For the analysis of the extracts, a *Bruker microflex LT* MALDI-TOF mass spectrometer was used. The prepared sample plate was introduced into the ion source through a lock system. Parameters used for the analyses (mass number range, laser energy, movement pattern of the sample plate) were set with the help of the general instrument control software (*flexControl – Bruker Daltonics*). The same program was used t perform mass number calibration as well (determination of the parameters of the time-of-flight vs. mass number function).

After this, recording of the mass spectra of the extracts was performed by the instrument automatically, in the order provided by us. At least 640 unique mass spectra in the 2 - 30 kDa range were prepared from each sample location, and a composite mass spectrum was recorded from these by the software.

3.5.3. Processing of measurement data, evaluation of mass spectra

To process measurement data, the *flexAnalysis* (*Bruker Daltonics*) software was used, which allows for the simultaneous processing of several, even 50 to 60 mass spectra. With the help of *flexAnalysis*, in the first step, a few simple operations were performed on the mass spectra (noise filtering – smoothing, baseline correction, finding the maxima of the peaks in the mass spectrum – identification of the mass numbers of the peaks). Following this, marker proteins selected during the preliminary tests were identified in the spectra of the extracts of the individual corn kernels, based on their mass numbers, and through these, "faulty" kernels were identified. The absence of the marker proteins of the paternal line, and the presence of the protein profile of the maternal line indicated self-pollination. A protein profile different from that of the maternal line indicates the presence of a foreign kernel (outcrossing) (**Figure 7**).

4. Results of genetic purity testing

The different genotypic backgrounds of hybrids result in genetic purities of varying degrees. In the case of most seeds, values were within the recommended limit value (>97%). During seed certification, it is mandatory to test the germination capacity and the plant pathology status as well. At the same time, genetic purity testing is not mandatory, but it is performed by seed distributors as a supplementary method, or they have it performed by suitable laboratories. The above-mentioned limit value is prescribed by the distributors. In practice, in most cases, a seed batch of 97-98% genetic purity is considered adequate quality.

In our work, coded maize hybrids were analyzed, whose genetic purity test results were known, and they were provided to us by the Seed testing Laboratory of the National Food Chain Safety Office (NÉBIH). Genetic purity inspection of the hybrids

was performed by the experts of NÉBIH using the generally known reference method – ultrathin polyacrylamide gel isoelectric focusing (*UTL-IEF or IEF, in short*). During this process, reference gels were prepared (father – mother – hybrid), and based on these the similarity of the protein patterns of the hybrids was determined, or the cause of the difference, which could be self-pollination, foreign fertilization (outcrossing) or extraneous corn in the seed batch. A basic conditions for the repeatability of the analyses performed by the reference method is adequate sampling, i.e, the composition of the samples taken for the certification of the seed batch at random has to be representative of the basic population. The laboratory sample has to be homogenized.

A major objective of our research was, in addition to the analysis of healthy seeds, to develop a genetic purity test for fungus-infected seeds by the MALDI-TOF method, because analytical results of such kernels, that can be obtained by the reference IEF method, are highly unreliable.

When applying the reference UTL-IEF method, 2 x 96 corn kernels were tested by the experts of NÉBIH, but we analyzed only 96 kernels per seed batch when using the MALDI-TOF method, because we wanted to know whether results similar to those of the reference method can be obtained when processing fewer corn kernels. Comparative data are summarized in ***Table 2***.

The data in ***Table 2*** show that – even if fewer corn kernels are processed – results obtained by our method developed for the MALDI-TOF technique show a very good agreement with results obtained by the reference method (UTL-IEF). Analysis of fungus-infected samples (5g and 6g) provided no meaningful results when using the reference method, so their analytical results could not be compared to results obtained by the large instrument technique. The minimal differences between the results that could be compared are due to two factors:

1. When calculating genetic purity (GT (%) = faulty kernels/analyzed kernels*100) the genetic purity values came from analytical samples consisting of different numbers of kernels, which slightly increased the calculated difference between the purity values.
2. Different populations were selected for the two different tests from the given hybrid maize seed batch;

To be mentioned, it was impossible to get genetic purity results in case of the fungus-fertilized kern (5g and 6g) using the reference method.

Hereinafter, mass spectra obtained by the analytical method developed for the MALDI-TOF technique, and the conclusions that can be drawn from them are described.

Figure 8 shows the mass spectra of a marker protein pair characteristic of a two-line hybrid. It can be observed that, in the case of the genetically inadequate, "faulty" kernel, the paternal marker protein is missing, indicating the possibility of self-pollination or foreign fertilization. There were also cases during the work where neither marker proteins appeared in the mass spectrum, so it was likely that the sample population to be analyzed contained a foreign kernel (*outcross*). ***Figure 9*** shows the gel photo of the same hybrid. On the image of the gel you can see that, when using the UTL-IEF reference method, the evaluation is not easy to perform, even in the case of healthy kernels.

Fungal infection or disease of the corn kernels can interfere with the evaluation of the gel obtained by UTL-IEF, used as a reference method. As a proof of this, the gel photo of a fungus-infected hybrid maze, obtained by the UTL-IEF method, is shown in ***Figure 10***.

In ***Figure 10***, it can be obvious to the skilled expert that bands of certain proteins disappear, while others appear in the gel chromatogram. This phenomenon is due to the basic principle of the reference method, because isoelectric focusing gel electrophoresis (IEF) is a forced flow method, separating proteins according to their isoelectric points. Separation is carried out on a polyacrylamide gel containing a mixture of amphoteric electrolytes (ampholytes). Due to the electric field, the ampholytes migrate, while creating a pH gradient. When a protein reaches the gel fraction with a pH value identical to its isoelectric point (pI), its charge is neutralized and it does not migrate further.

Since the results of the analyses performed by the MALDI-TOF technique are not affected by the pH of the extract, the above-mentioned effect does not prevail. Accordingly, there is no change in the marker protein profile when testing fungus-infected maize kernels (***Figure 11***).

5. Conclusions

Based on our results so far it was determined that, in the case of healthy hybrid maize kernels, results of our tests performed using the MALDI-TOF technique showed satisfactory agreement with the results of the reference method. Our MALDI-TOF mass spectrometric method provides an opportunity to analyze reserve protein profiles, i.e., to determine genetic purity from the kernels in the case of maize hybrid samples.

During the analyses performed using the reference method, only a few extraction agents can be used for the extraction of the reserve protein (water, 2-chloroethanol). In the case of the MALDI-TOF method, several extraction agents and additives (sodium chloride, dl-DTT) can be used in order for the extraction of marker proteins to be as efficient as possible. In our experience, in the case of maize samples, reserve proteins or marker proteins are best extracted when using alcoholic extraction buffers. However, al-

coholic extracts cannot be analyzed by the reference method.

Our method developed for the MALDI-TOF technique is much faster and simpler than isoelectric focusing performed on an ultrathin polyacrylamide gel (UTL-IEF), because evaluation of the results is supported by a software, and the searchability and possible reevaluation of the digitally stored results also have several advantages when compared to the conventional analytical technique. Thanks to the sensitivity of the method, even marker proteins that are present in small amounts can be detected.

Our experiments included the analyses of fungus-infected maize kernels. Fungal infection of the given kernels can also be recognized when using the reference method (IEF). However, on the gel chromatogram, characteristic protein bands (markers) disappear and show a blurry picture, so – based on this – the genetic purity of the given kernel cannot be determined with sufficient certainty. Running on the gel with isoelectric focusing is influenced by fungal infection in a hitherto unknown way. Therefore, we think that it is impossible to test fungus-infected maize seed batches using the reference method. Since the results of the analysis using the MALDI-TOF technique are not affected by fungal infection, this method can provide a solution to the inspection of infected batches. In our experiments, several infected maize kernels were analyzed. In all cases, marker proteins were clearly visible in the mass spectra. There is an increasing body of evidence to show that fungal infection does not cause a change in the profile of reserve proteins. It can be stated that if a kernel is fungus-infected, it can still be genetically appropriate. There were also batches treated against fungal infection among the maize lots analyzed, and testing of these did not present any difficulties when using the method developed by us.

Results (mass spectra) obtained during the genetic purity testing of hybrid maize samples using the MALDI-TOF technique are also suitable for the determination of varietal identity. With the help of the software background provided by MALDI-TOF, a database can be created from the data, that can later help reliable varietal identification, which is an indispensable prerequisite of variety protection.

6. References

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