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Characterization of Protein Fractions from Bt-Transgenic and Non-transgenic Maize Varieties Using Perfusion and Monolithic RP-HPLC. Maize Differentiation by Multivariate Analysis

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Protein fractions from transgenic Bt and non-transgenic maize varieties, extracted by the Osborne solvent fraction procedure, were characterized for the first time by perfusion and monolithic RP-HPLC in very short analysis times. Albumins and globulins from different transgenic Bt maizes as well as from their non-transgenic isogenic varieties were eluted in four peaks using perfusion RP-HPLC, whereas prolamins and glutelins were separated in seven peaks. Monolithic RP-HPLC enabled the separation of maize proteins in a large number of peaks showing 6 and 10 main peaks for albumins and globulins, respectively. Prolamins migrated at retention times higher than 5 min as seven peaks, whereas glutelins were separated in three main peaks appearing at retention times higher than 6.0 min. Moreover, chromatograms of the whole protein extracts showed 8 and 11 components for perfusion and monolithic RP-HPLC, respectively. A comparison of the chromatograms of the whole protein extracts relative to transgenic and non-transgenic varieties evidenced quantitative differences on the percentages of area, mainly for peaks 2 and 3 by perfusion RP-HPLC and for peaks 3 and 7 by monolithic RP-HPLC. A discriminant analysis based on these proteic profiles was carried out to classify and predict transgenic Bt maize lines, achieving 100% correct classification using perfusion RP-HPLC.

KEYWORDS: Bt-transgenic maize; non-transgenic maize; perfusion RP-HPLC; monolithic RP-HPLC; protein fractions; albumins, globulins, prolamins; glutelins

INTRODUCTION

Economic, environmental, and health benefits derived from the use of insect-resistant transgenic crops have extensively been described (*1*, *2*). In 2004, 81 million hectares were planted with genetically modified soybean (60% of the total transgenic crops grown), maize (23%), cotton (11%), and canola (6%) (*3*). In the year 2005, the increase of global area approved for biotech crops was 9.0 million hectares, equivalent to an annual growth rate of 11% (*4*). Bt maize occupied 11.2 million hectares, representing 14% of global transgenic area (*3*). The production of Bt maize is concentrated mainly in North and South America, whereas in Europe the production is scarce. In 2005, the planting of Bt maize in Portugal, France, and the Czech Republic brought to five the total number of European Union (EU) countries working modest areas of Bt maize, including Spain and Germany (*4*). Spain is the first state in the EU that has allowed the cultivation of Bt maize since 1998 and the only country in the EU with a significant area of transgenic maize crop (0.1 million hectares in 2005) (*5*).

Today, the transgenic Bt varieties inscribed in the Spanish Commercial Varieties' Register are 16, 5 lines with the event Bt-176 and 11 with the event MON 810 (Orden 7052/1998, BOE 26th March; Orden APA/520/2003, BOE 11th March; Orden APA/314/2004, BOE 16th February). In 2003, PR33P67 (from Pioneer), DKC6575 (from Monsanto), and Aristis Bt (from Nickerson) were registered as maize hybrids with the event MON 810 (Orden APA/520/2003, BOE 11th March) with total cultivated areas of 6000, 47, and 161 ha, respectively (*7*).

The transgenic Bt maize with the event MON 810 was genetically engineered to resist European corn borer (ECB) by producing its own insecticide. This line was developed by introducing the cry1Ab gene, isolated from the common soil bacterium *Bacillus thuringiensis* (Bt), into the maize cultivar Hi-II. The cry1Ab gene produces the insect control protein Cry1Ab, a δ-endotoxin for the ECB (*6*). The ECB, *Ostrinia*

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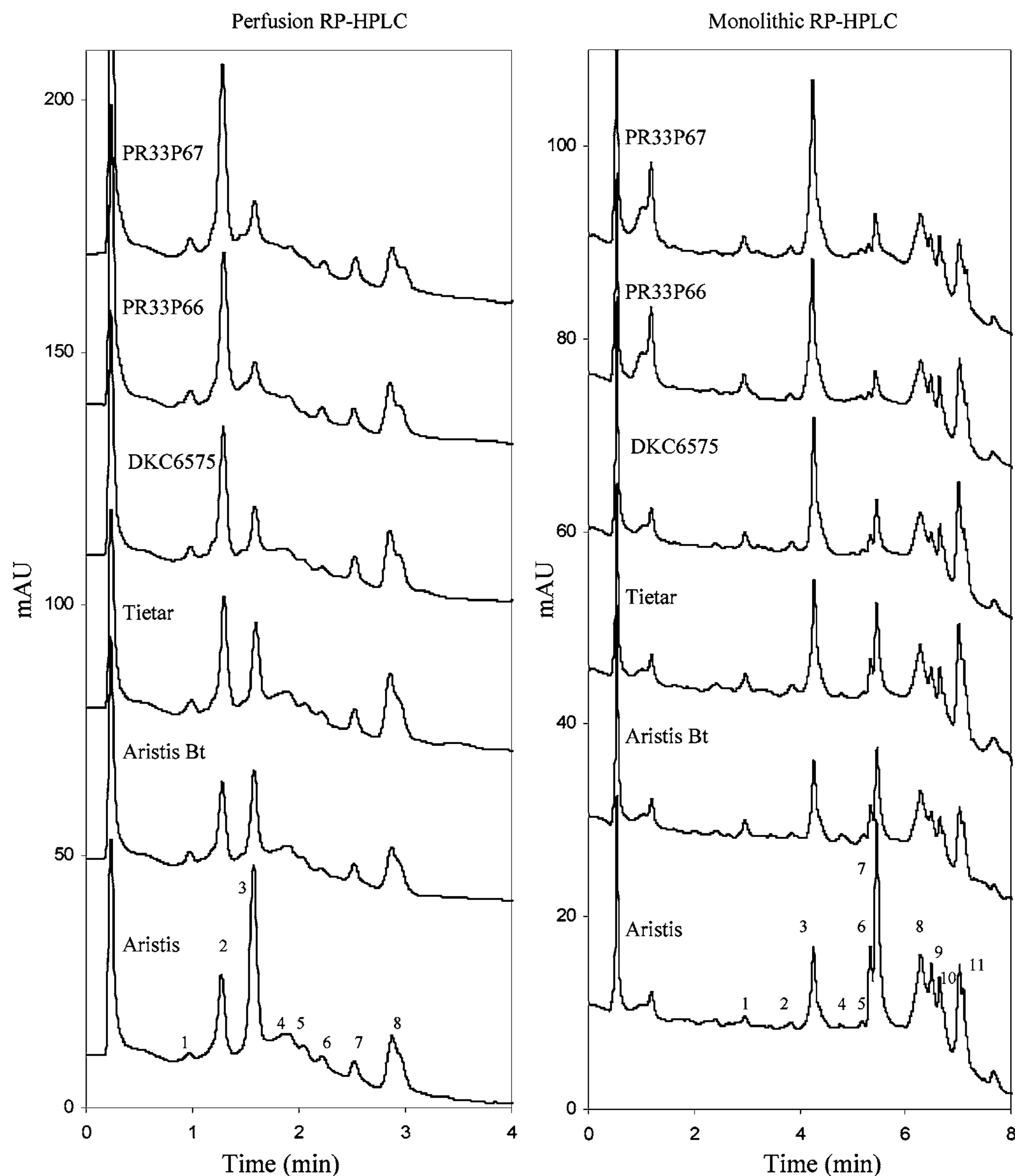

Figure 1. Perfusion and monolithic RP-HPLC chromatograms of whole protein extracts from isogenic and transgenic Bt maize lines.

nubilalis, is also the most damaging insect pest of maize in the United States and Canada with losses exceeding 1 billion dollars each year (*7*).

Regulations for the development, growth, and use of genetically modified organisms and derived ingredients are being implemented worldwide and with special emphasis in the EU countries. These new regulations demand reliable analytical methods to detect genetically modified organisms (GMO) in raw materials and food products (*8*). Methods for the detection of GMOs involve the analysis of DNA (e.g., PCR and Southern blotting) or the analysis of proteins (e.g., lateral flow strip, immunoassays, and Western blot) (*9*). García-Cañas et al. have reported a huge expertise in the detection of transgenic maize by the analysis of DNA using different PCR techniques and capillary gel electrophoresis with UV and laser-induced fluorescence detections (*10*−*13*).

Protein analysis constitutes another way to detect GMOs. High-performance liquid chromatography (HPLC) has been applied to the selection and identification of different plant genotypes (*14*). Some studies have been reported on the use of RP-HPLC for the characterization of proteins from genetically modified maize mutants (*15*). However, RP-HPLC methods for the characterization and identification of transgenic Bt maize have not been found in the literature.

Recently, our research group separated maize proteins by perfusion (*16*) and monolithic (*17*) RP-HPLC in very short analysis times (<4 min with the perfusion column and <8 min with the monolithic column) in relation to the high analysis times (40−90 min) reported with reversed-phased columns (*14*, *15*, *18*−*20*). These methods have successfully been applied to the characterization of commercial maize products (*16*, *17*) and the identification and classification of European and North American inbred and hybrid maize lines (*21*). These results encouraged us to test the applicability of these methods for the characterization of transgenic Bt maize.

Therefore, the aim of this work has been the characterization of albumins, globulins, and storage proteins (prolamins and glutelins) from transgenic Bt maize lines using perfusion and monolithic RP-HPLC. Whole protein extracts from transgenic Bt and non-transgenic maize varieties were also analyzed by both chromatographic methods and next classified by discriminant analysis.

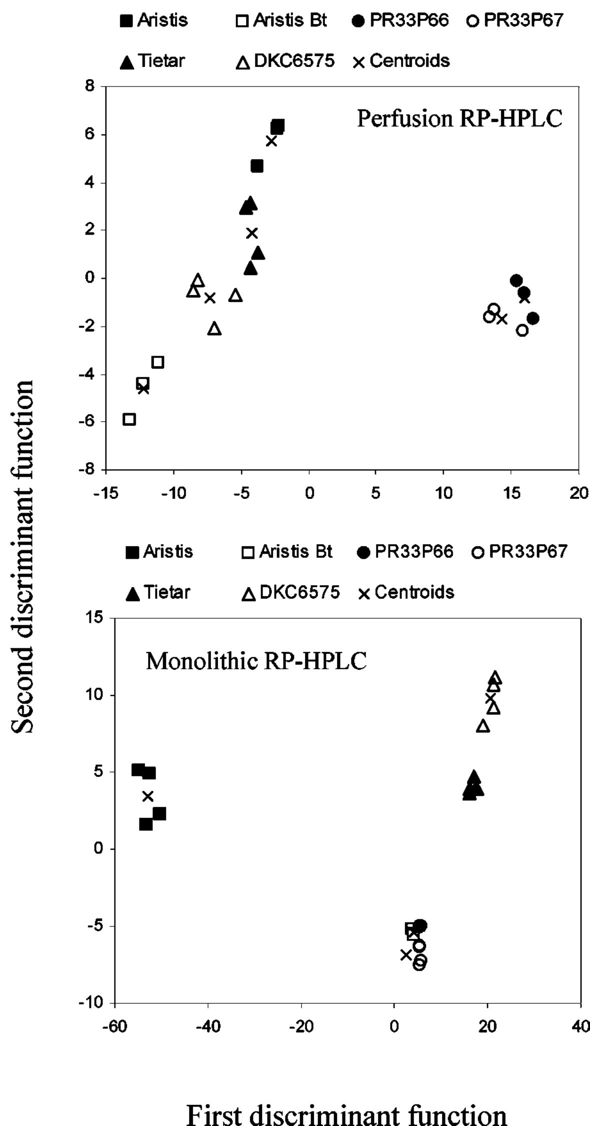

Figure 2. Distribution of isogenic and transgenic Bt maize lines analyzed by perfusion and monolithic RP-HPLC in the plane defined by the first two discriminant functions.

EXPERIMENTAL PROCEDURES

Chemicals and Samples. 2-Mercaptoethanol, tris(hydroxymethyl)-aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), 1-propanol (all from Merck, Darmstadt, Germany), ammonium acetate, chloride acid, and potassium chloride (all from Panreac, Barcelona, Spain) were employed for the extraction of maize proteins. HPLC grade acetonitrile (ACN) (Merck), Milli-Q water (Millipore, Bedford, MA), and trifluoroacetic acid (TFA) (Sigma, St. Louis, MO) were used for the preparation of mobile phases.

Corn gluten meal (CGM) with a purity of 60% was purchased from Sigma (St. Louis, MO). Transgenic Bt maize seeds with the event MON 810 (PR33P67) (from Pioneer Hi-Bred International, Inc.), DKC6575 (from Monsanto Co.), and Aristis Bt (from Nickerson Ltd.) and their non-Bt isogenic varieties (PR33P66, Tietar, and Aristis, respectively) were employed in this study.

Protein Fractionation. Transgenic and non-transgenic maize lines (30 kernels) were ground with an analytical mill (IKA Labortechnik, Staufen, Germany) during 3 min at ambient temperature. The dry matter content of maize flours was determined by drying at 130 °C to constant weight (AOAC method 925.10). The percent moisture of the kernels was about 7−9%.

Maize proteins were sequentially extracted according to the Osborne procedure (*22*) with modifications. Namely, 30 mg of each milled maize line was extracted twice with Milli-Q water (1 mL each time) and sonicated for 5 min in a bath sonicator (150 W, 50 Hz, FS-30, Fisher Scientific, Pittsburgh, PA). The mixture was centrifuged for 5 min at 3362*g* (Avanti J-25 centrifuge, Beckman Coulter) at 25 °C, and the supernatant (albumin fraction) was saved. The pellet was then extracted twice with 1 mL of Tris-HCl buffer (50 mM Tris-HCl, 50 mM KCl, and 5 mM EDTA) at pH 7.8 for 5 min and centrifuged as before. The supernatant corresponded to the globulin fraction. Afterward, the pellet was extracted twice with 1 mL of 50% (v/v) 1-propanol in water for 5 min and centrifuged. The supernatants were decanted, mixed, and saved as the prolamin fraction. Glutelins were extracted twice from the prolamin pellet with 1 mL of 50% (v/v) 1-propanol, and 1% (w/v) dithiothreitol in water for 5 min. After centrifugation for 5 min, the supernatants were mixed and saved as the glutelin fraction. All of the protein fractions obtained by this procedure were directly injected in the chromatographic system.

Table 1. Classification and Prediction of Isogenic and Transgenic Bt Maize Lines Analyzed by Perfusion and Monolithic RP-HPLC

	perfusion RP-HPLC		monolithic RP-HPLC	
maize line[a]	classification (%)	prediction (%)	classification (%)	prediction (%)
Aristis	100.0	66.7	100.0	50.0
Aristis Bt	100.0	100.0	50.0	50.0
PR33P66	100.0	66.7	75.0	75.0
PR33P67	100.0	66.7	75.0	75.0
Tietar	100.0	100.0	100.0	100.0
DKC6575	100.0	75.0	100.0	75.0
overall	100.0	80.0	83.3	70.8

[a] Four samples ($n = 4$) for each maize line were analyzed.

Pulverized maize (30 mg) was dissolved in 1 mL of the extracting solution consisting of 0.5% (v/v) 2-mercaptoethanol, 0.5% (w/v) ammonium acetate, and 45% ACN (v/v) in water, sonicated for 5 min, and centrifuged for another 5 min at 3362*g* (*16*, *17*). The supernatant was saved as the whole protein extract.

High-Performance Liquid Chromatography. A Hewlett-Packard 1100 series liquid chromatograph (Hewlett-Packard, Pittsburgh, PA) provided with a degassing system, a binary pump, a thermostated compartment for the column, an injection system, and a diode array detector was used. A POROS R2/H perfusion column (4.6 × 50 mm; 10 μm particle size) (Perseptive Biosystems, Framingham, MA) and a monolithic silica column Chromolith Performance RP-18e (4.6 × 100 mm) (Merck) were employed. Chromatographic conditions for the perfusion column were optimized previously by our research team (*16*): mobile phase A, 0.1% (v/v) TFA in Milli-Q water; mobile phase B, 0.1% (v/v) TFA in ACN; linear binary gradient, 5.0−50.2% B in 2.40 min, 50.2−65.4% B in 0.98 min, and 65.4−5.0% in 1 min; injection volume, 20 μL; flow rate, 3 mL/min; temperature, 25 °C; UV detection, 280 nm. The separation conditions for the monolithic column were those optimized previously (*17*): linear binary gradient, 5.0−26.4% B in 5.15 min, 26.4−87.5% B in 2.16 min, and 87.5−5.0% in 1 min; temperature, 35 °C. The injection volume, flow rate, mobile phase composition, and wavelength detection were as in perfusion chromatography. Data were recorded and processed with HP-Chemstation software.

Data Treatment. The area percentage for every peak was calculated as the average of three replicates (injected by duplicate). The integration was performed by setting the baseline from valley to valley. As the variables have the same units of measurement, the samples were not autoscaled. Linear discriminant analysis was performed using the computer program Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Separation of Maize Proteins from Transgenic Bt and Non-transgenic Maize Varieties by Perfusion and Monolithic RP-HPLC. Two different chromatographic methods using perfusion and monolithic columns previously optimized by our

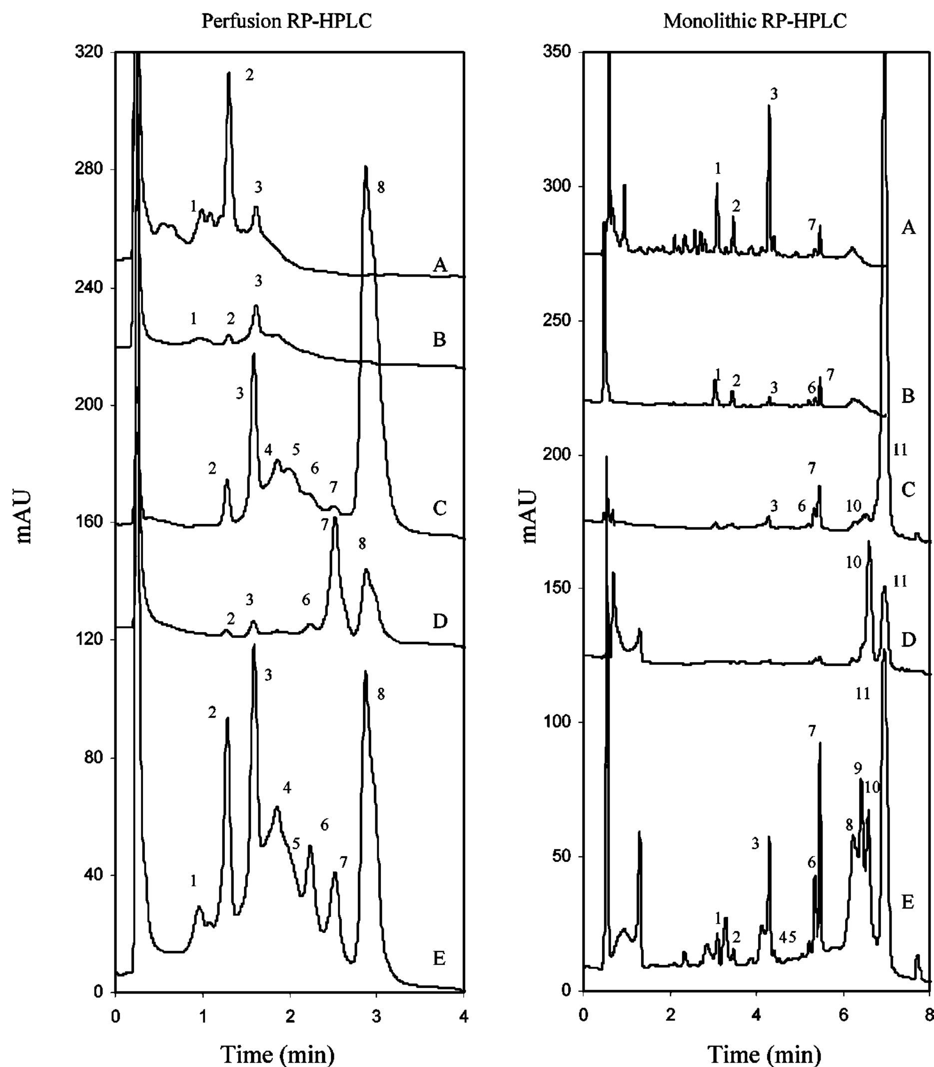

Figure 3. Perfusion and monolithic RP-HPLC chromatograms of albumin (**A**), globulin (**B**), prolamin (**C**), and glutelin (**D**) fractions and for the whole protein extract (**E**) from corn gluten meal.

research team (*16*, *17*) were applied to the separation of maize proteins from the whole protein extracts obtained from the three Bt-transgenic maize cultivars and their corresponding isogenic lines described above. **Figure 1** shows the perfusion and monolithic RP-HPLC chromatograms obtained from these transgenic and non-transgenic lines. For perfusion RP-HPLC, maize proteins eluted in eight peaks in less than 3 min, showing interesting differences between the maize lines. Thus, in Aristis and Aristis Bt, the signal obtained for peak 2 was smaller than that found for peak 3. Furthermore, the difference between the areas for these two peaks was higher for Aristis than for its transgenic line. For Tietar and its transgenic line (DKC6575) and for PR33P66 and its transgenic line (PR33P67), the signal for peak 2 was higher than that for peak 3. The ratio between the area percentage for peaks 2 and 3 calculated from **Figure 1** was smaller than 2.5 for Tietar and DKC6575 (ratios of 1.4 and 2.4, respectively) and higher than 3.0 for PR33P66 and PR33P67 (ratios of 4.1 and 3.5, respectively). These differences observed for peaks 2 and 3 could be useful for the differentiation of Bt maize cultivars.

The chromatograms obtained by injecting the aforementioned whole protein extracts, in this case in a monolithic RP-HPLC system, could be divided into three groups of peaks (**Figure 1**): one group at the beginning of the chromatogram (peaks 1−4), a second one at the middle (peaks 5−7), and, finally, a third group at the end (peaks 8−11). The characterization of isogenic and transgenic Bt maize lines was possible by observing the ratio between the signals corresponding to peaks 3 and 7. This ratio was smaller than 1.0 for Aristis and Aristis Bt (ratios of 0.5 and 0.9, respectively), whereas for the other lines the signal corresponding to peak 3 was higher than that found for peak 7. Ratios of 1.7 and 3.0 were found for Tietar and its transgenic line, respectively, and ratios of 7.8 and 6.8 were obtained for PR33P66 and its transgenic line, respectively. Good reproducibility was achieved for both columns. For duplicate injections, the RSD was better than 1% in peak area and in retention time, whereas for three replicate samples the RSD values were below 1% in retention time and 3−4% in peak area.

From these results, it was expected that chromatographic profiles obtained for the whole protein extracts could be useful to discriminate between non-transgenic and transgenic Bt maize lines using multivariate analysis. Among multivariate methods, discriminant analysis is considered to be an important tool for grouping samples wherein the origin of the samples is known (*23*).

In this study, multiple linear discriminant analysis was applied to the chromatographic data obtained by perfusion and monolithic analysis of whole protein maize extracts to provide a mathematical tool able to provide an adequate classification of this type of sample. Initially, the discriminant analysis was applied to perfusion RP-HPLC data. Four discriminant functions

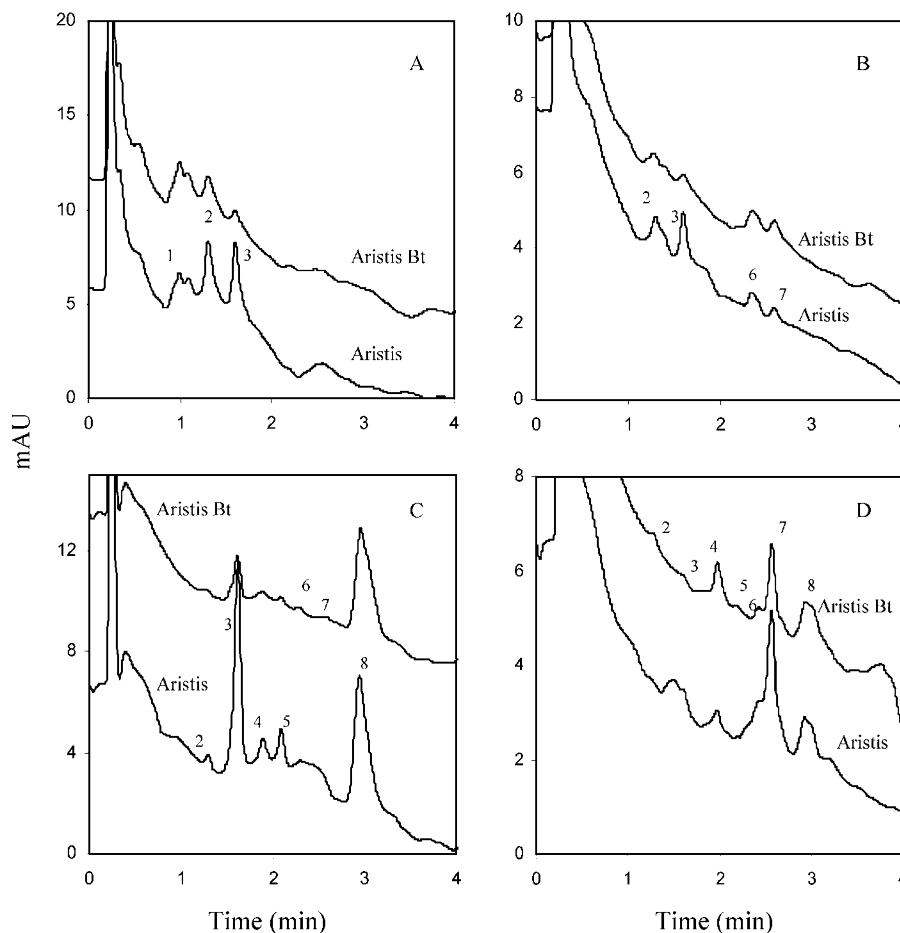

Figure 4. Perfusion RP-HPLC chromatograms of albumin (**A**), globulin (**B**), prolamin (**C**), and glutelin (**D**) fractions from Aristis and Aristis Bt.

were significant ($p < 0.01$), accounting for 93.4% of the total variance by the first two discriminant functions. The final model selected the following five peaks: 8, 4, 3, 2, and 6, in decreasing order of discriminating power. Peaks 2, 3, and 6 presented positive coefficients and peak 4 negative coefficients for both functions. Peak 8 was positive and negative for the first and second functions, respectively. The scatter plot of protein extracts analyzed by perfusion RP-HPLC in the plane defined by the first two discriminant functions is shown in **Figure 2**. Samples PR33P67 and PR33P66 were located on the right of the origin of the first discriminant function, whereas the other lines (Aristis, Aristis Bt, Tietar, and DKC6575) were located on the left. PR33P67 and PR33P66 were perfectly separated, although the distance between centroids was not very large. On the other hand, the second discriminant function was critical in the discrimination between Aristis and Aristis Bt and between Tietar and DKC6575. The transgenic lines (Aristis Bt and DKC6575) were located at negative values for the second function (with negative coefficients for peaks 4 and 8), and their isogenic lines (Aristis and Tietar) were located at positive values (with positive coefficients for peaks 2, 3, and 6). The classification table shows that 100.0% of the samples were correctly classified. The prediction capability of the model was evaluated by cross-validation achieving a percentage of prediction of 80.0% (**Table 1**).

A discriminant analysis was also applied to data obtained by monolithic RP-HPLC. Four discriminant functions were also significant ($p > 0.01$), the first two functions accounting for 98.9% of the total variance. The final model selected peaks 3, 7, 6, and 11, in decreasing order of discriminating power. Peak 7 (with a negative coefficient for the first function) discriminated Aristis from the other lines, which occurred on the positive side of the first function (with positive coefficients for peaks 3, 6, and 11). The scatter plot of protein extracts in the plane defined by the two first discriminant functions (see **Figure 2**) showed an inappropriate discrimination with respect to the first function, but the second function discriminated clearly DKC6575 and Tietar (located on the positive side of the second function) from PR33P66, PR33P67, and Aristis Bt (located on the negative side), which are partially overlapped. As shown in **Table 1**, a global percentage of correct classification of 83.3% and a global prediction of 70.8% were achieved.

Characterization of Maize Protein Fractions from Different Maize Varieties by Perfusion and Monolithic RP-HPLC. The chromatographic conditions described above were also applied to the characterization of maize protein fractions from different maize cultivars. For that purpose, the fractionation procedure was first applied to fractionate albumins, globulins, prolamins, and glutelins from CGM as well as from different transgenic and non-transgenic maize cultivars.

Perfusion and monolithic RP-HPLC chromatograms corresponding to the protein fractions extracted from the CGM are shown in **Figure 3**. Maize proteins in the whole extract showed eight peaks by perfusion RP-HPLC with the maximum signal for peaks 2, 3, and 8. The number assigned to the peaks found in the protein fractions was the same as that corresponding to peaks with the same retention times in the whole protein extract injected in the chromatographic system under the same conditions. CGM glutelins were eluted in five peaks corresponding to peaks 2, 3, and 6−8 in the whole protein extract. Prolamin proteins were eluted in two peaks appearing at the beginning of the chromatogram (peaks 2 and 3), together with a group of

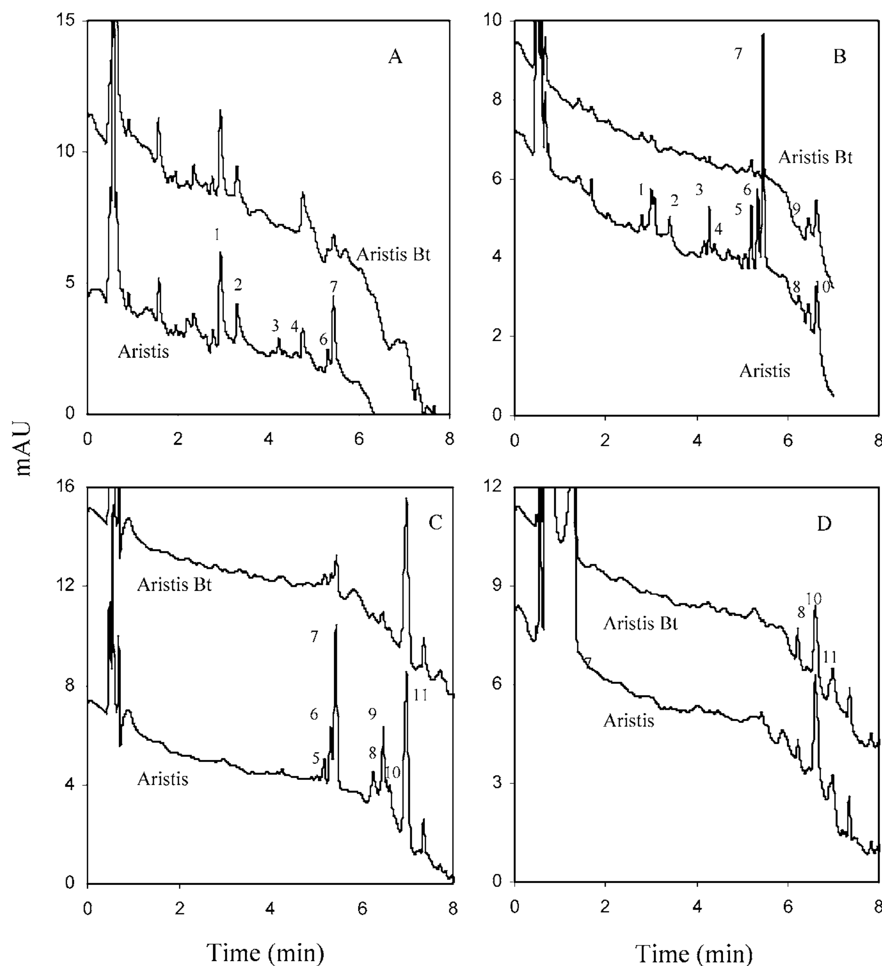

Figure 5. Monolithic RP-HPLC chromatograms of albumin (**A**), globulin (**B**), prolamin (**C**), and glutelin (**D**) fractions from Aristis and Aristis Bt.

peaks (peaks 4−7) at the middle of the chromatogram and a big peak at the end of the chromatogram (peak 8). On the basis of the retention times, these peaks corresponded to peaks 2−8 observed in the whole protein extract. The globulin chromatogram showed a profile with only three peaks at the retention times of peaks 1−3 in the whole protein extract. The albumin fraction was also eluted as three peaks corresponding to peaks 1−3 in the whole protein extract, peak 1 being partially defolded. From these results and those obtained previously by our research team when injecting the main zein fractions under the same conditions (*16*), it was possible to assign the main maize proteins eluting in the chromatographic peaks of the protein extracts. Peak 1 could be attributed mainly to albumins, whereas peak 2 eluted mainly in albumin and prolamin fractions. Peak 3 could be assigned mainly to prolamins (α-zein) (*16*), although it also appeared in the globulin and albumin fractions. Peaks 4 and 5 appeared only in the prolamin fraction and together with peaks 6 and 7 could be attributed to β- and γ-zeins (*16*), although these two last peaks also appeared in the glutelin fraction. Finally, peak 8 could be assigned to prolamins (α-zein), although this peak also appeared in the glutelin fraction.

CGM protein profiles obtained by monolithic RP-HPLC were totally different from those obtained with perfusion RP-HPLC (see **Figure 3**). Unlike perfusion chromatography, the monolithic chromatograms for the whole protein extract yielded four groups of peaks with retention times ranging from 2.0 to 3.9 min (first group), from 4.0 to 4.8 min (second group), from 5.0 to 5.8 (third group), and from 5.9 to 7.5 min (last group). The glutelin fraction from CGM was eluted as a doublet of peaks at the end of the chromatogram corresponding to peaks 10 and 11 observed in the whole protein extract. Prolamins were eluted in a high peak (peak 11) together with a doublet of small peaks (peaks 6 and 7). The globulin chromatogram showed five peaks corresponding to peaks 1−3, 6, and 7 in the whole protein extract. The chromatogram obtained for the albumin fraction was more complex, showing the maximum signal for peaks 1−3 and 7. From these results and those obtained previously by our research team when injecting the main zein fractions under the same conditions (*17*), it was possible to assign the main maize proteins eluting in the chromatographic peaks of the protein extracts. Peaks 1 and 2 could mainly be attributed to albumins, although they also appeared in the globulin fraction. Peak 3 was assigned to albumins, whereas peaks 6 and 7 could not be clearly attributed to any fraction because they appeared in albumin, globulin, and prolamin fractions. Peaks 8−11 were assigned to prolamins, although peaks 8 and 9 appearing in the whole extract could not be observed in any of the fractions from CGM. From these peaks, peaks 8−10 were attributed in a previous work to β- and γ-zeins and peak 11 was attributed to α-zeins (*17*). Finally, glutelins coeluted with prolamins in peaks 10 and 11.

Once the chromatographic profiles of the protein fractions from CGM were studied, perfusion and monolithic RP-HPLC methods were applied to the separation and characterization of these protein fractions from different transgenic and nontransgenic maize cultivars. As an example, perfusion and monolithic RP-HPLC patterns of albumins, globulins, prolamins, and glutelins from Aristis and Aristis Bt are shown in **Figures 4** and **5**, respectively.

The perfusion RP-HPLC chromatogram obtained for the albumin fraction from Aristis exhibited two large peaks

(2 and 3) and a group of peaks partially resolved at the beginning of the chromatogram, all of them migrating between 1 and 2 min. These peaks were found at the same retention times as peaks 1−3 in the whole protein extract of this line (**Figure 1**). Differences in the relative area between transgenic and non-transgenic maize lines were found, mainly for peaks 2 and 3. The chromatogram for the albumin fraction from Aristis showed higher percentages of area for peaks 2 and 3 (36.5 and 42.9%, respectively) than observed for Aristis Bt (9.4 and 14.5%, respectively). The comparison of the chromatograms corresponding to the albumin fractions of transgenic and non-transgenic lines with that of CGM albumin revealed similar profiles with the same number of peaks.

The globulin fraction was separated in four peaks with retention times ranging from 1.0 to 2.6 min. The first pair of peaks had the same retention times as peaks 2 and 3 in the whole protein extract, and the second one had the same retention times as peaks 6 and 7. Significant differences for the area percentages between Aristis and Aristis Bt were also observed, mainly for peaks 2 and 3 (23.9 and 47.9% for Aristis, respectively, and 11.9 and 18.5% for Aristis Bt, respectively). The first pair of peaks was also found in CGM globulin, whereas peaks 6 and 7 were not observed (**Figure 3**).

Aristis and Aristis Bt prolamin chromatograms showed profiles very similar to that of the prolamin fraction from CGM, although peaks 6 and 7 were poorly resolved in both maize lines. Only two peaks (3 and 8) accounted for >88% of the total area for Aristis as well as Aristis Bt. Peak 3 showed more intensity for Aristis (relative area = 34.8%) than for Aristis Bt (relative area = 11.9%), whereas peak 8 had a lower relative area (59.4%) for the isogenic line than for the transgenic line (84.1%).

Glutelins from Aristis and Aristis Bt were separated in three main peaks (4, 7, and 8) and other peaks poorly resolved. Note that peak 7 was expressed with higher intensity for Aristis (relative area = 60.6%) than for its transgenic line (relative area = 44.2%).

On the other hand, albumin, globulin, prolamin, and glutelin fractions from Aristis and Aristis Bt were also separated by monolithic RP-HPLC (**Figure 5**). Albumin chromatograms for maize lines showed that all major peaks eluted between 2.5 and 6.0 min. Significant differences for the relative area of peaks 2, 4, and 7 were found between Aristis and Aristis Bt. Peaks 2 and 7 presented higher area percentages for Aristis than for Aristis Bt, whereas peak 4 was expressed with more intensity for the transgenic maize line.

Globulins eluted in 10 peaks (1−10) with retention times ranging from 2.8 to 7.7 min. The signals provided by these peaks were higher for Aristis than for Aristis Bt, this difference being observed more clearly for the triplet of peaks 5−7. Prolamins were separated in three groups of peaks: a triplet (peaks 5−7) with retention times ranging from 5.2 to 5.5 min, a second group with three peaks at the middle of the chromatogram (peaks 8−10), and, finally, a big peak at the end of the chromatogram. Peak 11 presented the maximum signal accounting for a relative area of 58% for Aristis and 84% for Aristis Bt. The expression of both triplets was more intense for Aristis than for its transgenic line. Moreover, peaks 8−10 that did not appear in the CGM prolamin fraction appeared in the prolamin fraction from Aristis and Aristis Bt, corroborating the assignation of these peaks to prolamins (β- and γ-zeins). Finally, glutelins coeluted with prolamins in peaks 8, 10, and 11, showing the maximum signal for peak 10.

Conclusions. This work is one of the limited number of examples in which differentiation between transgenic and non-transgenic cultivars has been approached on the basis of proteic profiles. Perfusion and monolithic RP-HPLC analyses, together with discriminant analysis, were shown to be promising, especially for its simplicity, low cost, and short time consumption in comparison with the tedious and long analysis of proteins based on the their digestion and separation by gel electrophoresis. Good classification and prediction models were found for both chromatographic methods; however, perfusion RP-HPLC gave the better results with a power of classification of 100% and prediction of 80%. Perfusion and monolithic RP-HPLC methods were successfully employed for the characterization of albumin, globulin, prolamin, and glutelin fractions from transgenic Bt and isogenic maize cultivars. Significant differences in the relative peak areas were found among Bt transgenic and isogenic lines with both chromatographic methods and especially for the prolamin and globulin fractions of proteins. Nevertheless, further studies should be carried out to characterize more samples from other varieties of Bt maize and its isogenic lines and to study the effect of using other agronomic conditions different from those employed in this paper.

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