Research Article

Polyphenolic Profile of Maize Seedlings Treated with 24-Epibrassinolide

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High-performance thin-layer chromatography (HPTLC) combined with image analysis and pattern recognition methods were used for fingerprinting of phenolic compounds present in seedlings of two maize genotypes ZP 434 (new generation hybrid, drought tolerant) and ZP 704 (older generation hybrid, drought sensitive) treated with different concentrations of 24-epibrassinolide. This is the first report of TLC chromatographic profile of phenolics' mixtures in maize seed extracts influenced by brassinosteroid phytohormones. Nine samples of shoot of seedlings for the whole concentration range of phytohormones (5.2×10^{-7}–5.2×10^{-15} M), one sample of root of seedlings treated with 5.2×10^{-15} M 24-epibrassinolide, and the control samples of nontreated seedlings, for both genotypes, were analyzed. Phenolic profiles of root extracts indicate the absence of more polar compounds such as phenolic acids and glycosides present in shoot of seedlings. Also, hormones applied in higher concentrations have an inhibiting effect on the content of phenolics in ZP 434. Application of chemometric methods enables characterization of particular genotype of maize according to its phenolic profile.

1. Introduction

Phytochemical constituents found in plant-based foods such as secondary metabolites are known to have protective or disease preventive properties, acting as antioxidants, nutrient protectors, and anticarcinogens. Awareness of their various health and nutraceutical benefits increased the consuming of fruits, vegetables, and cereals. Comprehensive database on food phytochemicals and their health-promoting effects was published recently [1].

Cereal grains contribute to the total world food demand with more than 60% [2]. Among cereals, maize (*Zea mays*) is one of the commonly used in the human diet (maize flour), in animal nutrition and industrial production (starch, denatured alcohol, and lactic acid). This cereal, often referred to as "golden grain," is rich in carbohydrates and has low content of fat, which makes it a good source of energy. Besides being

a critical source of macro- and micronutrients, corn is also a rich source of many phytochemicals including carotenoids, phenolic compounds, anthocyanins, and tocopherols in human daily diets [3]. Numerous studies emphasize its health and nutraceutical benefits [4–7].

In order to increase the plant fitness, maize is often treated with different plant hormones, such as brassinosteroids, auxins, gibberellins, cytokinins, ethylene, abscisic acid, and jasmonic acids. Brassinosteroids (BRs) are natural plant growth regulators, present in low content in different organs (pollen, seeds, young vegetative tissue) in plant originating from all systematic groups. What sets BRs apart from other steroid hormones (acting in mammals and invertebrates, binding to nuclear receptors and direct activation of genes) is that their mechanism of action is associated with binding to receptors on the cell membrane, followed by a cascade of signaling and other metabolic processes up till gene activation [8].

Unlike other plant hormones and other physiologically active substances, BRs are effective in much lower concentrations (far below 10^{-6} M). Brassinosteroids influence every aspect of the growth and development of the plant, be it directly or indirectly, allowing the plant to reach its optimum form, taking into consideration the environmental conditions in which it is being grown [9]. In the context of the abovementioned, BRs mediate the response of plants to different stress factors, abiotic, biotic, and xenobiotic [10] and affect the secondary metabolism of plants [11].

In finding characteristic phytochemical patterns various qualitative and quantitative analytical methods are helpful, especially hyphenated techniques, which combine chromatographic and spectroscopic methods. High-performance liquid chromatography (HPLC) is usually a method of choice for such kind of studies. However, with development of high-performance adsorbent layers and sophisticated instrumentation for sample application, chromatogram development, derivatization, and chromatogram evaluation, high-performance thin-layer chromatography (HPTLC) became more popular [12]. The main advantages of HPTLC method over gas chromatography (GC) and HPLC are high sample throughput and a rapid low-cost analysis, due to the fact that many samples can be separated in parallel on the same plate; better precision and accuracy caused by simultaneous analysis of both samples and standards under the same conditions; and short time of analysis [13]. Careful choice of derivatizing agents in combination with chromatogram illumination under visible, 254- or 366-nm UV light, can tremendously enhance selectivity in visualization of target bands. Choosing appropriate scanning wavelength or storing information about colors by splitting a photo through red, green, and blue channel filter can further enhance selectivity [14].

One of the "flaws" of the method is the necessity of advanced knowledge of statistical procedures in order to extract the maximum amount of data from the vast quantity provided by HPTLC. Great amounts of information (variables or features) for a large number of samples (objects) require the use of chemometric procedures in order to efficiently extract the maximum useful information from the retention data. Based on the similarity/dissimilarity analysis or correlation matrix, a number of unsupervised and supervised chemometric methods could be performed [14, 15]. Choice of particular chemometric technique depends on its features and the nature of a problem to be solved.

In Serbia, maize is produced in significant amounts and is mainly used to produce flour, starch, and oil (extracted from the germs of ripe maize) for human nutrition, fodder for animal nutrition, and many other products. One of the most important abiotic stresses that seriously decreases final grain yield in maize is drought. Since the occurrence of drought is not predictable, breeders have to produce maize genotypes able to withstand stress and have stable yield under stressed conditions [16]. In that sense, two different maize hybrids, ZP 434 (new generation hybrid, drought tolerant) and ZP 704 (older generation hybrid, drought sensitive), were treated with different concentrations of 24-epibrassinolide in order to examine the influence of this plant hormone on

starting growth phase. The main goals of present paper are (a) determination of phenolic profile of maize extracts, (b) examination of the effects of different concentrations of phytohormones on the content of phenolic compounds, (c) the influence of the concentration of BRs on different part of the maize (shoot and root), and (d) characterization of particular genotype of corn.

2. Materials and Methods

2.1. Chemicals and Materials. Toluene and ethyl acetate were purchased from Merck (KGaA, Darmstadt, Germany). Formic acid and acetic acid were obtained from Zorka (Šabac, Serbia), while polyethylene glycol 4000 (PEG), and HPLC grade methanol was purchased from Sigma-Aldrich (Steinheim, Germany). 2-Aminoethyl diphenylborinate (NTS) was purchased from Fluka (Steinheim, Germany). All chemicals used for extraction procedure, for mobile phase composition, and for derivatization, whose purity is not previously emphasized, were of analytical purity grade. "Epin-Extra," as a commercial formulation of 24-epibrassinolide solution (produced by "NEST-M", Russia) consisted of 25 mg/L of 24-epibrassinolide, potassium metasilicate (0.1 g/L), wetting agents (PAV OP-7 or OP-10 PAV), and 500 mL/L of ethanol.

The SPE cartridges used for extraction and concentration of samples were Strata C18–E (500 mg/3 mL) obtained from Phenomenex (ThermoFisher Scientific). Ultrapure water (ThermoFisher TKA MicroPure water purification system, 0.055 μS/cm) was used to prepare extracts of maize. Syringe filters (13 mm, PTFE membrane 0.45 μm) were purchased from Supelco (Bellefonte, PA, USA).

2.2. Sample Preparation. Two different maize hybrids, ZP 434 and ZP 704 (obtained from the Maize Research Institute, Zemun Polje, Belgrade, Serbia), were treated with BRs of different concentrations.

2.2.1. Sample Treatment. Seeds (4×50) were germinated in plastic boxes, on filter paper sheets, topped at the beginning of experiment with 60 mL of "Epin-Extra" solution in concentrations: 5.2×10^{-7} M, 5.2×10^{-8} M; 5.2×10^{-9} M, 5.2×10^{-10} M, 5.2×10^{-11} M, 5.2×10^{-12} M, 5.2×10^{-13} M, 5.2×10^{-14} M, and 5.2×10^{-15} M of 24-epibrassinolide, under germination room conditions, at 30°C (day) and 20°C (night), with an 12 h light regime (110–160 μmol photons m^{-2} s^{-1})/12 h dark regime. After seven days, 4×25 uniformly grown seedlings were chosen for further analysis and separated to root and shoot. Samples were dried at 130°C in ventilation dryer and then milled on Perten 120 mill, Sweden. Working samples were achieved by mixing of four replications of 25 roots or shoots and they were kept in refrigerator at 4–8°C until chemical analysis.

2.2.2. Sample Preparation for TLC. Free phenolic compounds were extracted according to the following procedures.

Method 1. Approximately 1 g of sample was blended with 5 mL of ethanol-water (80 : 20, v/v) for 10 min using ultrasonic bath. The homogenate was centrifuged at 4000 rpm for

15 min. After centrifugation the supernatant was removed and extraction of the residue was repeated three times. The supernatants were pooled and then vacuum-evaporated to dryness at 45°C. The dry residue was diluted with 2 mL of water and these extracts were frozen and stored at 4°C until analysis.

Method 2. Approximately 1 g of sample was weighed and suspended in 10 mL of methanol containing 1% HCl, shaking for 2 h at ambient temperature, and centrifuged for 15 min at 4000 rpm. The extracts were combined and vacuum-evaporated to dryness at 40°C. The resulting precipitate was resuspended in 2 mL of 1% HCl/MeOH solvent and the extracts of supernatant fluid were kept at 4°C in the dark until further analysis.

Method 3. Approximately 1 g of sample was weighed and homogenized with 10 mL of 50% (v/v) aqueous methanol acidified with 0.1% HCl. Homogenized samples were extracted for 24 h at ambient temperature. The solution was purified through a C18 SPE cartridge, previously activated with 3 mL of methanol and 9 mL of ultrapure water. After sample introduction, the cartridge was washed with 6 mL of ultrapure water to remove all sugars and other polar constituents of corn. Phenolic fraction was eluted with 1.5 mL of methanol. The extracts were stored at 4°C until analysis.

Method 4. Approximately 0.5 g of sample was homogenized with 10 mL of 70% (v/v) acetone, ultrasonicated for 30 min at ambient temperature and centrifuged at 4000 rpm for 20 min. After centrifugation the supernatant was removed and extraction of the residue was repeated three times. The combined supernatants were vacuum-evaporated to dryness and dissolved in 5 mL of methanol. The extracts were stored at 4°C until analysis.

2.3. High-Performance Thin-Layer Chromatography. The 2 μL of maize extracts were applied in the form of 8 mm bands to the 20×10 cm silica gel HPTLC plates (Art. 5641, Merck, Darmstadt, Germany) by using Automatic TLC sampler 4 (ATS4, CAMAG, Muttenz, Switzerland). Development of chromatograms was performed with a mixture of toluene-ethyl acetate-formic acid (4 : 7 : 1, v/v/v) for less polar compounds (chromatographic system 1 (CS1)) and ethyl acetate-water-formic acid (17 : 2 : 2, v/v/v) for medium and highly polar compounds (chromatographic system 2 (CS2)), in the Twin Trough Chamber (CAMAG) saturated for 20 min (lined with filter paper). Developing distance was set to 80 mm and postdevelopment drying time was 2 min. Afterwards the plates were heated for 3 min at 100°C on TLC Plate Heater III (CAMAG), they were instantly dipped in 0.5% solution of NST in ethyl acetate for 1 s, by using Chromatogram Immersion Device III (CAMAG). In order to enhance and stabilize the fluorescent zones, after 5 min of air-drying, the plates were immersed in 5% solution of PEG 400 in dichloromethane for 1 s. Image capturing was performed at 366 nm with CAMAG video documentation system in conjunction with Reprostar 3 (CAMAG). Four apertures with exposure time of 30 ms and frame of −2 mm were applied. The photos were stored as TIF files for further image processing.

2.4. Data Acquisition and Statistical Analysis. Images of the plates were processed with the ImageJ processing program (http://imagej.nih.gov/ij/, ver. 1.47q, Rasband W. National Institutes of Health, USA) as it was described in our previous article [17]. Denoising of the images was done using 2 pixels median filter. Differences of the background intensity between images were not confirmed and baseline removal step was skipped. Normalization of the images was performed by scaling each sample to sum of intensity. The warping of the images was done with correlation optimized warping (COW) algorithm implemented in the PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a) (http://www.eigenvector.com/software/pls_toolbox.htm, Eigenvector Research, Inc., Wenatchee, W A 9880). The data were additionally preprocessed by using mean centering, which is the preferred option when the classification of samples is based on variables that are all measured in the same unit.

Principal component analysis (PCA) was carried out by the means of PLS ToolBox. A PCA was performed as an exploratory data analysis by using a singular value decomposition algorithm (SVD) and a 0.95 confidence level for Q and T^2 Hotelling limits for outliers.

3. Results and Discussion

The influence of different concentrations of 24-epibrassinolide (in concentrations 5.2×10^{-7}–5.2×10^{-15} M) on germination and starting growth phase of maize seeds of two different genotypes, through the content of phenolic compounds, was examined. The research involved the optimization of extraction methods of phenolics from maize samples, their separation, and evaluation using a TLC, processing of the chromatographic images and multivariate analysis of data.

3.1. Optimization of Method for Phenolics Extraction. Optimization of method for extraction of free phenolic compounds was performed on samples of maize seeds treated with 5.2×10^{-15} M of 24-epibrassinolide, simultaneously for both genotypes. Extracts were prepared according to the methods described in Experimental part (Methods 1–4) and subsequently analyzed by HPTLC using chromatographic conditions for the determination of phenolics in food samples previously described in [18]. The optimal results that include the largest number of chromatographic zones for both maize genotypes and both classes of phenolic compounds were obtained for Method 1. According to this, 9 samples of shoot for the whole concentration range of phytohormones, one sample of root treated with 5.2×10^{-11} M 24-epibrassinolide, and the control samples of nontreated seedlings, for both genotypes were prepared. Only one sample of root of maize seedlings was taken for analysis, because its weight in proportion to the mass of the rest of the seeds and shoot was significantly lower, and thus the concentration of 24-epibrassinolide in that part of the corn seedling was probably much higher and consequently induces changes in the contents of phenolics in this seedling parts. It was observed in preliminary experiments (data not shown) that the total
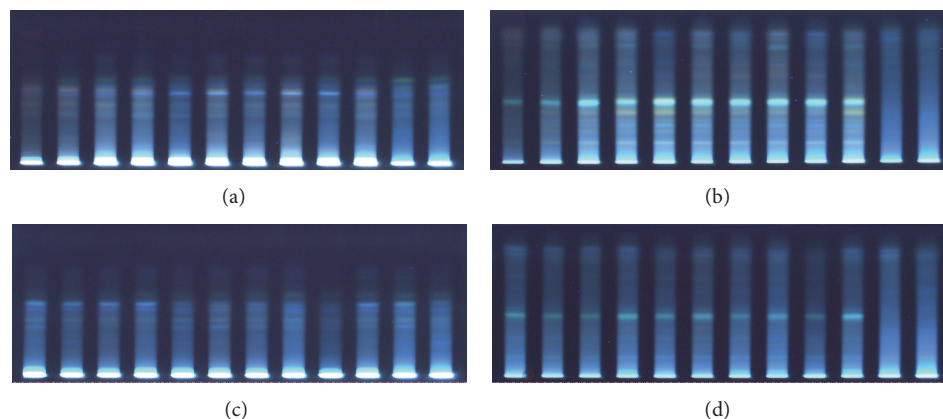
(a)

(b)

(c)

(d)

FIGURE 1: HPTLC chromatograms of maize extracts for ZP 434 (a, b) and ZP 704 (c, d) hybrids obtained in CS1 and CS2, respectively. Chromatograms sequence on each plate corresponds to sample order in Table 1.

TABLE 1: Maize samples.

Number	Maize	Label
1	Shoot treated with 5.2×10^{-7} M of BRs	S-7
2	Shoot treated with 5.2×10^{-8} M of BRs	S-8
3	Shoot treated with 5.2×10^{-9} M of BRs	S-9
4	Shoot treated with 5.2×10^{-10} M of BRs	S-10
5	Shoot treated with 5.2×10^{-11} M of BRs	S-11
6	Shoot treated with 5.2×10^{-12} M of BRs	S-12
7	Shoot treated with 5.2×10^{-13} M of BRs	S-13
8	Shoot treated with 5.2×10^{-14} M of BRs	S-14
9	Shoot treated with 5.2×10^{-15} M of BRs	S-15
10	Shoot-control	Sc
11	Root-control	Rc
12	Root treated with 5.2×10^{-11} M of BRs	R-11

mass of the seedlings, in particular mass of corn radicle in germinating seeds with higher concentrations of 24-epibrassinolide, was significantly lower compared to control and seedling germinated with lower concentrations of 24-epibrassinolide, suggesting a nonphysiological, phytotoxic effect of mentioned phytohormones, especially in root of the seedling of investigated genotypes of maize. It is known from [19] that the root of plants is more sensitive to the effects of brassinosteroid phytohormones compared to the shoot, but when we set up experiments it was not possible to assume such a distinct physiological response of used maize genotypes. Therefore the analysis of the content of phenolics in that part of seedling corn would give results that would not correspond to the natural physiological response of maize seedlings on the presence of brassinosteroids. In that sense, analysis of phenolics in root sample was served as a demonstration of the process of accumulation of phenolics in that part of maize seedlings under the influence of a 24-epibrassinolide. Analyzed samples are listed in Table 1.

3.2. HPTLC Phenolic Profile of Maize Extracts. Maize extracts are complex mixture that contains vast number of compounds which are very difficult to analyze by separation and

evaluation of all the constituents. In that sense, instead of focusing on individual compounds, a set of characteristic chromatographic signals could be used for comparison which leads to sample recognition. The entire chromatogram is treated as unique multivariate fingerprint, that is, multi-dimensional vector, without special identification of single peaks. For an assay of quality of maize depending on the type of hybrids and concentration of applied plant hormones, TLC chromatographic profile of its phenolics' mixtures was taken into consideration.

An HPTLC fingerprint of maize extracts was performed using chromatographic conditions previously developed by authors [17], with some modifications needing to adapt the method to the nature of analyzed matrix. Due to the complexity of maize extracts, HPTLC conditions were optimized in order to provide better separation of low, medium, and highly polar phenolic compounds. The experiment included two different chromatographic systems, CS1 and CS2, for fractions of different polarity.

Application of CS1 indicates the existing of a large number of more polar compounds which were strongly adsorbed on the silica gel (Figures 1(a) and 1(c)). In addition, a smaller number of more nonpolar flavonoids, such as flavonols, flavanones, and isoflavonoids, appear at higher R_F values with pattern dominated by orange, blue, and green colored zones. Profiles of less polar compounds were quiet different for extracts of two hybrids, while within the same genotype shoot and root profile were similar. Different intensities of certain zones could also be observed depending on the concentration of hormones used.

In order to better define the chromatographic profile of the more polar compounds present in maize extracts, second system, which contained a mobile phase with higher elution power, was applied. Application of CS2 resulted in chromatograms with vast number of sharp bands (Figures 1(b) and 1(d)). Mobile phase with relatively high elution power enables separation of the medium and very polar components, mainly polar phenolic acids, such as chlorogenic, ellagic, and gallic acids, as well as various flavonoid aglycones, apigenin, quercetin, kaempferol, and glycosides [20, 21]. All samples have a pattern dominated by one blue colored

zone on the R_F values of approximately 0.5, which could be considered as a characteristic feature of examined corn hybrids. Chromatograms of ZP 434 genotype beside previously mentioned zone which is more pronounced compared to ZP 704 also contained several less intensive orange zones on the lower R_F values which could be attributed to very polar glycosides. In addition, the content of more polar components is noticeably different in the extracts of shoot and root. Chromatograms of root extracts do not contain more polar compounds. These facts suggest that the same phytohormone, in two different organs of maize seedlings (radicle and shoot), induces the synthesis of various phenolic fractions. Although these data can be interpreted as a lack of discrimination of methods of chemical extraction and separation of phenolics from different organs of maize seedlings, we believe that these data can be interpreted primarily as induction of different synthetic routes for phenolics, due to various physiological functions of these compounds in different organs of maize seedlings. Similarly, the young leaves of plants contain higher content of some polyphenolic acids (e.g., ferulic acid [22]), flavonoids, and hydroxycinnamates [23], as well as anthocyanins [24], which all have antioxidant and protective function, up to obtaining full photosynthetic competence of leaves. Analysis of the impact of brassinosteroids on secondary metabolism of plants, in particular the synthesis of phenolics [11] is poorly documented in the literature, especially from the point of optimization of analytical methods.

Although chromatographic profile provided information regarding phenolics' composition, still it is not possible to ambiguously select the factors that are able to decidedly characterize the type of hybrids and the influence of concentration of brassinosteroids. In order to further analyze the obtained results, multivariate image analysis and pattern recognition method were applied.

3.3. Chromatograms Processing and Data Analysis. Stored images were split through RGB channels to increase selectivity and differentiate compounds according to their fluorescent colors [25]. All zones corresponding to phenolic compounds after derivatization give intensities of colors of the red (R), green (G), and blue (B) channels under 366 nm. This process increases selectivity and differentiates compounds according to their fluorescent colors. Differently colored phenolic compounds of maize, earlier not enough separated, could be evaluated by using channels of different color. Images at certain channel were processed with ImageJ software and raw data were exported for further chemometric data handling. The line profile plots of chromatograms obtained for CS2, for control maize samples of particular genotype, and adjusted to its three RGB channels are presented in Figure 2. The color value of a given point from the chromatographic plate depends on the channel at which it was observed. For the analyzed ZP 434 hybrid, the highest amount of information could be collected by using the blue channel for CS1 and green channel for CS2, and in the case of ZP 704 hybrid blue channel provides maximum information for both chromatographic systems. Previously mentioned channels were chosen for further analysis due to

better defined peaks, higher number of peaks, and higher intensity in comparison to the other two channels.

Information that was collected by decomposing a peak of certain colored zone into three components was used for observing the influence of concentration of BRs on phenolic profile of maize, on the one hand, and selection of compounds that are most suitable for characterization of particular genotype of maize, on the other hand.

Chromatographic profiles of shoot control (Sc) and root control (Rc) samples of both hybrids indicate differences in content of phenolic compounds when the development of chromatograms is conducted by CS2 (Figures 3(a) and 3(b)), contrary to the profiles obtained with CS1, which are almost identical (Figures 3(c) and 3(d)). Namely, root samples did not contain more polar compounds such as phenolic acids, aglycones, and glycosides, while the content of less polar flavonoids was identical but they were present in a smaller amount.

In shoot samples of ZP 434 treated with hormone concentration of 5.2×10^{-7} and 5.2×10^{-8} M, lower content of phenolics was noted, in comparison to the control sample. The samples treated with lower brassinosteroids' concentrations (5.2×10^{-9}–5.2×10^{-15} M) showed phenolic profile similar to the control shoot sample, pointing to the fact that hormone applied in higher concentrations has an inhibiting effect on the content of phenolics (Figure 3(e)). This trend was observed in both chromatographic systems. In shoot samples of ZP 704 hybrid treated with brassinosteroid type of phytohormone in concentration of 5.2×10^{-15} M the lowest content of phenolics was noted, while shoot sample with 24-epibrassinolide concentration of 5.2×10^{-14} M gave the profile with its highest content. Control samples, as well as shoot samples with other concentrations of phytohormone, were located between these two boundary values (Figure 3(f)). This trend was observed in both chromatographic systems used. In addition, it was determined that there is no difference in phenolics' content in control root sample and root sample from treatments with phytohormones.

In order to select compounds that are most suitable for characterization of particular genotype of maize PCA was applied on the matrix obtained by digitization of chromatograms (24 samples × 452 variables), for each channel in two chromatographic systems separately. Variables represent the intensities of pixels along the 452 length lines. Before applying the chemometric analysis, proper preprocessing of the signals was performed as is pointed out in Experimental part.

Comparing the obtained classification and the percent of total variances captured by all PCA models, it could be concluded that the best results were obtained by using blue channel of profiles obtained with CS1 and CS2. In that sense, we would present only these results. A PCA performed on data obtained with CS1 resulted in a four-component model which explains 94.34% of total variance. The first principal component, PC1, accounted for 67.57% of the overall data variance, and the second one, PC2, for 12.65%. Mutual projections of factor scores and their loadings for the first two principal components (PCs) are presented in Figure 4. Taking into account PC1 and PC2 score values (Figure 4(a)) two
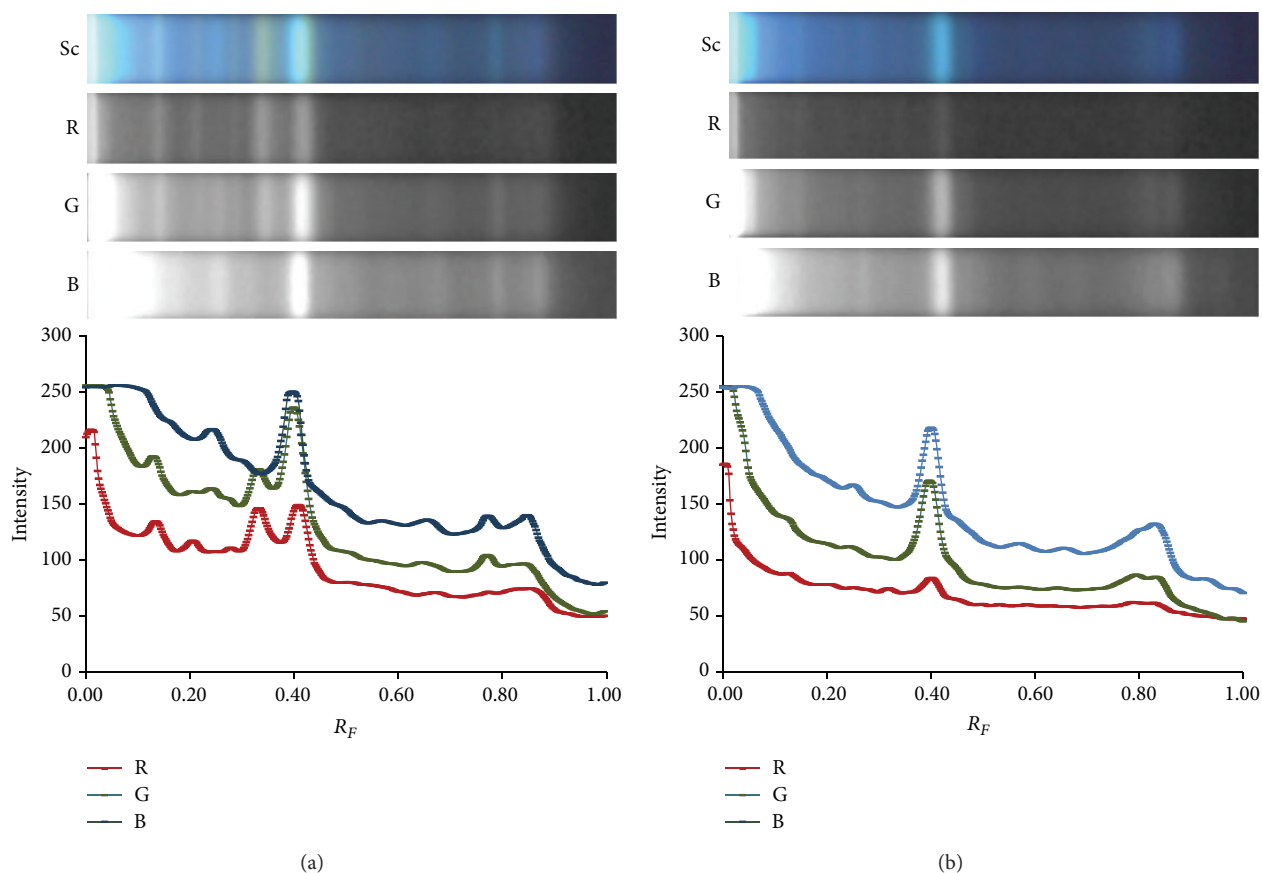
FIGURE 2: The line profile plots of chromatograms of shoot control samples of ZP 434 (a) and ZP 704 (b) hybrids obtained in CS2 and split through RGB channels (R: red, G: green, and B: blue). Labels on score plot correspond to those in Table 1.

distinctive groups corresponding to different corn hybrids are obtained. Within each group shoot and root samples are overlapped, demonstrating the similarity of content of less polar phenolic compounds in two parts of maize. Samples of ZP 434 hybrid are firmly clustered, exhibiting small internal variability. The samples of the other genotype are dissipated in a broader range of the PC1-PC2 score space, pointing out the higher variability among data. Two samples of ZP 434 hybrid from treatments with higher concentration of 24-epibrassinolide (5.2×10^{-7} and 5.2×10^{-8} M) and one sample of ZP 704 hybrid from treatment with 5.2×10^{-15} M of plant hormone are separated from the rest of the samples indicating their different content of phenolics compared to others. The corresponding loadings plot displays relationships between variables and can be used to identify variables that contribute to the positioning of the objects on the scores plot and hence influence any observed groups in the data set. The loading plots (Figures 4(b) and 4(c)) reveal that the zones with R_F values 0.07, 0.16, 0.46, and 0.52 are variables that have the most positive impact on PC2 direction and differentiate maize samples according to the genotype. Zones with R_F values 0.23, 0.37, 0.44, and 0.49 significantly affect the PC2 in a negative manner. Small influence of variables with 0.10, 0.33, 0.47, and 0.52 R_F values on PC1 was also observed.

A PCA performed on data obtained with CS2 resulted in a four-component model which explains 95.41% of total variance. The first principal component, PC1, accounted for 51.30% of the overall data variance and the second one, PC2, for 33.42%. Mutual projections of factor scores and their loadings for the first two principal components (PCs) are presented in Figure 5. Two groups corresponding to different maize hybrids are separated alongside the PC1 (Figure 5(a)). Within each genotype root samples are away from shoot samples, demonstrating dissimilarity of content of more polar phenolics in two parts of plant which is especially pronounced in ZP 434 hybrid. Two samples of ZP 434 hybrid from treatment with higher concentration of 24-epibrassinolide (5.2×10^{-7} and 5.2×10^{-8} M) are, again, separated from the rest of the samples indicating their different phenolics content compared to others. The loading plots (Figures 5(b) and 5(c)) reveal that the zones with R_F values 0.29, 0.36, and 0.41 are variables that have the most positive impact on PC1 direction and differentiate maize samples according to the genotype. Zones with R_F values 0.41 and 0.90 significantly affect the PC2 in a positive manner.

Variables that were marked as important for determination of particular genotype of maize for both chromatographic systems implied the significance of polar and
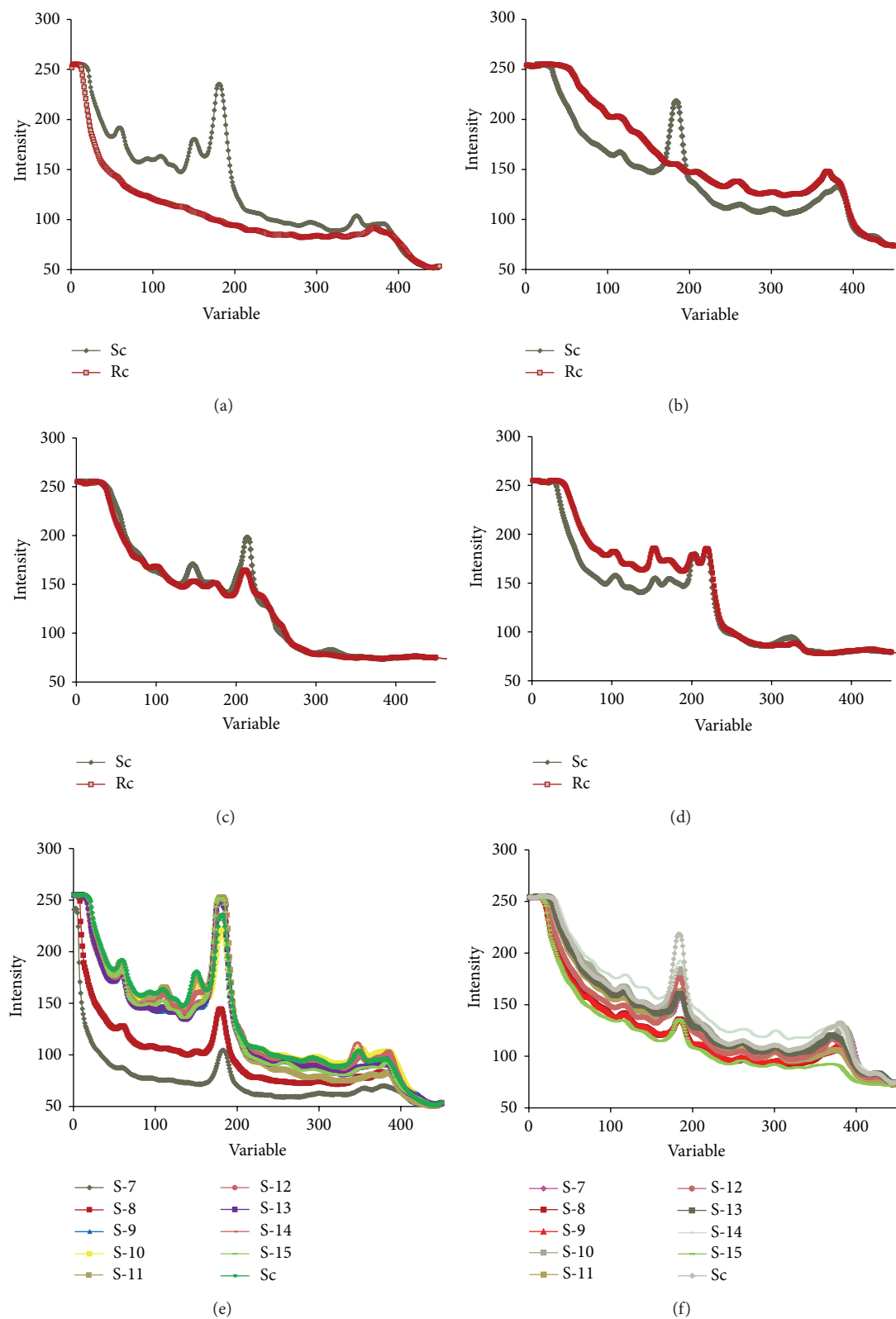
FIGURE 3: Phenolic profiles of shoot and root control samples for ZP 434 (a, c) and ZP 704 (b, d) hybrids obtained in CS2 and CS1, respectively, and shoot samples of ZP 434 (e) and ZP 704 (f) hybrids treated with brassinosteroid type of phytohormone in concentration of 5.2×10^{-7}–5.2×10^{-15} M. Labels on score plot correspond to those in Table 1.
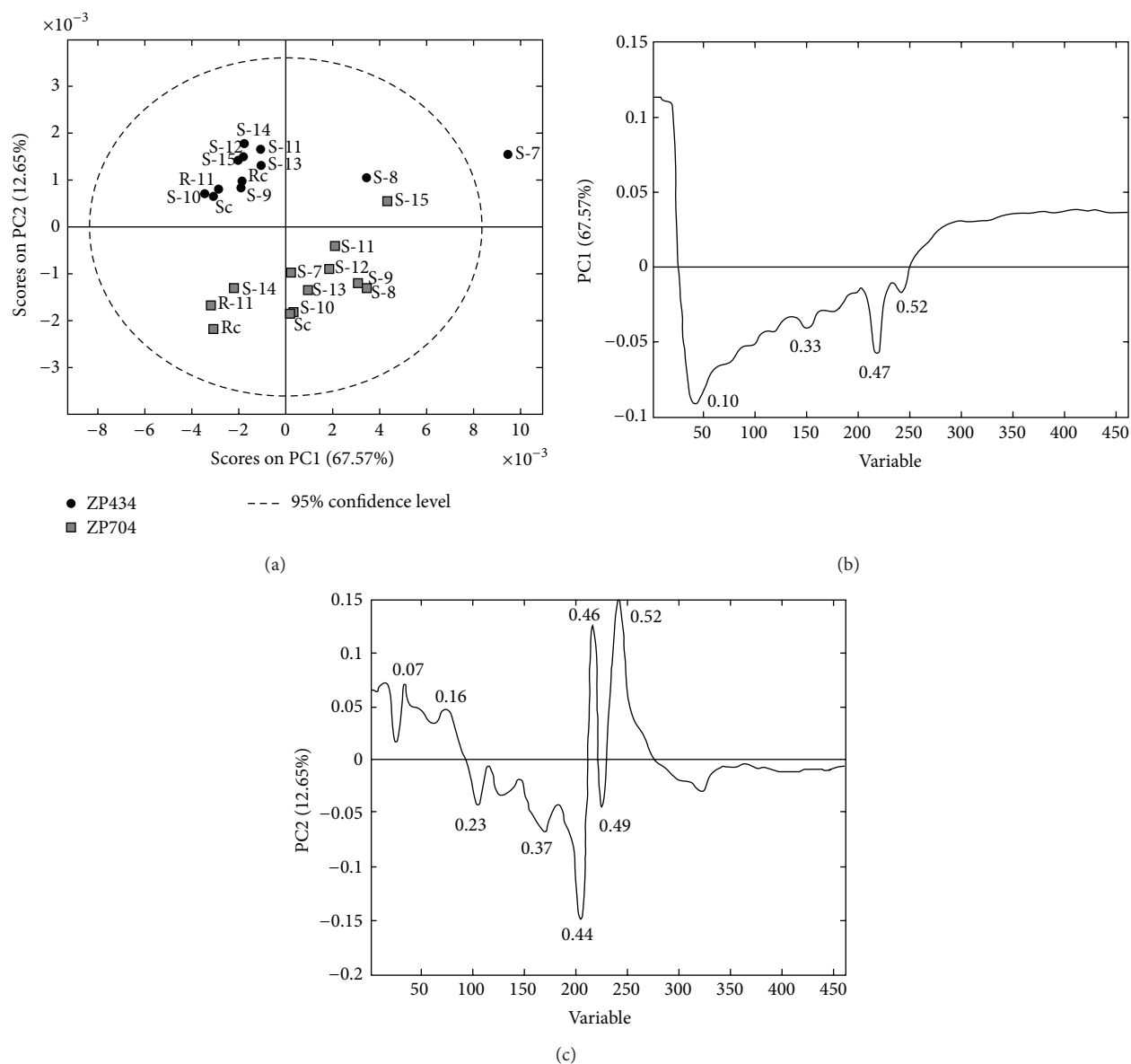
(a)

(b)

(c)

Figure 4: PCA analysis performed on data obtained in CS1, (a) mutual projections of factor scores, and (b, c) loadings for the PC1 and PC2, respectively, for a model corresponding to blue channel. Labels on score plot correspond to those in Table 1.

medium polar phenolic compounds present in maize extracts.

4. Conclusion

An efficient and reliable fingerprint TLC method combined with image analysis and pattern recognition methods was developed in order to determine phenolic compounds present in seedlings of two maize genotypes ZP 434 (new generation hybrid, drought tolerant) and ZP 704 (older generation hybrid, drought sensitive). Two seedling parts, shoot and root, were exposed to the different concentrations of 24-epibrassinolide (5.2×10^{-7}–5.2×10^{-15} M) during germination in order to examine the influence of this plant hormone on starting growth phase.

HPTLC conditions were optimized in order to provide better separation of low, medium, and highly polar phenolic compounds by using two different chromatographic systems. Profiles of less polar compounds were quiet different for extracts of two hybrids, while within the same genotype shoot and root profiles were similar. In addition, ZP 434 hybrid contained very polar compounds, such as glycosides which is more pronounced in comparison to ZP 704 hybrid, and root extracts were characterized with the absence of more polar compounds compared to shoot. Hormones applied in higher concentrations (5.2×10^{-7} and 5.2×10^{-8} M) have an inhibiting effect on the content of phenolics in new generation hybrid. Application of chemometric methods enables characterization of particular genotype of maize according to its phenolics profile. Variables that were marked as important for such determination implied the significance of polar
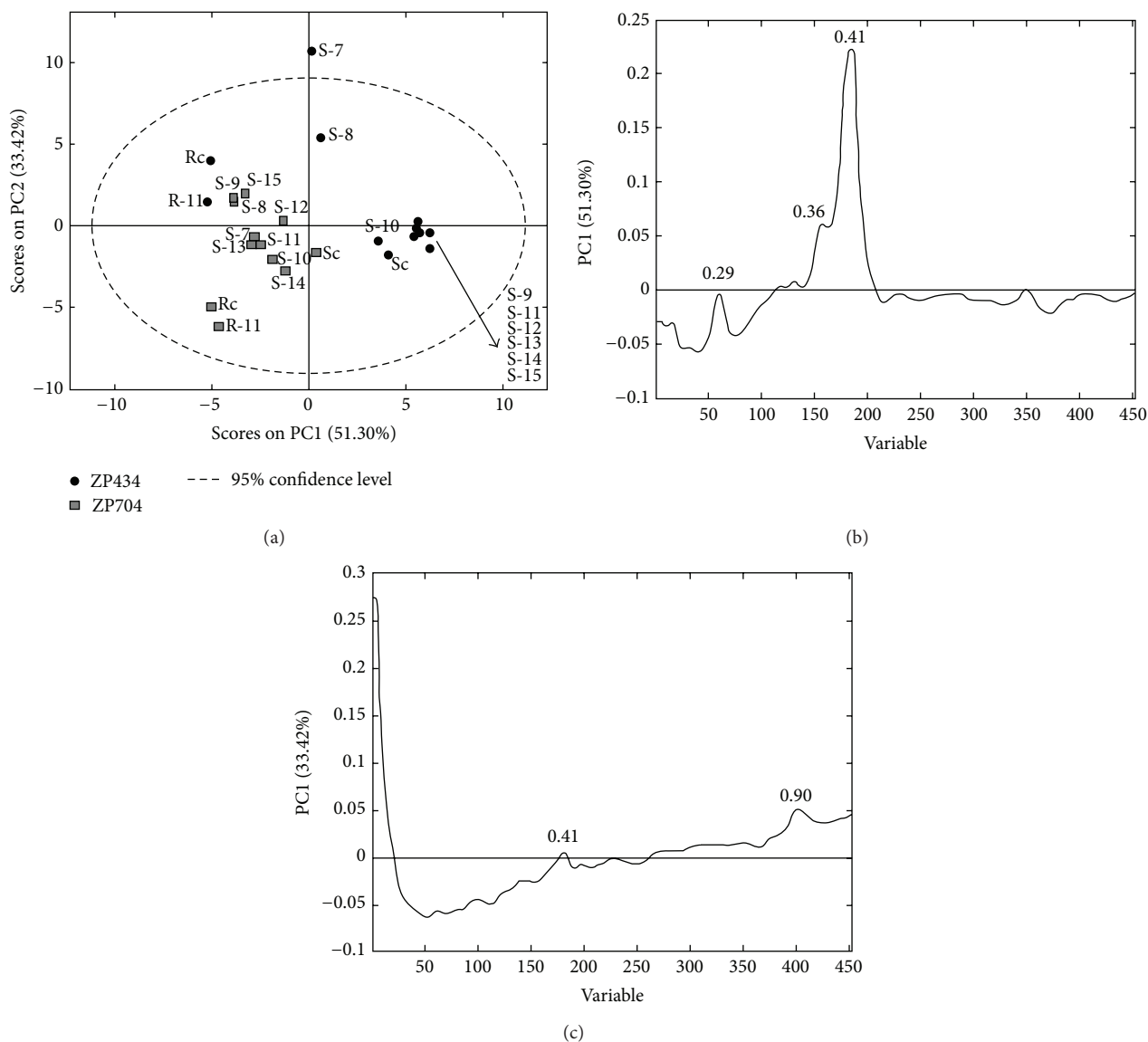
(a)

(b)

(c)

FIGURE 5: PCA analysis performed on data obtained in CS2, (a) mutual projections of factor scores, and (b, c) loadings for the PC1 and PC2, respectively, for a model corresponding to blue channel. Labels on score plot correspond to those in Table 1.

and medium polar phenolic compounds present in maize extracts.

This is a first report of TLC chromatographic profile of mixtures of phenolics in maize extracts originating from seedlings treated by brassinosteroid type of phytohormones.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

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