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Non-targeted Metabolomic Profiling of Maize Landraces (*Zea mays* L.) Combined with Chemometric Tools

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Authors' contributions

This work was carried out in collaboration between all authors. Author VGU designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors MR and MM supervised, corrected the draft and contributed in data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Grain samples of maize landraces were collected and subjected to Fourier Transform Infrared spectroscopy (FTIR) analysis combined with chemometric tools in other to discriminate them regarding the chemical composition. Principal component analysis (PCA) and hierarchical clustering (HCA) were applied on selected peaks of the spectral data. The most important chemical groups found in all maize landrace samples were monoterpenes, sesquiterpenes, tetraterpenes, aminoacids, polysaccharides, lipids and proteins. Trace signals of secondary metabolites were also found in cultivars according to year of harvest.

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1. INTRODUCTION

Commonly referred to as maize or corn, *Zea mays* ssp. *mays* is one of the world's most important crop plants, achieving a multibillion dollar annual revenue. In addition to its agronomic importance, maize has been a keystone model organism for basic research for nearly a century [1-2]. According to Dwivedi [1], the current industrial agriculture system may be the single most important threat to maize biodiversity. A serious consequence of biodiversity loss is the displacement of locally adapted maize landraces with adaptation traits to future climates by mono-cropping with genetically uniform hybrids and improved cultivars.

Maize landraces represent heterogeneous, local adaptations of domesticated species, and thereby provide genetic resources that meet current and new challenges for farming in stressful environments. These local ecotypes can show variable phenology and low-to-moderate edible yield, but are often highly nutritious. The main contributions of maize landraces to plant breeding have been traits for more efficient nutrient uptake and utilization, as well as useful genes for adaptation to stressful environments such as water stress, salinity, and high temperatures [1, 3-5].

Systematic maize landraces evaluation may define patterns of diversity, which will facilitate identifying alleles for enhancing yield and abiotic stress adaptation, thus raising the productivity and stability of staple crops in vulnerable environments [1, 3-5]. In other hand, regarding the evaluation tools, metabolomics has recently been claimed as a promising concept and valuable tool in biotechnology, given its extensive range of applications in functional genomics and, more globally, in the characterization of biological systems [6]. Indeed, the possible tasks include studying metabolic systems, measuring biochemical phenotypes, understanding and reconstructing networks and discriminating between samples [6]. In the targeted metabolomics approach, specific metabolites of known identity are profiled; good quantitative precision is typically obtained. On the other hand, untargeted metabolomics aims to simultaneously measure as many metabolites as possible in a biological specimen [7].

Taking into account that maize landraces are currently being replaced by new improved cultivars with a narrow genetic basis in Brazilian agriculture and with a purpose to add value of those important genetic materials, the present study was designed with the main goals of investigating the chemical composition of maize landraces using non-targeted metabolomic approach (Fourier Transformed Infrared Spectroscopy-FTIR) combined with chemometric tools (PCA and HCA)as a rapid diagnostic tool to ascertain changes in chemical composition.

2. MATERIALS AND METHODS

2.1 Maize Landraces

Maize landraces (*Zea mays* L.) were kindly provided by small farmers of the Anchieta County (the western part of Santa Catarina state, southern Brazil, 26°31′11″ S, 53°20′26″ W) and produced under agro-ecological management practices during three different harvests (2007, 2008, 2009) Table 1 presents the details of the cultivars used each year). Maize grains were collected, dried and crushed to obtain fine powder for later analysis by Fourier transform infrared spectroscopy (FT-IR).

2.2. Flour Sample Preparation

Maize grains (250g – dry weight) were selected from each maize genotype and ground to pass a 0.5mm sieve using a laboratory cyclone mill (MB Braeski C.Q).

2.3 Fourier Transform Infrared Spectroscopy

FTIR spectra of maize flours were recorded in a Bruker IFS-55 (Model Opus v. 5.0, Bruker Biospin, Germany) spectrometer with a DTGS detector equipped with a golden gate single reflection diamond attenuated total reflectance (ATR) accessory (45° incidence-angle). A background spectrum of the clean crystal was acquired and samples (100 mg) were spread and measured directly after pressing them on the crystal. The spectra were recorded at the absorbance mode from 4000 to 500 cm^{-1} at the resolution of 4cm^{-1}. Five replicate spectra were collected for each sample. For pre-processing, the spectra were normalized, baseline-corrected in the region of interest by drawing a straight line before resolution enhancement (*k* factor of 1.7). The assumed line shape was Lorentzian with a half width of 19 cm^{-1} [8-11].

Table 1. Maize landrace cultivars studied in this research during the three years

Year	Maize cultivar analyzed	Acronym used
2007	Cultivar7	CULT7
	Cultivar10	CULT10
	Cultivar11	CULT11
	Cultivar12	CULT12
	Cultivar13	CULT13
	Cultivar15	CULT15
	Cultivar17	CULT17
2008	Lingua De Papagaio	LP0
	Mato Grosso-Palha Roxa	MG0
	Movimento De Pequenos Agricultores	MPA10
	Palha Roxa	PR0
	Rajado 8 Carreiras	R8C0
	Rajado	RJ0
	Roxo	RX0
	Roxo De Emílio	RXE0
2009	Lingua De Papagaio	LP1
	Mato Grosso-Palha Roxa	MG1
	Movimento De Pequenos Agricultores	MPA11
	Palha Roxa	PR1
	Rajado 8 Carreiras	R8C1
	Roxo	RX1
	Roxo De Emílio	RXE1

2.4 Chemometric Analysis

FT-IR spectra were acquired and subjected to chemometric analysis using the R software [12]. Non-targeted metabolomics was used to find variations in the chemical composition of different cultivars, cultivated under different harvests and environmental conditions. FT-IR spectra were acquired in transmittance mode and then transformed to absorbance mode, as spectra objects in the ChemoSpec package [13] using the equation 1.

$$\left[A = -\log 10\left(T\right) \right] \quad \text{(Eq. 1)}$$

where A is the absorbance and T the transmittance

The spectra object was then converted to a hyperSpec [14] object using the bridging package hyperChemoBridge [15]. Before applying chemometric tools, the spectra were baseline corrected, intensity vector normalized and smoothed for subsequent analysis of PCA and HCA [6] for sample classification according to their biochemical status.

3. RESULTS AND DISCUSSION

3.1 Peak Selection and Multivariate Analysis

Results of the assignments for the most characteristic FTIR bands found in maize landraces and related chemical group are summarized in Table 2. The most important chemical groups found in all maize landrace samples were monoterpenes, sesquiterpenes, tetraterpenes, aminoacids, polysaccharides, lipids and proteins (Table 2).

The intensity of peaks is also shown in the Fig. 1 (A-C) for maize samples from 2007, 2008 and 2009 respectively. The selected peaks found in Fig. 1 were subjected for further multivariate analysis aiming to find similarities or differences between maize samples.

Principal component analysis of the 2007 maize landraces shown in the Fig. 2A. Differences between cultivars were observed. Cultivars (11 and 12) and (7, 17) grouped together. The total variance explained by the first 2 PCs was 94.4%, being 80.42% for PC1 and 13.98% for PC2. Cultivars 7 and 17 grouped together duo to similarities in amylose, amylopectin and lutein contents. Cultivar 10 grouped alone duo it's content in fatty acids.

PCA of 2008 samples is represented in the Fig. 2B. PC1 explained 43.09% and PC2 39.81%, respectively. A good separation was found between PRO, MPA10 and LP0 cultivars. PRO was most correlated to lipids or fatty acids and LP0 correlated to amylose, amylopectin, monoterpenes and proteins. PCA on 2009 maize samples is represented in Fig. 2C. PC1 explained 62.64% and PC2 (27.31%) of the total variance. The loading plot indicated that cultivars MG1, LP1, RX1 and RXE1 are similar in chemical composition duo to their values of amylose, amylopectin and fatty acids. Cultivars R8C1 and PR1 showed to be similar duo to their lower levels of proteins and lipids.

When FTIR data of 2007, 2008 and 2009 were subjected to hierarchical clustering (Fig. 3A-C) aiming to find similarities or differences between samples and look for important wave bands related to sample clustering, an interesting result can be observed and the main bands responsible for sample differences were found. Differences in the 2007 dataset were mainly due

to the region of carbohydrates (998, 1012 and 3000). Cultivar 15 grouped alone, and the most similar in chemical composition were cultivars 7 and 17, 11 and 12 respectively (Fig. 3A).

For samples of 2008 harvest, the region of proteins, amylose and amylopectin was most important in sample dissimilarity. PR0 and MPA10 were the most dissimilar cultivars (Fig. 3B). Two visible groups were found; being the first composed by LP0 and RXE0 and second group by RX0, MG0, RJO and R8C0. For maize landraces of2009 (Fig. 3C), proteins derived the sample clustering. MPA11 and LP1 were the most dissimilar cultivars confirming that observed previously in PCA analysis.

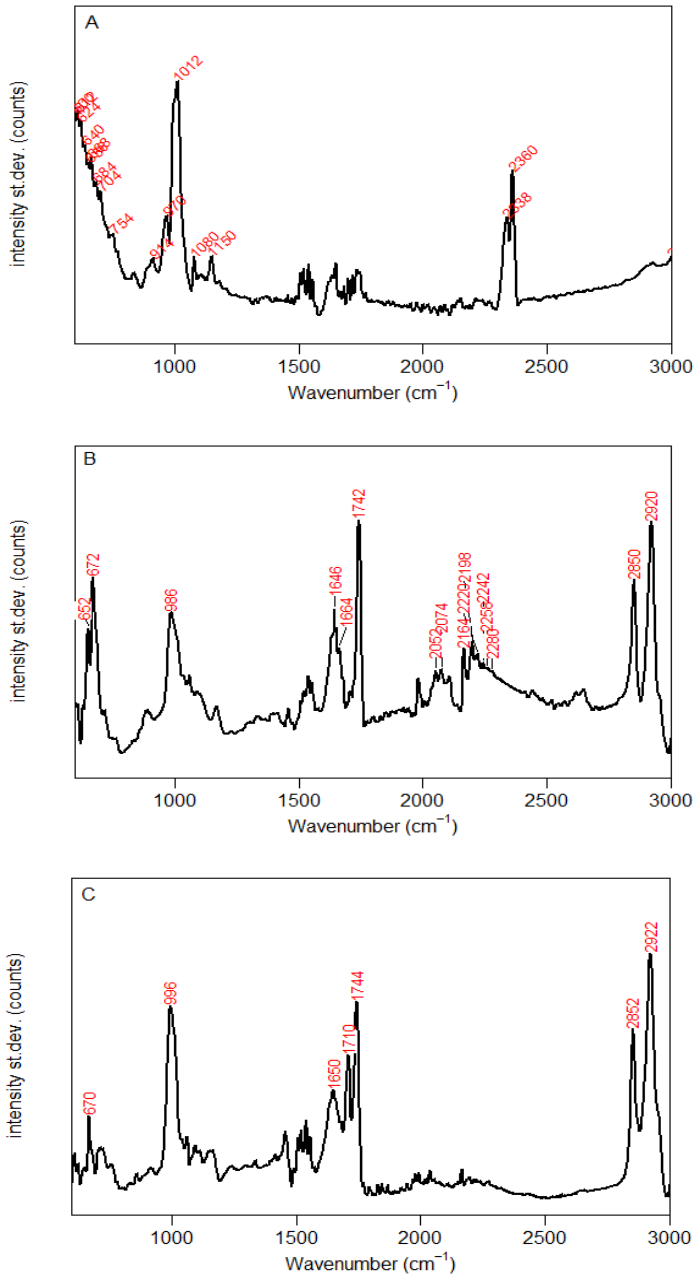


Fig. 1. Main peaks found in FTIR spectra of (A) maize landraces produced in 2007, (B) 2008, (C) maize landraces produced during 2009 harvest, respectively

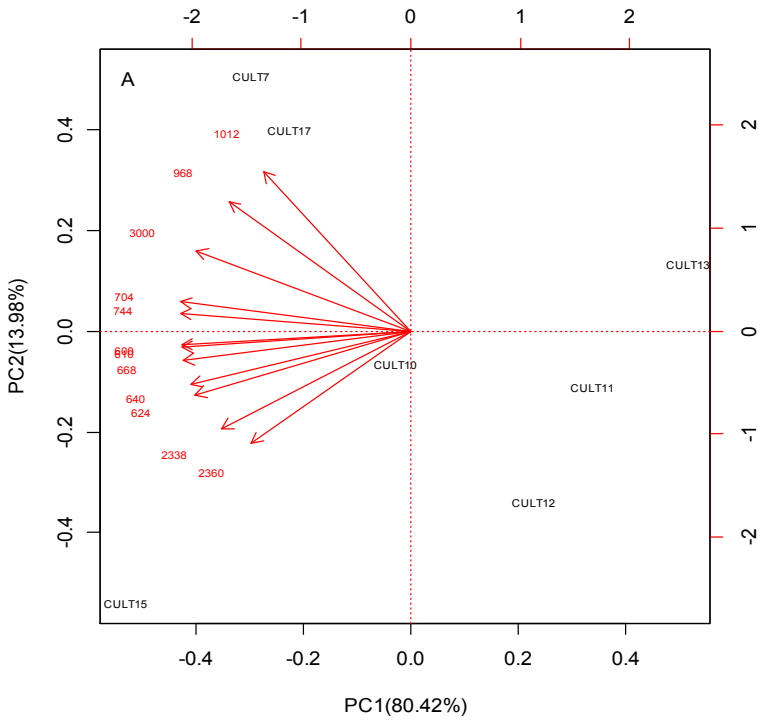
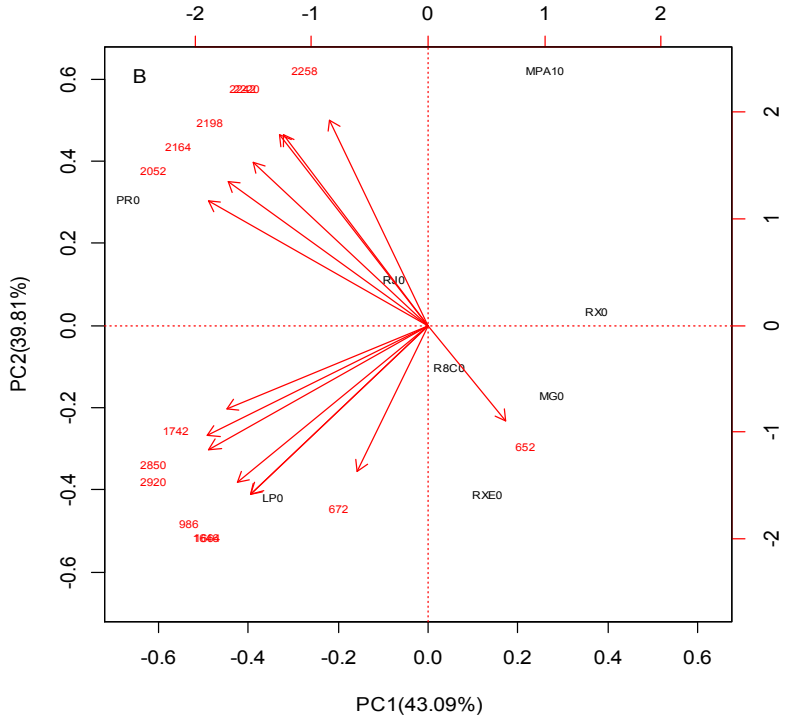


Fig. 2. Score and loading plots of Principal component analysis (PCA) of the whole spectral range (600-3000 cm^{-1}) showing the sample clustering according to their similarities. (A) Maze landraces produced during 2007 year



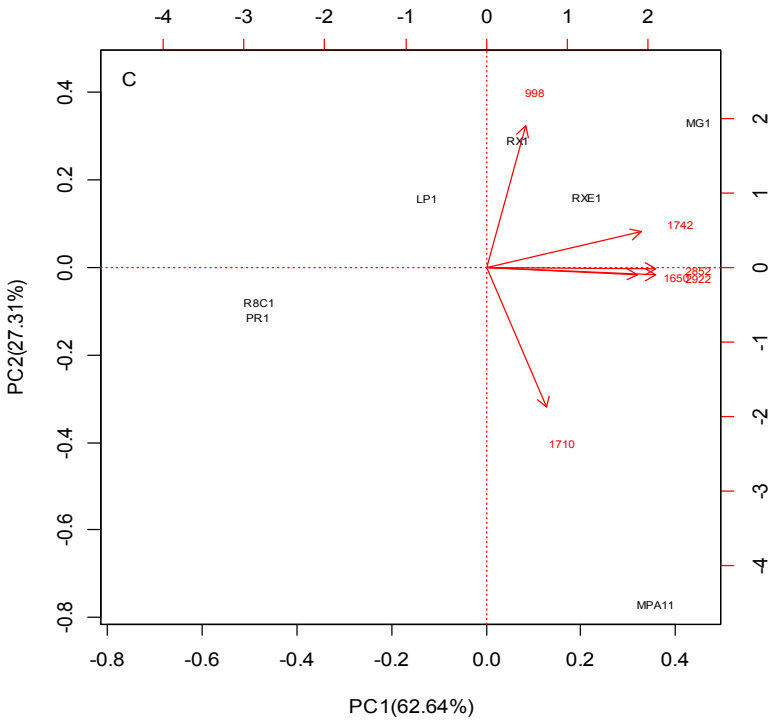


Fig. 2. Score and loading plots of Principal component analysis (PCA) of the whole spectral range (600-3000 cm^{-1}) showing the sample clustering according to their similarities. (B) Maze landraces produced during 2008 year, (C) 2009 cultivars

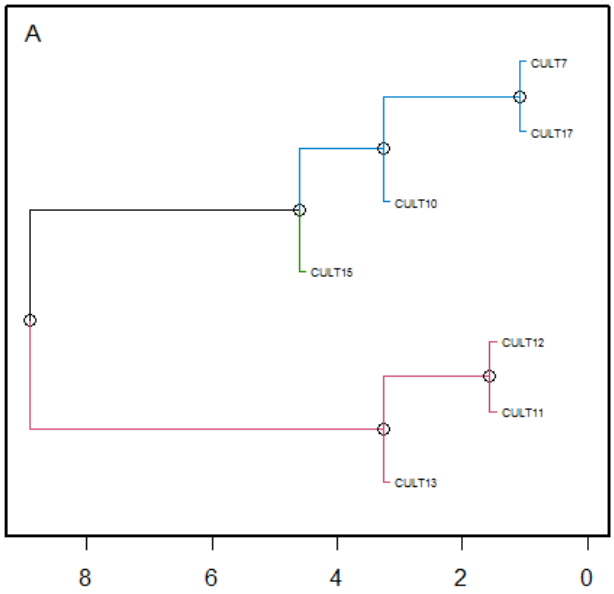


Fig. 3. Hierarchical cluster dendrogram (HCA) of the main peaks found in the spectra. (A) maize landraces produced during 2007 year

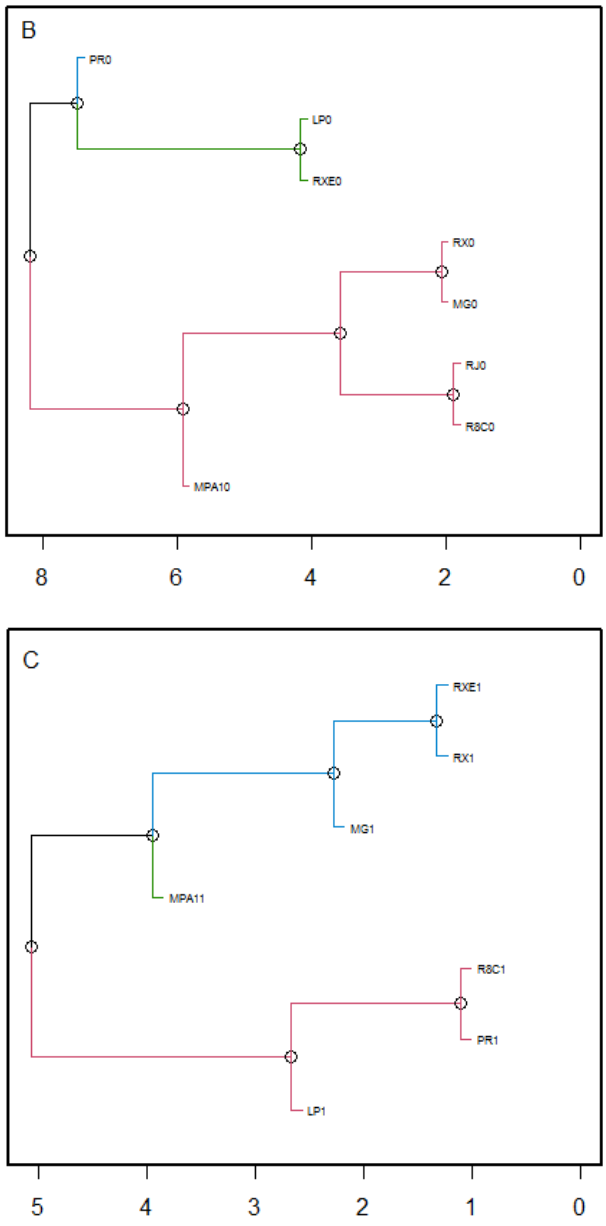


Fig. 3. Hierarchical cluster dendrogram (HCA) of the main peaks found in the spectra. (B) 2008, (C) maize landraces produced during 2009 year

Table 2. Assignment for the most characteristic ATR-FTIR bands found in maize landraces. Peaks and chemical group related is represented

Year of harvest	Chemical groups	Representative	ATR-IR wavenumber (1/cm)
			600
	Monoterpnes		610
	Sesquiterpenes		624
	Tetraterpenes	Lutein, beta-carotene	640
	Aminoacids	Methionine	668
			684
			704

Year of harvest	Chemical groups	Representative	ATR-IR wavenumber (1/cm)
2007			744
		Lutein	968
	Polysacharides	Amylose,amylopectin	998
		Amylose,amylopectin	1012
	Lipids/fatty acids		2338
			2360
			3000
	Monoterpenes		652
	Tetraterpenes		672
	Polysacharides	Amylose,amylopectin	986
	Proteins		1646
			1664
	Lipids		1742
2008			2052
			2164
			2198
			2220
			2242
			2258
	Lipids		2850
			2920
	Polysacharides	Amylose,amylopectin	998
	Proteins		1650
2009	Lipids		1710
			1742
			2852
			2922

4. CONCLUSIONS

FT-IR technique coupled with chemometric tools (PCA, HCA) were capable in finding changes in chemical composition of maize landraces produced over different years. Similarities in maize samples were also found duo to carbohydrate composition. The major bands in FT-IR are related to carbohydrates, lipids and proteins. Trace signals of secondary metabolites were also found in cultivars according to year of harvest.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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