academicJournals

Vol. 16(18), pp. 1061-1066, 3 May, 2017
DOI: 10.5897/AJB2017.15918
Article Number: 314492864061
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

*Full Length Research Paper*

# Fourier transform infrared spectroscopic analysis of maize (*Zea mays*) subjected to progressive drought reveals involvement of lipids, amides and carbohydrates

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Received 27 January, 2017; Accepted 18 April, 2017

**Changing climate means that there will be more episodes of drought, especially in arid regions. Drought stress is an important environmental factor that reduces crop productivity. Despite the vast amount of literature on drought stress in plants, there are still many uncertainties concerning the molecular responses under water deficit. In particular, if we wish to breed plants that are able to tolerate stress, identifying novel traits that contribute to this is desirable. In this study, Fourier transform infrared (FTIR) spectroscopy was used to investigate the response of maize to progressive drought. The objective was to determine whether FTIR spectroscopy is a technique that might be used in monitoring the molecular processes involved in drought responses in plants. Maize seeds were grown for three weeks in a controlled environment before drought was imposed by withdrawing water for up to 12 days. Principal component-discriminant function analysis (PC-DFA) indicated that water deficit elicited changes in the FTIR spectra within the wavenumber ranges of 3050 to 2800, 1750 to 1250 and 1250 to 900 cm$^{-1}$. With water deficit, the intensities of the absorption bands were reduced. PC-DFA loadings confirmed that the signals to the changes were lipids, amides and carbohydrates, respectively. This work shows that FTIR spectroscopy can provide valuable insights into drought responses of plants and maybe useful for identifying novel drought tolerance traits.**

**Key words:** Metabolic fingerprint, molecular changes, multivariate analysis, principal component-discriminant function analysis (PC-DFA), principal component analysis (PCA).

## INTRODUCTION

Drought can occur in different degrees, which can lead to a reduction in crop production. A plant's response mechanism is highly complex and is dependent on the plant species and duration of water deficit (Anjum et al., 2016). Drought causes massive economic losses (about $20 billion loss worldwide from 1900 to 2016) and is predicted to increase in the future, due to climate change (IPCC, 2007). Hence, understanding the drought tolerance mechanisms of plants will aid in the improvement of crops.

Maize is one of the most important crops in arid regions and a major cereal consumed worldwide (Ben-Iwo et al., 2016). The grains can be rich in protein, oil and starch (Kuhnen et al., 2010). Maize is not only important for feeding the growing human population but is also increasingly being used for biomass and biofuel purposes (Ben-Iwo et al., 2016). In order to improve its economic importance as sources of food and fuel, there is need to further understand how the crop tolerates stress, particularly drought stress, which reduces yield.

Fourier transform infrared (FTIR) spectroscopy is a method that measures the vibrations of molecular bonds and generates a spectrum that is considered the metabolic fingerprint of a sample (Amir et al., 2013; Kuhnen et al., 2010). It has been used as a tool for the analysis of plants, grain, soil, microalgae and in detecting microorganisms in food (Dean et al., 2010; Kamnev, 2008; Kuhnen et al., 2010; Lahlali et al., 2014, Winder and Goodacre, 2004). FTIR spectroscopy is a powerful and emerging tool that has the potential to be used to understand maize response to drought stress (Amir et al., 2013). To extract crucial information from a spectrum, multivariate analysis approaches such as principal component discriminant function analysis can be employed (Amir et al., 2013).

The objective of the study was to determine whether FTIR spectroscopy is a technique that might be used in monitoring the molecular processes involved in drought responses in plants. Principal component-discriminant function analysis (PC-DFA) and its loadings were employed to discriminate and detect changes in metabolites in response to drought.

## MATERIALS AND METHODS

### Plant growth conditions and drought treatments

Maize seeds (Cv Sundance) were supplied by Suttons Seeds (Paignton, UK). The seeds were sown in 3" small sized pots filled with John Innes No.1 soil in a growth chamber at the University of Manchester, United Kingdom with a 16 h photoperiod and a day/night regime at 23/15°C and relative humidity (RH) of 40-50%. Photosynthetic photon flux density was 130 μmolm$^{-2}$s$^{-1}$ (Light meter, SKYE instruments Ltd, UK provided by warm white LED lights; colour temperature = 2800 to 3000 K). Three weeks after planting (WAP), the maize seedlings were subjected to progressive drought stress for 12 days. Plant pots were weighed every two days during the period of drought stress until the 12$^{th}$ day. Soil water content (SWC) was estimated relative to water saturated controls using the formula: (FW-DW)/(AvFW-DW) × 100, where FW is the fresh weight of soil, DW is the dry weight of soil after drying to constant weight at 105°C and AvFW is the average fresh weight of the soils in the pots on day 0 of the experiment (Ogbaga et al., 2014). Twelve groups were used in the experiment for 1 plant x the 12 SWC conditions.

### Plant molecular analysis via Fourier transform infrared (FTIR) spectroscopy

Fully expanded leaf and shoot tissues from Days 0 to 12 and % soil water contents were excised 8 h into photoperiod with a pair of scissors, flash frozen in liquid nitrogen and lyophilized (freeze dried) with a Scan Vac Coolsafe freeze dryer (Vacuubrand, Wertheim, Germany) for 48 h as described by Ogbaga et al. (2016). FTIR spectroscopy was performed on 30 mg of dried homogenized tissues. The tissues were transferred to 2 ml micro centrifuge tubes and extracted in 600 µl of distilled water. 5 µl of homogenate was loaded onto the wells of a silicon 96 target plate (Bruker, MA, USA) and dried at 60°C. The Si plate was then placed in a Bruker Equinox-55 spectrometer and raw FT-IR data were recorded at an absorbance mode of 4000 – 600 cm$^{-1}$ wavenumber with a resolution of ~4 cm$^{-1}$ (Ogbaga et al., 2016).

### Principal component analysis (PCA) and principal component discriminant function analysis (PC-DFA)

PCA and PC-DFA analyses were performed the same way as described previously (Ogbaga et al., 2016). Raw FTIR data were recorded at an absorbance mode of 4000 to 600 cm$^{-1}$ wavenumber with a resolution of ~4 cm$^{-1}$. PC-DFA was used to analyze the data using R software (available at http://www.r-project.org/). PC-DFA scores and loading plots were visualized in Origin Pro 9 (OriginLab, Northampton, MA). PC-DFA a supervised multivariate technique was performed on the principal components (PCs) of the samples harvested on Days 0 to 12 at different soil water contents to determine the spectral regions of compounds (from PC-DFA loadings plots) that change with water deficit. PC-DFA is used to discriminate between samples based on their principal components with knowledge of which spectra were replicates. For clarity, the spectral regions of major compound classes are found at ~3050 to 2800 cm$^{-1}$ for lipids, ~1750 to 1250 cm$^{-1}$ for amides and ~1250 to 900 cm$^{-1}$ for carbohydrates (Ogbaga et al., 2016).

## RESULTS AND DISCUSSION

The % soil water content (SWC) fell progressively with time such that by the 10$^{th}$ day after drought stress was imposed, the soil was completely dry (Figure 1). The dryness was accompanied by a gradual drop in the FTIR spectra relative to the control (Figure 2). The absorption bands of the spectra dropped to approximately half of the original intensities by the 6$^{th}$ day when the SWC reduced to 15%. This decrease continued until the soil was completely dried (Figure 2). From the FTIR data, spectral differences were observed with water deficit. Chlorophyll fluorescence and stomatal conductance are established techniques used for non-destructive and non-invasive measurement of drought stress (Born et al., 2014). Here, the authors propose the use of FTIR spectroscopy as another method that can provide quick, high throughput

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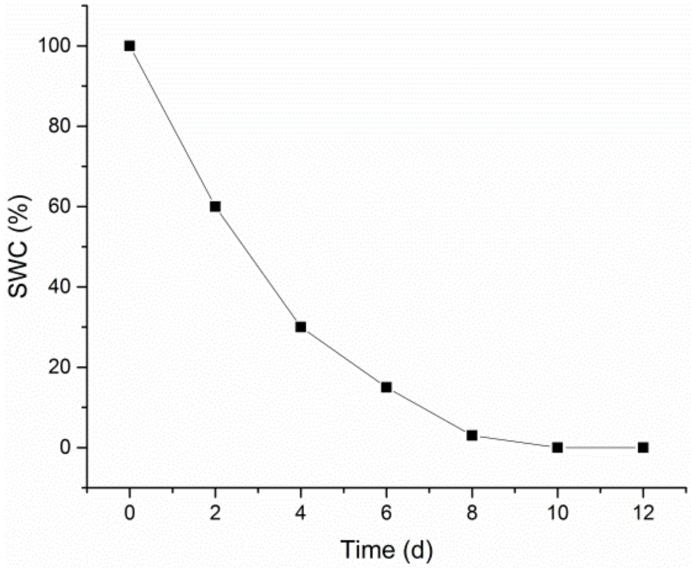


**Figure 1.** The change in pot water content relative to water saturation. Plants were grown for 3 weeks before drought imposed by withholding water for up to 12 days.

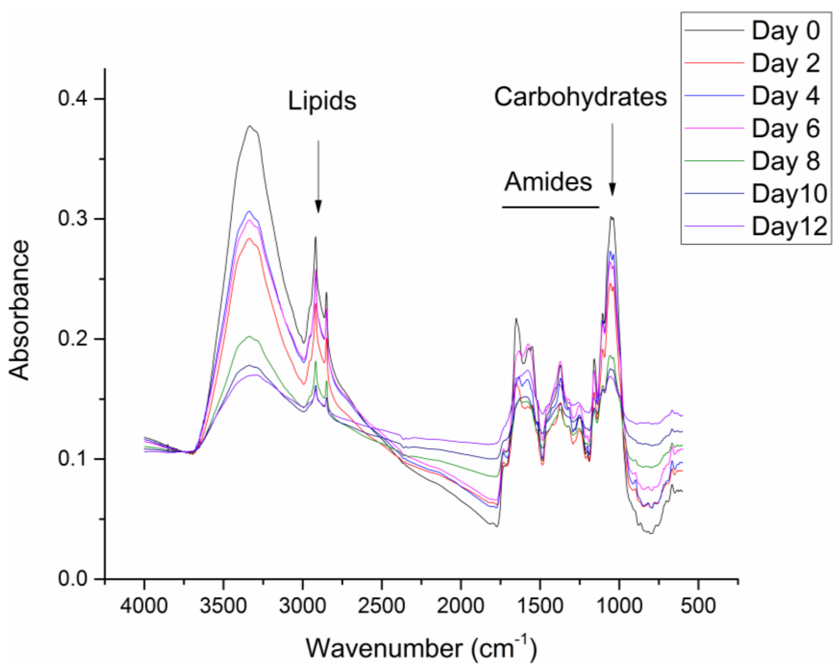


**Figure 2.** Average normalised FTIR spectra from days 0 to 12. FTIR spectra were normalised to wavenumber 3745 $cm^{-1}$.

data on drought stress.

In order to compare changes in lipids, amides and carbohydrates in the control and drought stressed plants, FTIR spectra were normalised at a wavenumber of 3745 $cm^{-1}$. The absorption band positions from the spectra are those corresponding to lipids, amides and carbohydrates and are similar to our earlier study which suggested that these metabolites were involved in prolonged drought in sorghum (Ogbaga et al., 2016). The intensities: 3050 to 2800, 1750 to 1250 and 1250 to 900 $cm^{-1}$ bands corresponding to lipids, amides and carbohydrates, respectively, were higher in control plants as compared to

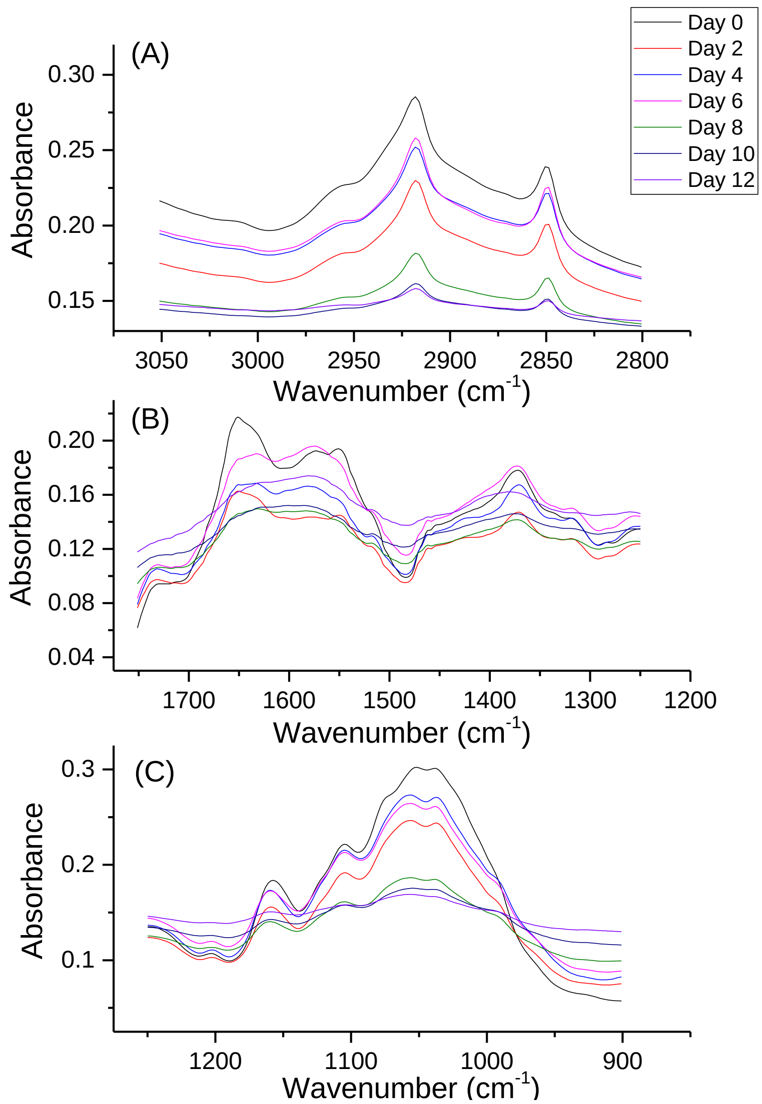


**Figure 3.** Average lipid (3050 to 2800 cm$^{-1}$) absorbance (A), average amide (1750 to 1250 cm$^{-1}$) absorbance (B) and average carbohydrate (1250 to 900 cm$^{-1}$) absorbance (C) of maize grown from days 0 to 12.

the stressed plants (Figure 3). Under drought the lowest bands were seen on the 12$^{th}$ day for lipid and carbohydrate regions. However, there was a differential response in the intensities observed at amide region with water deficit (Figure 3). The absorption bands from the spectra in this study suggest that the same metabolites detected in sorghum during prolonged drought are also involved in progressive drought in maize. In addition, reduction in the intensities of the bands detected with increasing drought indicated a reduction in lipid and carbohydrate content and also changes in the composition of the proteome (Lahlali et al., 2014; Athar et al., 2016). Thus, information on drought-specific responses could be extracted from the spectra.

Figure 4 shows the PC-DFA scatter plot with an initial increase in LD2 on y-axis up to the 4$^{th}$ day. From the 6$^{th}$ day, changes in the plots reduced and were explained by both LD2 and LD1 (Figure 4). The loading profiles of the changes in PC-DFA shown in Figure 4 are captured in Figure 5. The loading profiles confirmed that the signals associated with progressive drought stress in the maize plants were indeed lipids, amides and carbohydrates (Figure 5). Changes in lipid region could reflect lipid signalling in response to drought stress or the regulation of enzymes involved in lipid signalling such as phospholipase C and phospholipase D (Zhao, 2015). In terms of structure, lipids are rich in $CH_2$ functional group, while the amide groups are rich components of proteins mostly containing N-H structures. Carbohydrates on the other hand, are dominated by vibrations of C-O-C or C-O

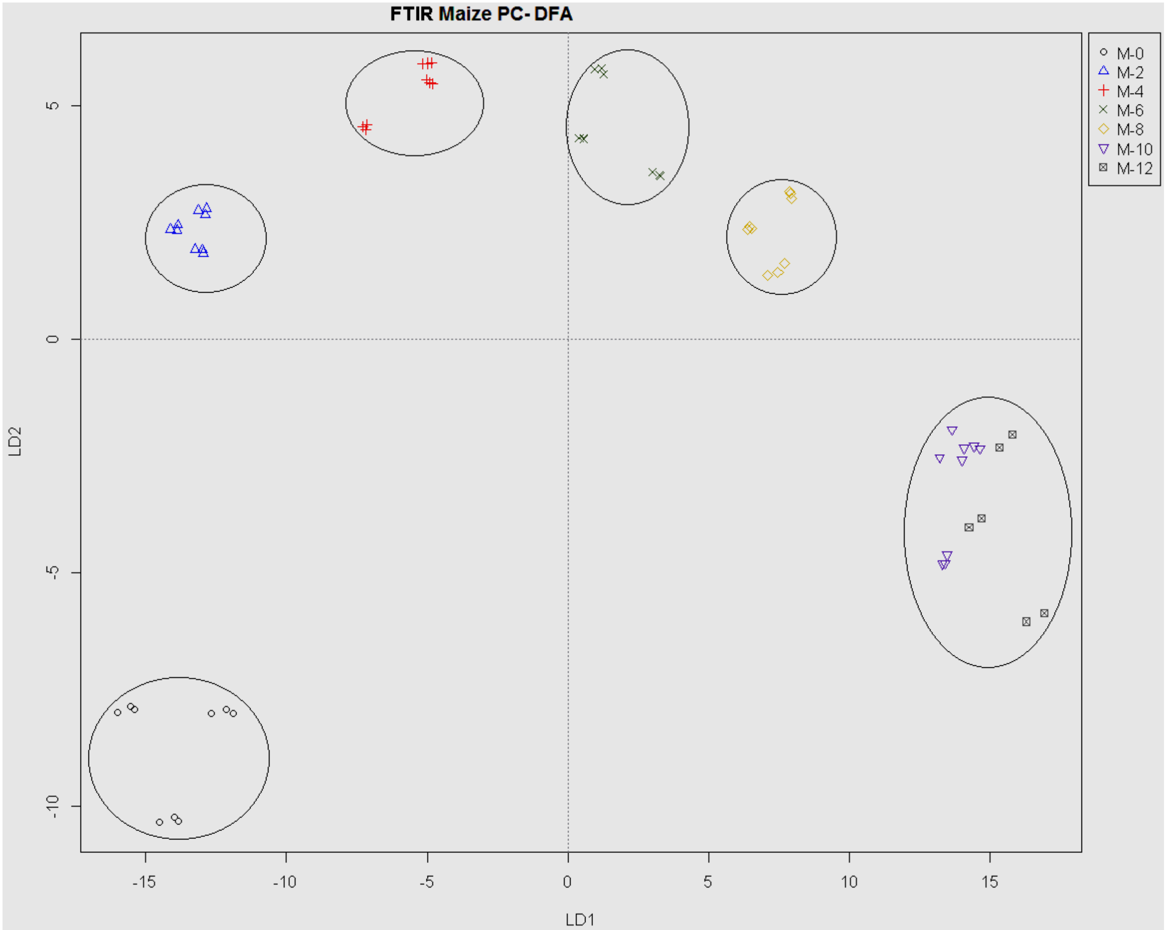


**Figure 4.** Principal component discriminant function analysis (PC-DFA) of maize (M) on days 0, 2, 4, 6, 8, 10 and 12. LD1- Linear discriminant 1, LD2- linear discriminant 2.

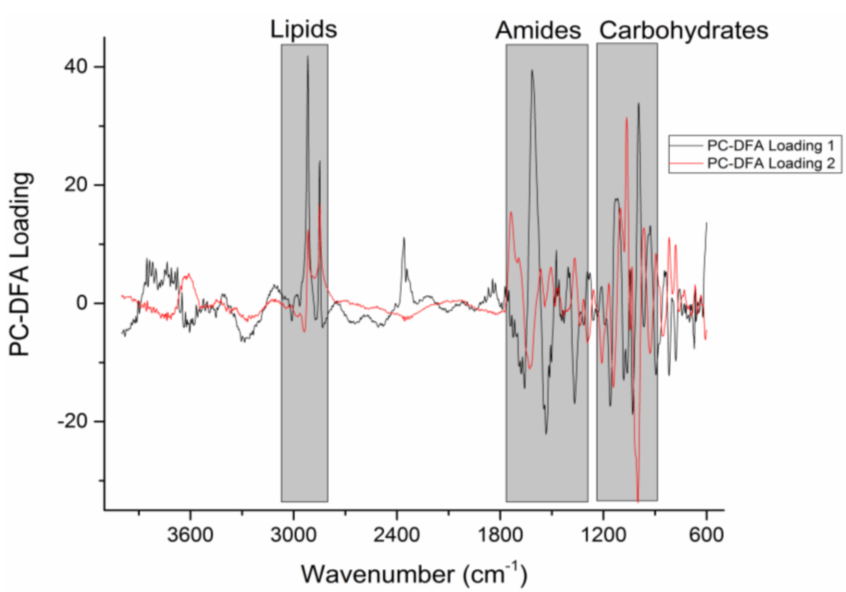


**Figure 5.** Principal component discriminant function (PCDF) loadings. The shaded regions from left to right represent lipids, amides and carbohydrates.

functional groups (Beekes et al., 2007).

## Conclusion

The study indicated that the signals in the wavenumber ranging from 3050 to 2800, 1750 to 1250 and 1250 to 900 $cm^{-1}$, associated with lipids, amides and carbohydrates respectively, changed with water deficit in maize. As FTIR is a fast tool, it can be used as a probe for the estimation of drought and a rapid measure of molecular changes in drought stressed plants.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ABBREVIATIONS

**FTIR,** Fourier transform infrared spectroscopy; **LD1,** linear discriminant 1; **LD2,** linear discriminant 2; **PCA,** principal component analysis; **PC-DFA,** principal component-discriminant function analysis; **SWC,** soil water content.

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