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# Water Stress Effect on Cell Wall Components of Maize (*Zea mays*) Bran

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**Abstract**

In México, around 82% of the total production of maize is grown under rainfed conditions leading to a water stress environment which affects physiologic and biochemical process of the plant. Maize bran is a composited plant material consisting mainly in aleurone layer, testa and pericarp; the cell walls of these tissues are composed of proteins, non-starch polysaccharides, phenolic acids and lignin which are potential bioactive substances for human nutrition. In this research it was investigated the effect of water stress on cell wall components in the bran of three genotypes of maize by applying irrigation and water stress treatments. The content of protein, lignin, arabinoxylans, total phenols and phenolic acids was performed in the bran of 'Cebú', 'DK2027' and 'DK2034' genotypes. Water stress applied through grain development stage increased protein levels of 'Cebú', 'DK2027' and 'DK2034' in 4.05, 16.13 and 0.40% respectively. Respecting to lignin content, water stress increased levels at 1.28, 2.26 and 4.24% for 'Cebú', 'DK2027' and 'DK2034', respectively. Arabinoxylans content also increased in water stress treatment at levels of 1.28, 2.26 and 3.66% in 'Cebú', 'DK2027' and 'DK2034'. On the other hand, water stress treatment decreased the levels of total phenols and hydroxycinnamic acids in the three maize hybrids analysed. Reduction of total phenols was 35.34, 5.59 and 31.57% for 'Cebú', 'DK2027' and 'DK2034', respectively. In addition, the levels of *t*-ferulic, *c*-ferulic and *p*-coumaric acids decreased 17.74, 23.93, 29.83% in 'Cebú', 8.92, 8.62, 24.03% in 'DK2027' and 13.66, 11.03, 10.38% in 'DK2034' respectively.

***Keywords:*** arabinoxylans, hydroxycinnamic acids, lignin, protein, total phenols, water stress, *Zea mays* bran

## Introduction

Maize crop is important in nutrition and economy of the Mexico population because it is considered the centre of origin, domestication and diversification of this cultivation with around 60 different varieties in this territory (Kato *et al.*, 2009). According to the Mexican Agriculture and Fishery Information Service (SIAP) data, in 2012 around 82% (6, 616,152 ha) of the total production area with maize was rainfed established. In these conditions, maize crop frequently go through periods of water deficit which affects anatomic, morphologic, physiologic and biochemical process on plants (Farooq *et al.*, 2009). On the other hand during development of plants, water stress affects anatomic, morphologic, physiologic and biochemical processes increasing the levels of reactive oxygen species, which has direct and indirect effects on secondary metabolism and therefore on antioxidant compounds synthesis (Farooq *et al.*, 2009; Sharma *et al.*, 2012). The objective of this study was to determine changes in levels protein, lignin, arabinoxylans, total phenols and hydroxycinnamic acids in the bran of three maize genotypes grown under water stress.

## Materials and Methods

### *Plant material and experimental conditions*

Seeds of the non-drought tolerant maize hybrids 'Cebú', 'DK2027' and 'DK2034' were donated by Monsanto Company and cultivated in the research field of Universidad Autónoma de Nuevo León, Agronomy Faculty with a geographic localization in 25° 52' 13.5"N and 100° 02' 22.5"W. Maize was grown during 2013 spring-summer cycle, sowing was performed on February 25th on clay soil and water constants correspondent to field capacity and permanent wilt point were

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27 and 12% respectively. Experimental units involved 4 furrows of 5 m long and 0.8 m spaced from each other and it was established a 0.1 m distance between plants in simple line. In irrigation (IR) treatment, field capacity was kept above 60% during the whole cycle, while in the water stress (WS) treatment irrigation was suspended permanently from R1 phase (visible stigma, reached on May $10^{th}$) through R6 phase (physiologic maturity, reached on June $30^{th}$), all samples were harvested on July $10^{th}$ when water content was around 14-15%.

*Bran preparation*

Grains of each experimental unit was soaked in water for 2 h and ground in an electrical mill. Later, each sample was suspended in 5 L of water; the bran was removed manually and recovered with a sieve and after that, defatted with acetone for 4 h. Finally samples were dried overnight at 40 °C and ground to a particle size minor to 0.5 mm (mesh 35).

*Protein, lignin and arabinoxylans*

Protein content was determined by Kjeldahl method (N x 6.25) (AOAC, 1990) using a Labconco Micro-Kjeldahl digestion and distillation system (Kansas City, MO, USA). Acid detergent lignin concentration (Goering and Van Soest, 1970) was determined by ANKOM 200/220 fibre analyzer (Fairport, NY, USA), using Ankom F57 filter bags with a pore size of 30 µm. For arabinoxylans content, maize bran was suspended sodium phosphate buffer (0.1 M) and treated with $\alpha$-amylase, protease and amyloglucosidase according to Bunzel *et al.* (2004) for starch and protein degradation. Arabinoxylans extraction was carried out according to Carvajal-Millan *et al.* (2006) with modifications. Briefly, maize bran was treated with NaOH 0.5 M for 4 h at room temperature, followed by precipitation with ethanol for 4 h at 4 °C, after that arabinoxylans were recovered by filtration and finally freeze dried.

*Total phenols and hydroxycinnamic acids*

Extraction of phenolics was carried out by alkaline hydrolysis followed by diethyl ether extraction and finally dissolved in 80% methanol according to the procedure described by Kim *et al.* (2006). Total phenols were performed using Folin-Ciocalteu analysis according to Chun and Kim (2004). Levels of phenolics were calculated based on calibration curve with ferulic acid in concentrations from 0 to 200 mg $L^{-1}$ and results were expressed as micrograms equivalent of ferulic acid per gram of sample (µgEAF $g^{-1}$). Hydroxycinnamic acids were analysed according to Zhao *et al.* (2005) using a HPLC equipment Agilent Technologies 1260 Infinity with G1311C quaternary pump and G4212B diode array detector (detection at 325 nm) and a $C_{18}$SupelcoNucleosil column (5 µM, 250 x 3 mm). Levels of hydroxycinnamic acids were calculated based on a calibration curve with standards of *t*-ferulic, *c*-ferulic, *p*-coumaric, caffeic and sinapic acids, in concentrations from 0 to 50 mg $L^{-1}$and results were expressed as micrograms of each phenolic acid per gram of sample (µg $g^{-1}$).

*Statistical analysis*

A randomized complete block design in locations was used; irrigation and water stress conditions represented each location. Four repetitions of 'Cebú', 'DK2027' and 'DK2034'treatments were established. Data analysis was carried out with Minitab 14.0 (Minitab Inc., 2004) and for simple comparison, Tukey test with $\alpha$=0.05 was used.

Table 1. Effect of water stress on protein, lignin and arabinoxylans in the studied maize hybrids

| Genotype | Treatment | Component (%) | | |
|---|---|---|---|---|
| | | Protein | Lignin | Arabinoxylans |
| 'Cebú' | IR | 14.06 $^{a}$ | 0.102$^{a}$ | 23.40 $^{a}$ |
| | WS | 14.63 $^{a}$ | 0.107 $^{a}$ | 23.70 $^{a}$ |
| 'DK2027' | IR | 13.14$^{b}$ | 0.104 $^{a}$ | 19.85 $^{a}$ |
| | WS | 15.26 $^{a}$ | 0.119 $^{a}$ | 20.30 $^{a}$ |
| 'DK2034' | IR | 14.91 $^{a}$ | 0.125 $^{a}$ | 17.20$^{a}$ |
| | WS | 14.97 $^{a}$ | 0.134 $^{a}$ | 17.83$^{a}$ |

Means within a column for each hybrid with different letter are significantly different at P≤0.05.

Table 2. Effect of water stress on the total phenols and hydroxycinnamic acids in the studied maize hybrids

| Genotype | Treatment | Total Phenols (µgFAEg$^{-1}$) | Hydroxycinnamic Acids (µgg$^{-1}$) | | |
|---|---|---|---|---|---|
| | | | *t*-Ferulic | *c*-Ferulic | *p*-Coumaric |
| 'Cebú' | IR | 18931±707$^{a}$ | 6560±77$^{a}$ | 1989±25$^{a}$ | 828±17$^{a}$ |
| | WS | 11483±433$^{b}$ | 5396±48$^{b}$ | 1513±70$^{b}$ | 581±16$^{b}$ |
| 'DK2027' | IR | 16598±625$^{a}$ | 6265±63$^{a}$ | 1833±24$^{a}$ | 824±15$^{a}$ |
| | WS | 15671±886$^{a}$ | 5706±116$^{b}$ | 1675±24$^{b}$ | 626±20$^{b}$ |
| 'DK2034' | IR | 16643±558$^{a}$ | 5827±79$^{a}$ | 1759±57$^{a}$ | 983±27$^{a}$ |
| | WS | 11388±464$^{b}$ | 5031±61$^{b}$ | 1565±72$^{a}$ | 881±15$^{a}$ |

Means within a column for each hybrid with different letter are significantly different at P≤0.05.

## Results and Discussion

*Protein, lignin and arabinoxylans*

Water stress applied through grain development stage increased protein in levels of 'Cebú', 'DK2027' and 'DK2034' increased 4.05, 16.13 and 0.40%, respectively. Respecting to lignin content, water stress increase their levels at of 1.28, 2.26 and 4.24% for 'Cebú', 'DK2027' and 'DK2034' and arabinoxylans content also increase in water stress treatment at levels of 1.28, 2.26 and 3.66% in 'Cebú', 'DK2027' and 'DK2034' (Table 1).

Results found in the present study for protein are different than those reported by Ali *et al.* (2010), who reported that protein content of whole maize grain decreased significantly due to water stress on 'Agaiti-2002' and 'EV-1098' genotypes in 13.89 and 19.25% respectively. In the same way, Aydinsakir *et al.* (2013) found that protein content in maize grain was influenced by water deficit, with a decreasing level around 61.53 and 38.46% in 'Ant-190' and 'Safak' varieties, respecting to a full irrigation treatment.

Our data in lignin content, are different to conclusions of other studies where proteomic and metabolonic analysis of lignin synthesis in maize were analysed; Vincent *et al.* (2005), analysed the lignin content of the 'F2' ans '$I_0$' maize lines by thioacidolysis and they found that the lignin content was lower in leaves of plant subject to water deficit than in those of well-watered plants and that was consistent with the levels of caffeic acid/5-hydroxyferulic 3-*O*-methyltransferase which is a key enzyme involved in lignin synthesis; in addition, Alvarez *et al.* (2008) concluded that water stress reduces lignin biosynthesis of the maize root xylem vessels of the cultivar 'FR697' and they support this conclusion based in the analysis of metabolites which showed an increase of free monolignols in xylem sap.

To best of our knowledge there are not data available on the effect of water stress on arabinoxylans content in maize, but there are some studies of the effect this abiotic stress on wheat arabinoxylans. The present results had the same behaviour of the 'Superb' spring wheat variety studied by Zhang *et al.* (2010) but they obtained higher levels of arabinoxylans than the current ones, ranging from 9.76 to 12.6% at different temperature regimes. In addition, Rakszegi *et al.* (2014) also observed increasing levels of arabinoxylans in Magma and Fatima-2 wheat varieties with 27.05 and 28.65%, respectively.

*Total phenols and hydroxycinnamic acids*

Water stress treatment decreased the levels of total phenols and hydroxycinnamic acids in the three maize hybrids analysed. Reduction of total phenols was 35.34, 5.59 and 31.57% for 'Cebú', 'DK2027' and 'DK2034', respectively. In addition, the levels of *t*-ferulic, *c*-ferulic and *p*-coumaric acids decreased 17.74, 23.93, 29.83% in 'Cebú', 8.92, 8.62, 24.03% in 'DK2027' and 13.66, 11.03, 10.38% in 'DK2034' respectively. Predominant hydroxycinnamic acid from bran extracts was *t*-ferulic (from 67 to 72%) followed by *c*-ferulic (from 20 to 21%) and *p*-coumaric (from 7 to 11%) for both irrigation and water stress treatments, while caffeic and sinapic acids were found in traces or not detected in samples (Table 2).

To the best of our knowledge, there are not data available about the effect of water stress on total phenols and hydroxycinnamic acids of maize bran, but there are few reports that have published data of these assays from another anatomical part of the maize plant and products obtained from maize grain. Hura *et al.* (2008) evaluated the content of total phenols and *t*-ferulic acid in leaves of maize genotypes after two weeks of drought treatment and they found a total phenols decrease around 57 and 45% for 'Ankora' and 'Nova' genotypes, which is higher than the present findings. In addition they observed that decrease of *t*-ferulic acid was around 35 and 31% after drought treatment in the studied hybrids which is in the range of the present study. Ali and Ashraf (2011) studied the effect of drought treatment on the secondary metabolites of maize leaves and they found decreasing levels around 27 and 25% in total phenols for 'EV-1098' and 'Agaiti-2002' maize cultivars, being these results lower than the current data. Alvarez *et al.* (2008) analysed xylem sap of 'FR697' maize cultivar after 12 days of drought treatment and they observed the same pattern of hydroxycinnamic acids composition of the present study, reporting that *t*-ferulic acid was the predominant with a decreasing of 46% of this compound after drought treatment and this result was higher than the current experiment, in addition *p*-coumaric acid increased 286% which is a different behaviour comparing with the current results. Vuletić *et al.* (2014), analysed the effect of osmotic stress in root cell walls of the maize inbred line 'VA35' and they found that content of total phenols diminished 30%, which is in agree with this results, but they also found a different behaviour in the analysis of hydroxycinnamic acids since they observed an increase of 49 and 18% in *t*-ferulic acid and *p*-coumaric respectively.

## Conclusions

This study indicated that water stress had a positive effect on the content of protein, lignin and arabinoxylans, increasing their levels and had a negative effect on the content of total phenols and hydroxycinnamic acids. Further studies are necessary to determine the factors responsible of this behaviour.

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