05 May 2023

The Director
University of Missouri South African Education Program (UMSAEP)
213 Hulston Hall
Columbia
MO 65211

Dear Prof. Uphoff,

REPORT: 2021-2022 UMSAEP Award (N. Ludidi)

This serves as my report of the activities associated with the award made to me at the University of the Western Cape (UWC) under the University of Missouri South African Education Program for a research visit undertaken in October 2021 to May 2022.

Summary

The funding was granted for me and my then PhD student (now Dr. Ali Elnaeim Elbasheir Ali) to visit the laboratories of Profs. Robert Sharp and Scott Peck (both at the University of Missouri – Columbia, MU) to compete work done on maize between Prof. Sharp and I, and to complete work done on sorghum as part of the PhD thesis of Ali while initiating research work on drought and heat stress in pearl millet (an indigenous

University of the Western Cape, Robert Sobukwe Road, Bellville 7535, Republic of South Africa

Professor of Biotechnology
Plant Stress Tolerance Laboratory
Department of Biotechnology

nludidi@uwc.ac.za
+27219592750
+27789762376

University of the Western Cape, Robert Sobukwe Road, Bellville 7535, Republic of South Africa

Citation: Kolo, Z.; Majola, A.; Phillips, K.; Ali, A.E.E.; Sharp, R.E.; Ludidi, N. Water Deficit-Induced Changes in Phenolic Acid Content in Maize Leaves Is Associated with Altered Expression of Cinnamate 4-Hydroxylase and p-Coumaric Acid 3-Hydroxylase.

On the one hand, decreased p-coumaric acid content (Figure 2B) was associated with a 0.4-fold increase in C4H enzymatic activity (the enzyme participating in the biosynthesis of p-coumaric acid; Figure 3A). On the other hand, increased caffeic acid content (Figure 2C) was associated with a 0.6-fold increase in C3H enzymatic activity (the enzyme participating in the biosynthesis of caffeic acid; Figure 3B). The level of the phenolic acids and enzymatic activities described herein did not differ significantly between the leaves of well-watered temporal and developmental control plants (Figures 2 and 3A,B).

Figure 3. Changes in the enzymatic activities of C4H (**A**) and C3H (**B**) in relation to effects of water deficit on the expression of genes encoding a cinnamate 4 hydroxylase (**C**) and a p coumarate 3 hydroxylase (**D**) in maize leaves. Data represent means of three independent experiments in tissue obtained from the third youngest leaf of each treatment. C4H is cinnamate 4 hydroxylase and C3H is p coumarate 3 hydroxylase. Error bars with different letters represent significantly different means SD at a confidence level defined by p 0.05.

Upon observing differences in *p*-coumaric acid and caffeic acid contents and enzymatic activity for C4H and C3H, we investigated if water deficit alters the expression of the maize genes encoding C4H and C3H. Use of the $2^{-\Delta\Delta T}$ method in the qPCR data to calculate transcript accumulation showed a 0.6-fold increase in C4H gene expression in response to water deficit (Figure 3C), whereas C3H gene expression increased 1.8-fold in the leaves in response to the water deficit treatment (Figure 3D).

Water deficit caused significant changes (p 0.05) in all the assessed parameters except for the amount of cinnamic acid except (Table 1). The highest change was in the level of expression of p-coumarate 3-hydroxylase and the associated metabolite, namely caffeic acid (Table 1).

Table 1. Comparison of means of measured physiological, biochemical and molecular changes in maize grown in well-watered (temporal), well-watered (developmental) or water deficit conditions.

	RWC	Evans Blue Uptake	Cinnamic Acid	p-Coumaric Acid	Caffeic Acid	Ferulic Acid	C4H Activity-Acid	C4H	C4H	C4Acid C4H

p-coumaric acid produced by the C4H activity and this results in lowered *p*-coumaric acid

concentrations recommended by the manufacturers. The water potential of the Promix Organic was adjusted with water to −0.03 MPa for growing plants under well-watered temporal or developmental control conditions and −0.45 MPa for growing plants under water deficit conditions at the time of transplanting, on the basis of measurements taken

4.2.4. Measurement of Free Phenolic Acids

The third youngest leaf from three different plants in each treatment was used to measure p-coumaric acid, caffeic acid and ferulic acid content. For these measurements, 100 mg of freshly weighed leaf tissue from each sample was extracted by grinding the leaf tissue inwasi0(was)T9 (the)-250.999 (leaf)]TJ 1.013 0 (leaf)]TJ 1.013 0 (leaf)]TJ 1.0131ceth8n.5i0 (lea