Drought Response in Selected Tropical Inbred Maize Lines and Relative Expression of PARP2 Gene under Limited Water Conditions

Joshua Kiilu Muli1,2, *, Nancy Budambula1, Cecilia Mweu3, Sylvester Elikana Anami3

1Department of Biological Sciences, University of Embu, Embu, Kenya
2School of Biological Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
3Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Email address:
Joshmulison@gmail.com (J. K. Muli), muli.joshua@embuni.ac.ke (J. K. Muli)
*Corresponding author

To cite this article:
Joshua Kiilu Muli, Nancy Budambula, Cecilia Mweu, Sylvester Elikana Anami. Drought Response in Selected Tropical Inbred Maize Lines and Relative Expression of PARP2 Gene under Limited Water Conditions. Plant. Vol. 6, No. 1, 2018, pp. 8-15. doi: 10.11648/j.plant.20180601.12

Abstract: Drought is the leading single factor that limits maize production thus inhibiting the crops genetic potential. In response to drought, maize and other plants synthesize Poly ADP-Ribose (PAR) protein. This process is controlled by the Poly ADP-Ribose Polymerase (PARP) genes and consumes cellular energy, leading to plant death. This study evaluated four tropical inbred maize (Zea mays L.) lines; CML 216, CML 144, A04 and E04 for their response to growth limiting water stress and their relative expression of PARP2 gene under drought and non-drought conditions. The leaf lengths and growth rates of the fourth leaf were monitored for 21 days post emergence while fresh and dry weights of drought stressed and non-stressed seedlings were recorded a month after emergence of the fourth leaf. The relative expression of PARP2 gene was determined using rtPCR after isolating RNA from drought stressed and non-stressed maize seedlings. There was no significant difference in the mature lengths of the fourth leaf in the four genotypes when the maize seedlings were not subjected to drought and when subjected to severe drought stress. However, subjecing maize seedlings to mild drought resulted in a significant difference in the mature leaf lengths based on the different genotypes (P= 0.0066). The growth rate of maize seedlings based on the fourth leaf was observed to be affected by drought, with a higher mean growth rate (1.74 cm day⁻¹) registered in seedlings which were not subjected to drought and those subjected to moderate drought (1.78 cm day⁻¹). A slower growth rate (1.37 cm day⁻¹) was observed in seedlings subjected to severe drought stress. Fresh and dry weights of maize seedlings were also observed to be significantly different based on the level of drought exerted (P = < 0.0001) and the genotype (P = < 0.0001). The expression of PARP2 gene was found to be directly proportional to the level of drought stress exerted. Results from this study suggest how tropical maize genotypes respond to drought.

Keywords: Drought, Growth Rate, Maize, PARP

1. Introduction

Maize is an important crop which is grown throughout the world for use as food and feed. World production estimates by 2013 stood at about 1,018,111,958 tonnes, with the main producers being the United States, China, Brazil, Argentina and Ukraine. The entire African continent contributed only 6.9% of the slightly over 1 billion tonnes global harvest. In Africa, maize is cultivated on over 37 million hectares (Ha) of the total 231 million hectares of arable land. Third to wheat and rice, the importance of maize as a food crop in the world and especially Africa cannot be overlooked. It contributes significantly to food either as grain or flour although it has other uses including ethanol production and as feed for animals [1]. Currently, the average maize yield in Africa amounts to about 2.09 tonnes of grain per hectare, which is way below the crops’ potential [2]. Among the
2. Materials and Methods

2.1. Plant Materials

Four white seeded tropical inbred maize lines; CML 216, CML 144, E04 and A04 used in the study were planted and bulked in plots in the research fields of the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The seeds of all the inbred maize lines were provided by the University of Agriculture and Technology (JKUAT). The seeds had germinated, and after the emergence of the first leaf, individual seedlings were transferred into small plastic pots containing a measured amount of soil. The soil had been air dried and filled into the small pots to fill two-thirds of the perforated pots. These pots were then put in containers with water overnight to allow them absorb the maximum amount of water. The following day, the pots were removed from the water containers and seedlings transplanted into them. Pots were then allowed to lose the excess water for another 24 hours to field capacity and the weights of the pots determined. The amount of water in the soil was determined by the following equation:

Water content (g) = (pot weight+wet soil)-(pot weight+dry soil)

This determined water content was maintained throughout as the water content for the control experiments. Water content for moderate stress experiments was maintained at 50% that of the controls and water content for severe stress maintained at 25% that of the controls. After seedling transfer, pots containing seedlings to be subjected to drought were dried down to the desired weights for both moderate and severe stresses by withholding watering. Pot weights were monitored on a daily basis and water was carefully added only after the target weight had been reached. Water was added only to readjust pot weights to the target level and compensate for evaporation. Water applied to readjust pot weights was always applied at the edges of the pots, never directly in the plant vicinity and was always done at 10 pm every day [10]. A completely randomised design with three replications was used.

2.2. Drought Stress Experiments

Well dried seeds harvested after self-pollinating inbred lines were soaked in distilled water overnight and planted in vermiculite in a large plastic pot. After the 5th day when all seeds had germinated, and after the emergence of the first leaf, individual seedlings were transferred into small plastic pots containing a measured amount of soil. The soil had been air dried and filled into the small pots to fill two-thirds of the perforated pots. These pots were then put in containers with water overnight to allow them absorb the maximum amount of water. The following day, the pots were removed from the water containers and seedlings transplanted into them. Pots were then allowed to lose the excess water for another 24 hours to field capacity and the weights of the pots determined. The amount of water in the soil was determined by the following equation:

Water content (g) = (pot weight+wet soil)-(pot weight+dry soil)

This determined water content was maintained throughout as the water content for the control experiments. Water content for moderate stress experiments was maintained at 50% that of the controls and water content for severe stress maintained at 25% that of the controls. After seedling transfer, pots containing seedlings to be subjected to drought were dried down to the desired weights for both moderate and severe stresses by withholding watering. Pot weights were monitored on a daily basis and water was carefully added only after the target weight had been reached. Water was added only to readjust pot weights to the target level and compensate for evaporation. Water applied to readjust pot weights was always applied at the edges of the pots, never directly in the plant vicinity and was always done at 10 pm every day [10]. A completely randomised design with three replications was used.

2.3. Plant Growth Analysis

Plant growth was monitored daily by measuring the length of the fourth leaf (Oumaya et al., 2006). The distance between the base and tip of the leaf was measured for all plants at 10 pm. These measurements continued until no further growth was recorded for all plants in the four maize genotypes, by the 21th day after leaf emergence. After the fourth leaf reached maturity, all the collected data points were entered in excel sheets. Leaf elongation rate per day was determined by obtaining the difference in leaf length from one day to the next and the result divided by one. The resulting data was used to determine the performance of each genotype at the different stress levels.

2.4. RNA Isolation and cDNA Synthesis

Maize leaf tissues were cut and 50 mg was placed in into 2 ml Eppendorf tubes containing two metal beads. The tubes were immediately placed in a container with liquid nitrogen. The samples were ground into fine powder, 1 ml of trizol...
reagent was added and mixed vigorously by brief vortexing. The mixture was incubated for 5 minutes at room temperature. Thereafter, 0.2 ml of chloroform was added to each tube then shaken vigorously by hand and incubated for 5 minutes at room temperature. After incubation, the samples were centrifuged for 15 minutes at 12000xg at room temperature and the upper aqueous phase transferred to a fresh 1.5 ml Eppendorf tube. To these tubes, 0.5 ml of isopropyl alcohol was added, mixed and incubated at room temperature for 5 minutes then centrifuged at 12000xg for 10 minutes at room temperature. The supernatant was discarded and the RNA pellet washed twice with 1 ml of 75% chilled ethanol by mixing and vortexing for 15 seconds then centrifuging at 12000xg for 5 minutes at room temperature. The pellet was air-dried for 5 minutes and dissolved in 60 µl DEPC water then stored at -20°C [12].

Synthesis of cDNA from the isolated RNA was done according to the user manual (Thermo Fisher Scientific, Waltham, USA). The RNA was treated with DNase to eliminate any traces of genomic DNA. To 1 µg of RNA, 1 µL of 10X Reaction Buffer with MgCl₂ and 1 µL of RNase-free DNase I were added and the volume adjusted to 10 µL. The mixture was incubated for 30 minutes at 37°C and 1 µL 50 mM EDTA added and incubated at 65°C for 10 minutes to inactivate DNase I. The prepared RNA was used as template for reverse transcriptase, where 5 µg of the RNA was added to a reaction mix of 1 µL Oligo (dT) 18 primers and nuclease-free water to a volume of 12 µL, 4 µL 5X Reaction Buffer, 1 µL RiboLock RNase Inhibitor, 2 µL 10 mM dNTP Mix and 1 µL RevertAid M-MuLV RT. The mixture was incubated for 60 minutes at 42°C and the reaction terminated by heating at 70°C. The resulting cDNA used as a template for PCR.

Reverse Transcriptase Polymerase Chain Reaction (rtPCR)
The standard PCR conditions were used to detect genes in cDNA (Cooper and Hausman, 2013). PCR reagents were mixed in a 200 µl tube to a final reaction volume of 25 µl (Table 1). PCRs were done using the Eppendorf Mastercycler Pro (Eppendorf AG, Hamburg) programmed as shown in Table 2. Difficult PCRs were carried out by optimizing reaction conditions, in particular the annealing temperature, using the cycler’s gradient PCR feature. The sequences of the primers used were TCCACACACGTTCAGCAGTT for the forward primer and TGTACACGTA TCGCCGTTTC for the reverse primer.

### Table 1. Composition of master mix for PCR amplification.

| Reagent                  | Final Concentration | Final volume (µl) |
|-------------------------|---------------------|------------------|
| Buffer (× 10)           | × 1                 | 5                |
| dNTPs (10 mM)           | 0.5 mM              | 2.5              |
| MgCl₂ (50 mM)           | 2.5 mM              | 2.5              |
| Primer1 (2 µM)          | 0.25 µM             | 6.25             |
| Primer2 (2 µM)          | 0.25 µM             | 6.25             |
| Taq (5 U/µl)            | 1 U/rxn             | 0.5              |
| Template (10 ng/µl)     | 1 µg                 | 2                |
| dH₂O                    | To 25 µl            |                  |

### Table 2. Conditions for PCR reactions.

| Step                      | No. of cycles | Temperature (°C) | Time  |
|---------------------------|---------------|------------------|-------|
| Initial denaturation      | 1             | 95               | 5 min.|
| Denaturation              | 9             | 95               | 30 sec.|
| Annealing                 | 9             | 48.9             | 30 sec.|
| Extension                 | 4             | 72               | 30 sec.|
| Final extension           | 1             | 72               | 8 min.|

#### 2.5. Data Analysis

Data on growth parameters such as leaf length of stressed and unstressed plants as well as other parameters such as fresh weights and dry weights of seedlings were stored in excel sheet and analysed using ANOVA at 95% confidence interval with SAS statistical computer software. Mean separation was done using Tukey’s pairwise comparison test at 5% probability level.

#### 3. Results

##### 3.1. Effect of Drought Stress on Mature Leaf Lengths of Maize Seedlings

The growth patterns of the fourth leaf of all the tropical maize under unstressed/control conditions, mild drought stress and severe drought stress were similar. Differences were only observed at the time when the plants reached maximum leaf length and in the mature lengths of the plant's fourth leaves. Under control conditions, inbred line CML 144 was the first to attain maximum leaf length by the 11th day, followed by genotype E04 which attained maximum leaf length by the 12th day post leaf emergence. The fourth leaf of genotype CML 216 achieved maximum length on the 13th day post-emergence (DPE) while the last genotype to attain maximum leaf length of the fourth leaf under control conditions was A04 on the 14th DPE (Figure 1).
Figure 1. Leaf lengths of four inbred lines under control conditions (A), moderate drought (B) and severe drought (C).

Table 3. Mature leaf lengths and growth rates of the fourth leaf under different drought stress levels.

| Growth rates | Mature leaf lengths |
|--------------|---------------------|
| Line         | Control | Mild drought | Severe drought | Control | Mild drought | Severe drought |
| CML216       | 1.77±0.24<sup>a</sup> | 1.65±0.17<sup>b</sup> | 1.26±0.06<sup>a</sup> | 38.83±3.18<sup>a</sup> | 36.06±1.66<sup>b</sup> | 27.94±0.63<sup>a</sup> |
| CML144       | 1.60±0.26<sup>b</sup> | 1.86±0.22<sup>ab</sup> | 1.39±0.13<sup>a</sup> | 41.00±0.99<sup>a</sup> | 41.25±0.69<sup>ab</sup> | 30.38±0.92<sup>a</sup> |
| A04          | 1.56±0.22<sup>b</sup> | 1.66±0.20<sup>b</sup> | 1.42±0.09<sup>a</sup> | 35.42±0.23<sup>a</sup> | 37.00±0.50<sup>ab</sup> | 30.79±0.33<sup>a</sup> |
| E04          | 2.02±0.29<sup>a</sup> | 1.97±0.21<sup>a</sup> | 1.43±0.08<sup>a</sup> | 43.94±3.99<sup>a</sup> | 43.17±1.34<sup>a</sup> | 31.28±1.08<sup>a</sup> |

Values with the same letter in the same column were not significantly different by Tukey's pair-wise comparison (P < 0.05).

When mild/moderate drought stress was exerted, the maximum leaf length of genotype CML 216 was attained on the 13<sup>th</sup> DPE. All the other genotypes experienced a delay in onset of maximum leaf length. CML 144 attained maximum leaf length on the 15<sup>th</sup> DPE while genotypes E04 and A04 attained maximum leaf length on the 16 DPE (Figure 1). When the maize genotypes were subjected to severe drought stress, further delay in the attainment of the maximum length of the fourth leaf was observed. Inbred maize lines CML 216, A04 and E04 attained maximum leaf lengths on the 20<sup>th</sup> DPE while CML 144 attained maximum leaf length on the 18<sup>th</sup> DPE (Figure 1). ANOVA revealed that there was no significant difference in the mature leaf lengths of all maize genotypes under control conditions (p = 0.2063) as well as the mature leaf lengths of all maize genotypes under severe drought stress (p = 0.0702). However, under moderate stress, it was observed that there was a significant difference in the mature leaf lengths of the four maize genotypes under study (P = 0.0066).

Under control conditions, mature leaf lengths ranged from 43.94 cm for maize inbred line E04 to 35.42 cm for maize inbred line A04, with genotypes CML 144 and CML 216 registering intermediate mature leaf lengths of 41.00 cm and 38.83 cm respectively. Under mild drought stress, the longest mature leaf length of 43.17 cm was observed in genotype E04, followed by genotypes CML 144 and A04 whose mature leaf length was 41.25 cm and 37.00 cm respectively. CML 216 under mild stress registered the least mature leaf length of 36.06 cm. The mature leaf lengths under severe drought stress were 31.28 cm for E04, 30.79 cm for genotype A04, 30.38 cm for genotype CML 144 and 27.94 cm for inbred maize line CML 216 (Table 3). Irrespective of the genotype, ANOVA also revealed that there was a significant difference in the mature lengths of the fourth leaf (p < 0.0001) based on the level of stress exerted. Although there was no significant difference in the mature length of the fourth leaf under control and mild drought stress, mature leaf lengths at these stress levels were significantly different from those observed under severe drought stress. Leaf folding and drooping were observed in seedlings subjected to moderate and severe drought stresses and the severity of the folding of leaves increased with the level of stress and the day temperature.

3.2. Relative Growth Rate of the Fourth Leaf of the Maize Seedlings

Under all drought conditions, growth rate followed a similar trend. All genotypes showed accelerated growth up to a certain maximum after which the rate of growth started to decelerate. Maize plantlets which were not subjected to drought stress had a relatively higher growth rate than those subjected to moderate and severe drought stress and achieved maximum growth early. Under control conditions, the onset of maximum growth rate ranged from 7<sup>th</sup> to 10<sup>th</sup> DPE. CML144 was the first to achieve maximum growth rate by the 7<sup>th</sup> DPE (5.3 cm day<sup>-1</sup>), followed by E04 (5.6 cm day<sup>-1</sup>) and CML 216 (4.5 cm day<sup>-1</sup>) on the 8<sup>th</sup> DPE while A04 achieved maximum growth rate (5 cm day<sup>-1</sup>) at the 10<sup>th</sup> DPE. Analysis of variance revealed that there was a significant difference in the growth rate of the fourth leaf based on the
different genotypes under control conditions \((p = 0.0034)\). The highest mean growth rate was observed in genotype E04 (2.02 cm day\(^{-1}\)), followed by genotypes A04 (1.77 cm day\(^{-1}\)), CML 144 (1.59 cm day\(^{-1}\)) and CML 216 (1.56 cm day\(^{-1}\)) (Table 3).

After subjecting seedlings to moderate stress, the rate of growth reduced. Although maximum growth rate was achieved at the same time as that of the controls, the rate of growth at that time was relatively lower. CML 144 had a maximum growth rate of 4.04 cm day\(^{-1}\), while genotypes E04 and CML 216 had maximum growth rates of 4.11 cm day\(^{-1}\). Genotype A04 at moderate drought stress had a maximum growth rate of 3.72 cm day\(^{-1}\). Again, analysis of variance revealed that subjecting maize seedlings to moderate drought stress impacted a significant difference in the growth rate of the fourth leaf based on the different genotypes \((p = 0.0021)\). High mean growth rate were observed in genotypes E04 (1.97 cm day\(^{-1}\)) and CML 144 (1.86 cm day\(^{-1}\)), while inbred lines CML 216 and A04 had the least growth rates of 1.67 cm day\(^{-1}\) and 1.65 cm day\(^{-1}\) respectively.

Subjecting maize seedlings to severe drought stress caused a marked reduction in the rate of growth in all genotypes. Maximum growth set in later except for CML 144 whose maximum growth rate was observed at the 7\(^{th}\) DPE but reduced steadily up to the 19\(^{th}\) DPE when growth stopped. CML 216 showed maximum growth rate at the 12\(^{th}\) DPE (2.08 cm day\(^{-1}\)) while A04 showed maximum growth rate at the 13\(^{th}\) DPE (1.89 cm day\(^{-1}\)). All genotypes under severe stress showed a slow and prolonged growth rate compared to moderate drought and control conditions. Analysis of variance revealed that there was no significant difference in the mean growth rate of the fourth leaf of maize seedlings based on the different genotypes. Mean growth rates ranged from 1.43 cm day\(^{-1}\) for genotype E04 to 1.26 cm day\(^{-1}\) for genotype A04. Genotypes CML 216 and CML 144 had intermediate growth rates of 1.42 cm day\(^{-1}\) and 1.39 cm day\(^{-1}\) when subjected to severe drought stress.

### 3.3. Impact of Drought on Fresh and Dry Weights of Seedlings

Drought stress was observed to impact the fresh and dry weights of maize seedlings differently. When maize seedlings were not subjected to drought stress, it was observed that CML 144 seedlings had the highest fresh weight (16.39 g), followed by A04 (15.70 g) and E04 (15.57 g) while CML 216 had the least fresh weight (11.97 g). These fresh weight measurements were taken 30 days post emergence of the fourth leaf. Analysis of variance revealed that the fresh weights of genotypes CML 144, A04 and E04 were significantly different from that of genotype CML 216 \((p = 0.0008)\). After subjecting seedlings to moderate drought stress, ANOVA also revealed that there was a significant difference in the fresh weights of seedlings \((p = < 0.0001)\) based on the different genotypes. Genotype A04 registered the highest fresh weight after subjection to moderate drought stress (12.56 g), followed by E04 (11.68 g) and CML 144 (10.49 g) while CML 216 had the least fresh weight (8.08 g).

After maize seedlings were subjected to severe drought stress, ANOVA also revealed that there was a significant difference in the fresh weights based on the different genotypes \((p = < 0.0001)\). The highest fresh weights of maize seedlings under severe stress were observed in genotypes E04 (6.78 g), A04 (5.67 g) and CML 144 (5.37 g), with genotype CML 216 registering the least fresh weight (1.78 g) under severe drought stress (Table 4). Irrespective of the genotype, ANOVA also revealed that there was a significant difference in the fresh weights of maize seedlings based on the different drought stress levels \((p = < 0.0001)\). The highest fresh weight was observed in seedlings under control conditions (14.90 g), followed by those subjected to moderate drought stress (10.70 g) and the least registered by seedlings subjected to severe drought stress (4.89 g).

After seedlings were dried, it was observed that there was no significant difference in the dry weights of seedlings which had not been subjected to drought stress. Dry weights of unstressed maize seedlings ranged from 1.79 g for genotype A04 to 1.48 g in CML 216. However, subjecting maize seedlings to moderate and severe drought had a significant impact on their dry weights. Maize seedlings subjected to moderate drought had dry weights ranging from 1.67 g in A04 to 0.96 g in E04 while CML 144 and CML 216 registered dry weights of 1.34 g and 1.03 g respectively. Analysis of variance revealed that there was a significant difference in the dry weights of the different maize genotypes after subjection to moderate drought \((p = < 0.0001)\). Very low dry weights were registered when maize seedlings were subjected to severe stress. Genotypes A04 and CML 144 registered dry weights of 0.79 g and 0.70 g respectively while E04 and CML 216 registered dry weights of 0.42 g and 0.33 g respectively (Table 4). ANOVA revealed that based on the stress level and irrespective of the genotype, there was a significant difference in the observed dry weights of the maize seedlings \((p = < 0.0001)\). The mean dry weight under control conditions was 1.65 g, while that of moderate drought stress was 1.25 g and 0.56 g under severe drought stress.

### Table 4. Fresh and dry weights in grams of 4-week-old seedlings after subjection to different drought conditions.

| Line   | Freshweight | Milddrought | Severedrought | Dryweight | Control | Milddrought | Severedrought |
|--------|-------------|-------------|---------------|-----------|---------|-------------|---------------|
|        |             | Control     |               |           | Control | Milddrought | Severedrought |
| A04    | 15.70±0.99\(^a\) | 12.56±0.45\(^b\) | 5.67±0.27\(^b\) | 1.79±0.13\(^a\) | 1.67±0.08\(^a\) | 0.79±0.04\(^a\) |
| CML 144| 16.39±0.88\(^a\) | 10.49±0.59\(^b\) | 5.37±0.32\(^a\) | 1.77±0.20\(^a\) | 1.34±0.09\(^a\) | 0.70±0.03\(^a\) |
| CML 216| 11.97±0.62\(^a\) | 8.08±0.15\(^b\) | 1.78±0.74\(^b\) | 1.48±0.10\(^a\) | 1.03±0.09\(^b\) | 0.33±0.04\(^a\) |
| E04    | 15.57±0.29\(^a\) | 11.68±0.82\(^a\) | 6.78±0.19\(^a\) | 1.58±0.09\(^a\) | 0.96±0.05\(^b\) | 0.42±0.03\(^b\) |

Values with the same letter in the same column were not significantly different by Tukey’s pair-wise comparison \((P < 0.05)\).
3.4. Relative Expression of PARP2 Gene Under Drought Stress

As determined by agarose gel electrophoresis of the isolated maize RNA, good quality high concentration RNA was obtained for further molecular work. After cDNA conversion of the obtained RNA, RT-PCR using gene specific primers designed from PARP2 exon gene regions revealed that there were differences in expression of the gene under different drought conditions in the different genotypes under study. Based on the bands observed on 1.5% agarose gel, it was possible to amplify the PARP2 gene in all the genotype samples obtained from severe drought and moderate stress samples. Amplification of the PARP2 gene from samples obtained from moderate drought stress in all the four maize genotypes was also possible. In genotypes CML144, E04 and A04, relative expression of PARP2 gene was only detected under moderate and severe drought conditions (Figure 2). Contrary to expectations, RT-PCR revealed amplification of the gene under unstressed conditions for Genotype CML 216 in one of the samples (Figure 3) in addition to expression at moderate and severe stress conditions. All other genotypes did not exhibit amplification of the gene at control conditions.

By comparison, it was observed that expression of the gene under moderate stress was relatively lower than that observed at severe drought stress based on the brightness of the observed bands. The RT-PCR performed using the PARP2 gene primers from converted cDNA from drought stressed and unstressed plants produced an amplification of the expected band size of 938 bp.

![Figure 2](image1.png)
*Figure 2. Agarose gel electrophoresis of the PCR products obtained after amplification of cDNA form maize genotype E04 using PARP2 primers. SS samples were from plants under severe drought stress, MS samples were from plants subjected to moderate stress while C samples were from plants which were not subjected to drought stress.*

![Figure 3](image2.png)
*Figure 3. Agarose gel electrophoresis of the PCR products obtained after amplification of cDNA form maize genotype CML 216 using PARP2 primers. SS samples were from plants under severe drought stress, MS samples were from plants subjected to moderate stress while C samples were from plants which were not subjected to drought stress.*

4. Discussion

This study established that drought stress affects certain aspects of maize growth. Leaf length, growth rate, fresh weight and dry weight were observed to be significantly lower in plants subjected to severe drought stress compared to those that were not subjected to drought. Although it was observed that different maize genotypes responded differently to drought stress, the final outcome of drought damage in all genotypes was similar such as the reduction in growth rate, fresh weight and dry weight. Previous studies showed that drought stress hinders the growth and development of maize. Khan et al. [2001] observed that components of growth in maize variety YHS 202 that were affected by drought included height, leaf area index, root structure, biomass, fresh weight, dry weight and diameter of the stem. Aslam et al. [2015] reported cases of leaf folding and drooping in maize plants subjected to drought stress levels of 50% and 25% of field capacity, a phenomenon which was also observed in our study. Leaf folding and drooping is thought to be a genetically controlled strategy by the plants to withstand drought. It is controlled by several genes among them ZMNF-YB2 in maize [16].

In this study, it was observed that the rate of growth in all the maize genotypes was higher in plants that were not subjected to drought stress than those subjected to moderate and severe drought stress. Reduction in the rate of growth due to onset of drought stress can be attributed to turgor loss in expanding cells and metabolic regulation in the plant. Loss of turgor in expanding cells leads to inhibition of cell division. Regulation in metabolism due to onset of drought is an adaptive mechanism by the plant to restrict increase in
size of transpiring leaf area under drought [17]. Reduction in fresh weight as well as dry weights of plants as drought progressed in this study could be attributed to several factors. These factors include; leaf rolling and drooping under drought stress, leaf wilting which leads to blocking of stomata and reduced gas exchange in plants experiencing drought stress. All these factors lead to reduction in the plant leaf area exposed to sunlight hence reduced photosynthesis. This eventually leads to a reduction in the plants biomass as a result of reduced food accumulation and carbon assimilation [18].

The role of poly (ADP-ribose) polymerase (PARP) in stress tolerance and energy homeostasis in plants has been described and it is now agreed that this protein is involved in the plant's ability to tolerate or succumb to DNA damage. Ionising radiations induce the expression of PARP1 gene in plants while accumulation of toxic metals like cadmium as well as dehydration trigger the expression of PARP2 [19]. Dehydration stresses that can trigger expression of PARP2 gene in plants range from biotic to abiotic stresses. These include insect damage, fungal and bacterial pathogens, cold stress, light stress, mechanical damage and drought stress [20]. This explains why there was an expression of the PARP2 gene in this study under conditions where drought was absent in genotype CML 216. This finding suggests that other forms of stress had set in to trigger the expression of the PARP2 gene. It has been shown that PARP2 gene in plants is the most important of all PARP genes in enabling the plant respond to DNA damage and induce immune response [21]. As observed in this study, dehydration stress at moderate and severe drought stress triggered the expression of this gene in maize lines CML 216, CML 144, E04 and A04.

From this study, it was observed that the maize line E04, which had the longest leaves under control and moderate drought stress had the lowest expression of PARP2 gene. The maize line E04 was therefore able to maintain energy homeostasis by reducing the breakdown of NAD⁺, hence conserving energy. This explains why E04 plants had the longest leaves compared to other plants and suggests an inverse relationship between PARP2 gene expression and drought survival as well as good expression of physiological traits. When plants experience drought stress, there is an increase in expression of PARP genes, which leads to a rapid breakdown of the NAD⁺ pool. As a result, re-synthesis of NAD⁺ is stimulated, leading to use of three to five molecules of ATP for every molecule of NAD⁺ synthesized. This eventually leads to depletion of ATP and onset of apoptosis [19]. To counter this damaging effect of the expression of PARP genes, chemical inhibitors and genetic mutations have been employed. Inhibition of expression of PARP genes leads to over-expression of other genes that respond to stimuli, abiotic stresses, JA, ABA, lipids and secondary metabolites. Therefore, it has been shown that PARP mutants can tolerate abiotic stresses unlike other plants expressing PARP genes [22].

5. Conclusion

This study established how tropical inbred maize lines CML 144, CML 216, E04 and A04 respond to growth limiting drought stress. The study further showed that these maize genotypes respond differently to drought stress, with devastating effects of water limitation observed under severe drought conditions in all genotypes. It was also observed that fresh and dry weights of maize reduce with increasing onset of drought. The expression of PARP2 gene was directly proportional to the level of drought experienced by maize plants. At high levels of drought stress, there is high expression of the PARP2 gene while at low levels of drought stress, the gene is expressed at low levels or not expressed at all.

References

[1] Ranum P, Peña-Rosas JP, and Garcia-Casal MN. 2014. Global maize production, utilization, and consumption. Ann NY Acad Sci 1312:105–112.

[2] FAO. 2015. FAOSTAT database collections. Food Agric Organ United Nations. Rome.

[3] Ribaut J-M, Betran J, Monneveux P, and Setter T. 2009. Drought Tolerance in Maize, p. 311–344. Hand book of maize: its biology.

[4] Heisey PW, and Edmeades GO. 1997. World Maize Facts and Trends 1997 / 98 Maize Production in Drought-Stressed Environments : Technical Options and Research Resource Allocation/Agricultural Economics. Mexico.

[5] Caims JE, Sonder K, Zaidi PH, Verhulst N, Mahuku G, Babu R, Nair SK, Das B, Govaers B, Vinayan MT, Rashid Z, Noor JJ, Devi P, San Vicente F, and Prasanna BM. 2012. Maize production in a changing climate. impacts, adaptation, and mitigation strategies/Advances in Agronomy, 1sted.

[6] Lamb RS, Citarella M, and Teotia S. 2012. Functions of the poly (ADP-ribose) polymerase superfamily in plants. Cell Mol Life Sci 69:175–189.

[7] Hashida SN, Takahashi H, and Uchimiy H. 2009. The role of NAD biosynthesis in plant development and stress responses. Ann Bot 103:819–824.

[8] Vanderauwera S, De Block M, Van de Steene N, van de Cotte B, Metzlaff M, and Van Breusegem F. 2007. Silencing of poly (ADP-ribose) polymerase in plants alters abiotic stress signal transduction. Proc Natl Acad Sci U S A 104:15150–15155.

[9] Ragot M, Gay G, Muller JP, and Durovray J. 1999. Efficient Selection for Adaptation to the Environment through QTL Mapping and Manipulation in Maize, p. 128–130. In Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-limited Environments. CIMMYT, Mexico D. F.

[10] Verelst W, Bertolini E, De Bodt S, Vandepoele K, Demeulemaere M, Pè ME, and Inzé D. 2013. Molecular and physiological analysis of growth-limiting drought stress in brachypodium distachyon leaves. Mol Plant 6:311–322.
[11] Bouchabké O, Tardieu F, and Simonneau T. 2006. Leaf growth and turgor in growing cells of maize (Zea mays L.) respond to evaporative demand under moderate irrigation but not in water-saturated soil. Plant, Cell Environ 29:1138–1148.

[12] Sah SK, Kaur G, and Kaur A. 2014. Rapid and Reliable Method of High-Quality RNA Extraction from Diverse Plants. Am J Plant Sci 5:3129–3139.

[13] Cooper GM, and Hausman RE. 2013. The Cell: A Molecular Approach, 6th ed. ASM Press, Washington D. C.

[14] Khan MB, Hussain N, and Iqbal M. 2001. Effect of water stress on growth and yield components of maize variety YHS202. J Res (Science), Bahauddin Zakariya Univ Multan, Pakistan 12:15–18.

[15] Aslam M, Maqpool M, and Cengiz R. 2015. Effects of drought on maize, p. 5–18. In Drought Stress in Maize (Zea Mays L.). Springer International Publishing.

[16] Entringer GC, Guedes FL, Oliveira AA, Nascimento JP, and Souza JC. 2014. Genetic control of leaf curl in maize. Genet Mol Res 13:1672–1678.

[17] Blum A. 2011. Plant Water Relations, Plant Stress and Plant Producción, p. 11–52. In Plant Breeding for Water-Limited Environments. Springer Science+Business Media, Berlin, Germany.

[18] Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Carvalho I, Faria T, and Pinheiro C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. Ann Bot 89:907–916.

[19] De Block M, Verduyn C, De Brouwer D, and Cornelissen M. 2005. Poly (ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. Plant J 41:95–106.

[20] O’Kane D, Gill V, Boyd P, and Burdon R. 1996. Chilling, oxidative stress and antioxidant responses in Arabidopsis thaliana callus. Planta 198:371–377.

[21] Song J, Keppler BD, Wise RR, and Bent AF. 2015. PARP2 Is the Predominant Poly (ADP-Ribose) Polymerase in Arabidopsis DNA Damage and Immune Responses. PLoS Genet 11:1–24.

[22] Schulz P, Jansseune K, Degenkolbe T, Meret M, Claey H, Skirycz A, Teige M, Willmitzer L, and Hannah MA. 2014. Poly (ADP-Ribose) polymerase activity controls plant growth by promoting leaf cell number. PLoS One 9:1–15.