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

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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 21days 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, subjecting 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 1billion 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

causes of the low yield of maize in Africa is drought in addition to other abiotic and biotic factors.

Drought, like other stresses in maize causes adverse crop losses. It is the leading single cause of severe food shortage in developing countries whose consequences exceed those of conflicts and floods [3]. Since water resources are constantly getting depleted, there is need to develop drought tolerant maize lines as well as assess drought response in the existing lines. Drought is responsible for yield losses of up to 15%, 53% and 30% in SSA when it occurs at pre-flowering, flowering and post flowering in maize [4]. Accompanied by heat, drought stress in maize results in crop damage as a result of the destruction of cellular organization. This damage includes protein denaturation, increase in fluidity of lipid membranes, inactivation of enzymes and inhibition of protein synthesis [5]. The outward manifestation of drought damage in maize includes reduced growth rate, reduction of fresh and dry weights as well as subsequent drying and death of plants.

When plants experience drought, the Poly ADP-Ribose (PAR) protein is synthesized by the Poly (ADP-Ribose) Polymerase *(PARP)* enzyme. The synthesis of this protein is an energy depleting process and can lead to different responses such as cellular defence under mild stress, DNA repair under moderate stress and cell death under severe stress [6]. There are two pathways which lead to the formation of PARP. Both pathways are energy consuming hence one of the main causes of cell death under severe drought stress. The pathways involve depletion of nicotinamide adenine dinucleotide (NAD^+) as a substrate and subsequent utilization of ATP [7]. In transgenic *PARP*-deficient plants, disruption of the gene results in a broad spectrum abiotic stress tolerance. The reduction of ATP consumption avoids extensive respiration in the mitochondria and therefore inhibits the formation of Reactive Oxygen Species (ROS) which form under almost all abiotic stress conditions [8]. This study evaluated how tropical inbred maize lines respond to different levels of drought stress, as well as determined the expression of maize *PARP2* gene under different drought levels through rtPCR.

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 Kenyatta University Plant Transformation Laboratory. The inbred lines CML 144 and CML 216 are generally late maturing, resistant to maize streak virus and insect pests, are tolerant to acidic soils as well as adapted to the tropical climate [9]. These lines do not grow very tall hence are easy to pollinate. A04 and E04 are Kenyan inbred lines commonly used as parental lines of hybrids and are successful in highland environments.

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 21st 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 $MgCl_2$ 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 TGTACACGTATCGCCGTTTC for the reverse primer.

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

Reagent	Final Concentration	Finalvolume (µl)
Buffer (× 10)	× 1	5
DNTPs (10 mM)	0.5mM	2.5
$MgCl_2$ (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)		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	25	95	30 sec.
Annealing		48.9	30 sec.
Extension		72	30 sec.
Final extension	1	72	8 min.

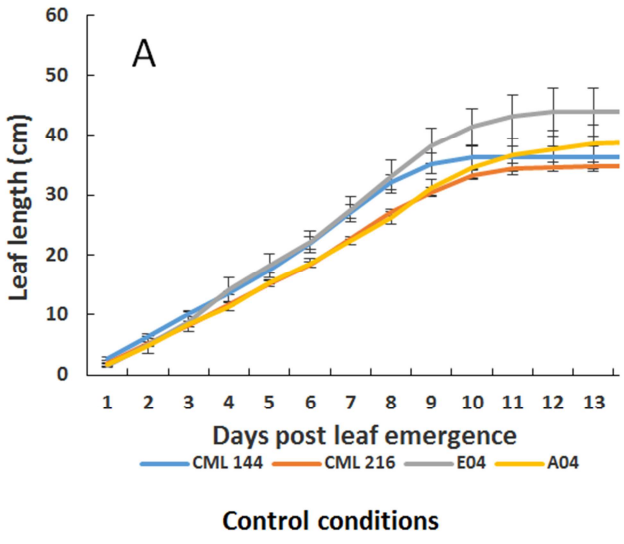
2.5. Data Analysis

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).



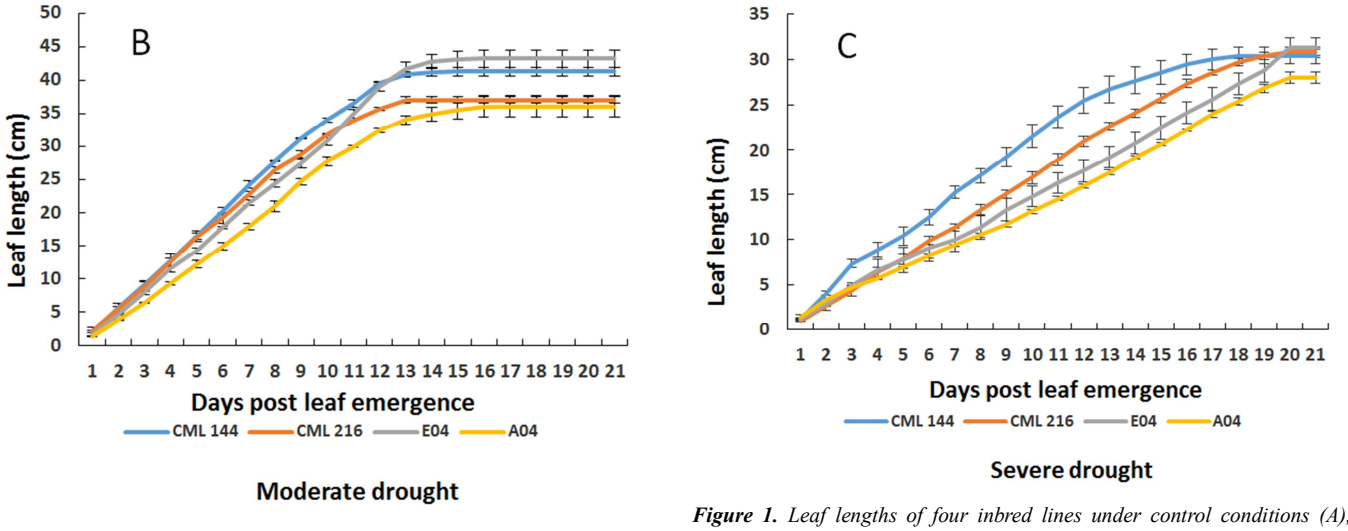


Figure 1. *Leaf lengths of four inbred lines under control conditions (A), moderate drought (B) and severe drought (C).*

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

Growthrates				Matureleaflengths		
Line	**Control**	**Mild drought**	**Severe drought**	**Control**	**Mild drought**	**Severe drought**
CML216	1.77±0.24^{ab}	1.65±0.17^{b}	1.26±0.06^{a}	38.83±3.18^{a}	36.06±1.66^{c}	27.94±0.63^{a}
CML144	1.60±0.26^{b}	1.86±0.22^{ab}	1.39±0.13^{a}	41.00±0.99^{a}	41.25±0.69^{ab}	30.38±0.92^{a}
A04	1.56±0.22^{b}	1.66±0.20^{b}	1.42±0.09^{a}	35.42±0.23^{a}	37.00±0.50^{bc}	30.79±0.33^{a}
E04	2.02±0.29^{a}	1.97±0.21^{a}	1.43±0.08^{a}	43.94±3.99^{a}	43.17±1.34^{a}	31.28±1.08^{a}

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 13th DPE. All the other genotypes experienced a delay in onset of maximum leaf length. CML 144 attained maximum leaf length on the 15th 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 20th DPE while CML 144 attained maximum leaf length on the 18th 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 excreted. 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 7th to 10th DPE. CML144 was the first to achieve maximum growth rate by the 7th DPE (5.3 cm day^{-1}), followed by E04 (5.6 cm day^{-1}) and CML 216 (4.5 cm day^{-1}) on the 8th DPE while A04 achieved maximum growth rate (5 cm day^{-1}) at the 10th 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 7th DPE but reduced steadily up to the 19th DPE when growth stopped. CML 216 showed maximum growth rate at the 12th DPE (2.08 cm day^{-1}) while A04 showed maximum growth rate at the 13th 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			Dryweight		
	Control.	Milddrought	Severedrought	Control.	Milddrought	Severedrought
A04	15.70±0.99^{a}	12.56±0.45^{a}	5.67±0.27^{a}	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^{a}	5.37±0.32^{a}	1.77±0.20^{a}	1.34±0.09^{b}	0.70±0.03^{a}
CML 216	11.97±0.62^{b}	8.08±0.15^{b}	1.78±0.74^{b}	1.48±0.10^{a}	1.03±0.09^{bc}	0.33±0.04^{b}
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^{c}	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.

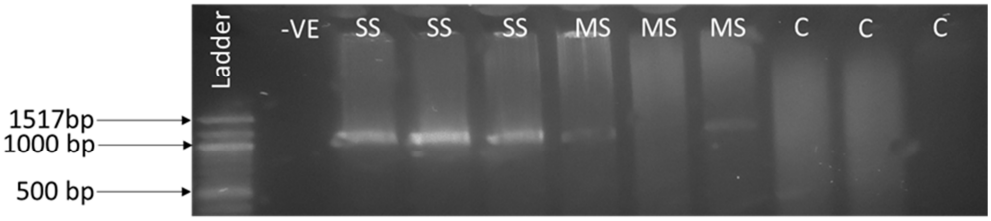


Figure 2. *Agarose gel electrophoresis of the PCR products obtained after amplification of cDNA form maize genotype E04 using PARP2 primers. SS samples were from plants under severe drought stress, MS samples were from plants subjected to moderate stress while C samples were from plants which were not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

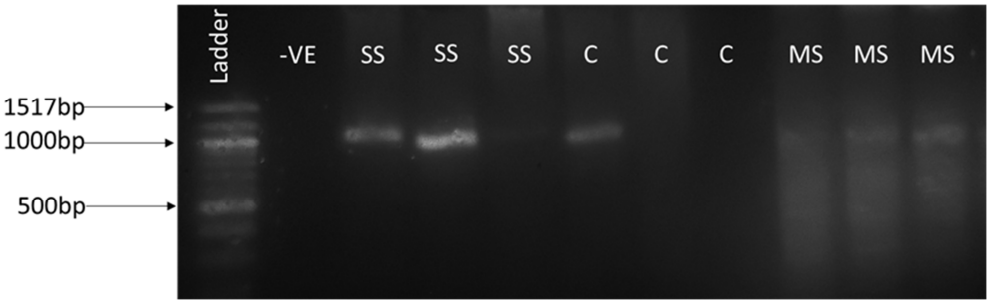


Figure 3. *Agarose gel electrophoresis of the PCR products obtained after amplification of cDNA form maize genotype CML 216 using PARP2 primers. SS samples were from plants under severe drought stress, MS samples were from plants subjected to moderate stress while C samples were from plants which were 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.

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