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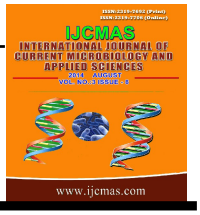

Original Research Article

Using Molecular Markers to Study Mechanics Responsible For Drought Tolerance in Some Genotypes of Sorghum

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A B S T R A C T

Keywords

Sorghum, drought tolerance, protein, specific-PCR-markers,

Five lines of sorghum namely, LXROG 13, GZ3423-3-5-4-1, GZ7865-6-9-7-3, GZ4534-7-4-3-1 and GZ1324-8-6-5-2 with different reaction of drought tolerance were grown under normal and drought irrigation in green house in the farm of Mansoura university during 2013 season to study the ability of drought tolerance in sorghum plants using SDS-Protein electrophoresis, Isozymes electrophoresis and Specific-PCR analysis through using RTGIS 5 primer as index for high level of maximum root length in cells, GTLO 14 primer as index for hygroscopic cells, AMR 14 Primer as index for waxy layers in leaves of sorghum and HSC12 primer as index for Glycine betaine in cells for drought tolerance in sorghum . In light of previous results turned out to be the lines, GZ3423-3-5-4-1, GZ4534-7-4-3-1 and GZ1324-8-6-5-2 were highly tolerance of drought under Egypian conditions.

Introduction

In nature or crop fields, water is often the most limiting factor for plant growth. If plants do not receive adequate rainfall or irrigation, the resulting drought stress can reduce growth more than all other environmental stresses combined. Drought can be defined as the absence of rainfall or irrigation for a period of time sufficient to deplete soil moisture and injure plants. Drought stress results when water loss from the plant exceeds the ability of the plant's roots to absorb water and when the plant's water content is reduced enough to interfere with normal plant processes. A plant responds to a lack of water by halting growth and reducing photosynthesis and other plant processes in order to reduce water use. As water loss progresses, leaves of some species may appear to change color—usually to blue-green. Foliage begins to wilt and, if the plant is not irrigated, leaves will fall off and the plant will eventually die. Drought lowers the water potential of a plant's root and upon extended

exposure, abscisic acid is accumulated and eventually stomatal closure occurs. This reduces a plant's leaf relative water content. The time required for drought stress to occur depends on the water-holding capacity of the soil, environmental conditions, stage of plant growth, and plant species. Plants growing in sandy soils with low water-holding capacity are more susceptible to drought stress than plants growing in clay soils. A limited root system will accelerate the rate at which drought stress develops. A root system may be limited by the presence of competing root systems, by site conditions such as compacted soils or high water tables, or by container size (if growing in a container). A plant with a large mass of leaves in relation to the root system is prone to drought stress because the leaves may lose water faster than the roots can supply it. Newly installed plants and poorly established plants may be especially susceptible to drought stress because of the limited root system or the large mass of stems and leaves in comparison to roots.This proposal is focused on supporting increases in the yield and yield stability of sorghum through research, specifically on the topic of development and deployment of water management and water use efficiency. The decision to grow sorghum is frequently based on its drought tolerance. The importance of drought tolerance in agriculture is likely to grow agriculture uses 69% of the world's available water supply, and 46% of available water in the USA. Many parts of the world, including some parts of the USA, face "water scarce" conditions in the future. The development of drought-resistant crops by conventional breeding has been hampered by low heritability, and by large 'genotype x environment' interactions. Conventional sorghum breeding has only utilized a small subset of the available germplasm was hypothesize that a substantial degree of phenotypic variation in responses to drought exists and remains among a broad sampling of sorghum genotypes. The proposed activities build upon a detailed physiological and agronomic characterization of a 'diversity panel' of 384 geneotypes that broadly samples worldwide sorghum diversity, let by our cooperators H. Upadhyaya (germplasm curator), v. Vadez (plant physiologist), and C. T. Hash (sorghum breeder) at ICRISAT. Numerouse –omics approaches offer the means to develop testable hypotheses about possible relationships between specific genes or gene families and drought response. Our work will identify specific genes, DNA markers, and possibly even nucleotides, that are diagnostic of particular drought responses. These DNA markers may accelerate progress in sorghum improvement through either marker-assisted selection or through identification of specific genes that make singularly large contributions to sorghum drought tolerance. Because the diversity panel to be studied broadly samples worldwide sorghum diversity, molecular-level results are expected to have a very broad relevance to the sorghum gene pool generally, and phenotypic results are expected to identify a broad sampling of drought tolerant germplasm, different subsets of which are likely to be adaptable to different regions. The benefits of using such germplasm lines might be determined quickly, by their evaluation in hybrid combinations with wiedly-used inbreds already adapted to respective target regions.

Materials and Methods

Five lines of sorghum namely, LXROG 13**,** GZ3423-3-5-4-1**,** GZ7865-6-9-7-3, GZ4534-7-4-3-1 and GZ1324-8-6-5-2 with different reaction of drought tolerance were grown under normal and drought irrigation in three replicates for each condition of irrigation

under green house conditions in may 2013.

Normal irrigation:-The first irrigation after 21 day from sowing,the second irrigation after 21 day from the first one, one irrigate every 15 day and prevent irrigation before harvesting with 21 days.

Drought irrigation:- The first irrigation after 21 day from sowing and the second irrigation after 21 day from the first one only Until harvest.

Table.1 The pedigree and reaction for Drought tolerance in sorghum

Geno types	Pedigree	Drought tolerance	Grain shape	Duration (Day)
P1	LXROG 13	Moderate	Short	160
P2	GZ3423-3-5-4-1	Tolerance	Short	155
P3	GZ7865-6-9-7-3	Moderate	Long	145
P4	GZ4534-7-4-3-1	Tolerance	Medium	165
P5	GZ1324-8-6-5-2	Tolerance	Medium	149

Traits studied

Molecular Genetic Studies

SDS-Protein electrophoresis

Sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) was used to study the protein banding patterns of the five lines of sorghum under normal and drought conditions. Protein fractionation was performed according to the method of Laemmli (1970) as modified by Studier (1973).

Isozymes electrophoresis

Native – polyacrylamide gel electrophosis (native-PAGA) was conducted according to Stegemann et al., (1985) to identify isozyme variations between normal and drought conditions on the five lines of sorghum using two isozymes systems; peroxidase and polyphenol oxidase, respectively.

After electrophoresis, gels were stained according to their enzyme systems with the appropriate substrate, chemical solutions and then in cubated at room temperature in dark for complete staining. For peroxidase, benzidine – dihydrochloride HCL of 0.125 gm and 2 ml glacial acetic acid and was completed with distilled water up to 50 ml. Gel was placed into this solution and five drops of hydrogen peroxidase was added. The gel was incubated at room temperature until bands appear (Brown, 1978).For polyphenol oxidase, 100 ml of sodium phosphate buffer 0.1 M at PH 6.8.15 mg cathecol and 50 mg sulfanilic acid were used. The gel was placed into this solution and incubated at 30℃ for 30 min until bands appeared.

Specific-PCR analysis

DNA was extracted for the leaves of the selected plants of five sorghum lines which different reaction for drought tolerance in sorghum namely:-

RTGIS 5 primer as index for high level of maximum root length in cells

The sequences as follows:
AAAACCTAATTTTTTTCCGGGCTAAC
CCAAATTTTTCCGGGCCGCAAAACCC
GGGGTTTTTTTTCCGTCCCTAAAACCC
GGGTTTCCACCTAAAGGTTCCCTTTG
GGGGGAAATCCCCCCAAAATTTTTTA
AAGGGGCATAAACCTGGGAAATTTA
ACCGATCCTAAATTTAAGGGGGGTAA
AAAAAACCCCTTTAAAGGGGAACTA
ACCGGGGGGAAAATTTTAAACCTTAA
AAAACCCTGGAAACCTAACGAAAAC

CCTAAAGGGGGGGAAATTTACATTTG
GGGAACCTAGGAAACCCTAGGTTAAC
CCTAGGGGAAAAATCCCTAAAACCC
GGGGAACCTTTTAAGGGCCCGGGAA
ACCCTTTTAAAAAGGAACCT

GTLO 14 primer as index for hygroscopic cells in sorghum and the sequence as follows

AACCTTTACCCCAAACCGGGAAAAAC
CCTACCCAGTTTTTGGGAAACCCTTA
AGGGAAAACCCCTTTTAAAAGGGGA
AATAAGGTTAACCCTACCCTTGTAAC
CCCCCTTTAAGGGAACTGGGGGAAAA
AAACCTTTACTAAACCCTTTAGGGGA
AAAAGGGAAACCTAGGGAACCCACT
AAACCCTTTTTAAGGGCCTCTTTAAA
ACCCGGCCCAACGAAAAGGGAACCC
ATTTTTTTG

AMR 14 Primer as index for waxy layers in leaves of sorghum and the sequences as follows

TAGTAACCTAGAAACCGGAAGAACC
CGGGAAATTTAATCCCCCTTAACCGG
GAAACCGGGAAAACCCATTTTAAAG
GGGGAAACCCTTAGGGAAATTTAGTT
TCCCCCGGGAAGGGAACCAAAAGGG
GTTTTAACCCCGTAACCCGGGAAATT
TCCCATTTTTTAAAAGGGGCCCTAAA
AGGGAAACCCCCATTTTTAACCCCCC
AAATTTAAAAGGGGGAAACCCTTTAA
AACCCGGGT

HSC12 primer as index for Glycine betaine in cells and the sequences as follows

TTTAAAGGGGGGAAACCCCCAAAAA
CCCCAGGGTTTTGGGGTGGGAAACCC
AATTTTCCCCCCAAAAGGGGCCCCAA
TTTAGCGAATTTTTCCCGGGAACCCC
CTCCCAAAAGGGTTTAAACCGGAGGG
AAACCCCAATTGGAAATCAAAACCC
GGATTTAACCCTTCCCCAAAGGGGTT
TAAGGGGTGTTTTAAAGGGAAACCCG
GGGAAAAACCTGGGCCCATCCCCGTT
GGGGAATTTTAACCCAAATT

Table.2 Gel electrophoretic buffers

TBE buffer	10 x
Tris	10.80 g
Boric acid	5.50 g
EDTA	0.74 g
H20 (dd)	Up to 100 ml
Loading buffer:	
Tris	10.89
Boric acid	5.59
EDTA	0.74
H20 (dd)	up to 100 ml

according to the method of Graham and Henry (1997).

Agarose Gel Electrophoresis

PCR amplification products were analyzed using 1.5% agorose gel electrophoresis in 1 × TBE buffer and stained with ethidium bromide. The run was performed at 100 V in Bio Rad submarine. The bands of amplified DNA ladder of 100 to 2000 base pairs and photographed with gel documentation system. Williams et al. (1990). Graham, Henry (1997) and Sharma et al. (2003).

Gel Analysis

Gels were photographed under ultra violet with polaroid film 667 and scanned with Bio-Rad video densitometer model 620 at awafe length of 557 soft ware data analysis for Bio-Rad model 620 USE densitometer and computer were used.

Results and Discussion

Molecular Markers

SDS-Protein electrophoresis

The electrophoretic banding patterns of proteins extracted from the leaves of sorghum under normal and drought conditions in (fig. 1 and table 3). Thirteen bands ranging from 15 to 215 bp; were polymorphic with 85% polymorphism varied between normal conditions compared with drought conditions.

The bands number (1, 2, 3, 4, 5, 6, 10, 11) with molecular weights of (215, 150, 114, 110, 80, 75, 30, 25) KDa respectively, were appeared in all parents under all conditions which means that these bands were commonly bands in these cultivars.

On the other hand; the bands number (7, 8, 9, 12, 13) with molecular weights of (60, 40, 35, 20, 15) KDa, respectively were appeared also in all the parents under normal and drought conditions, except, the band number (7) in (P_3) under drought conditions, the band number (8) in (P_3) under normal conditions, the band number (9) in (P_5) under normal conditions, the band number (12) in (P_3) under normal conditions and the band number (13) in (P_5) under normal conditions, respectively.

The appearance of the bands number (8, 9, 12, 13) with molecular weights of (40, 35, 20, 15) KDa in the parents number (P_3, P_5, P_3, P_5) under drought conditions only, respectively may be due to manufacture specific protein which responsibility and powering for drought tolerance in sorghum under Egyptian conditions. This increasing of density and intensity of the bands number (1, 2, 3, 4, 5, 7) with molecular weights of (215, 150, 114, 110, 80, 60) KDa, respectively in the parents number (P_1, P_2 and P_4) under drought conditions may be due to highly ability of drought tolerance in these parents. These results confirmed that there was consistent difference in protein banding patterns between all cultivars of sorghum under normal and drought conditions and this modification of gene expression is due to high conservative genes found in plants, which due to highly tolerance of drought. These genes might have a crucial role in the response to different stresses, as well as the main role of systemic signals generated by the tissue exposed to drought tolerance in drought. These results were in agreement with those reported by El-Fadly et al. (2007), Al-Whaibi (2010) and Cherian and Fereira (2010).

Isozymes electrophoresis

Peroxidase (pox) isozymes

The electrophoretic patterns of peroxidase enzymes under normal and drought conditions are showed in (fig. 2 and table 4.)

A total number of four bands were exhibited; all bands were appeared in all parents of sorghum under normal conditions. The bands number 2 and 3 were monomorphic and detected in all parents which their density of bands were high under normal conditions, while, the four bands of peroxidase isozymes were appeared in the five parents except the bands number 2 and 3 which disappeared in the parents number one and four respectively. The preview results indicated that few bands disappeared or newly appeared with highly density under drought conditions as compared to the normal conditions. The reason for the decrease in peroxidase activity after water stress may be due to damage of the protein which control to drought tolerance in sorghum. The results

showed that (P2, P3 and P5) were highly tolerance of drought when its were sufficient compatibility to express different reaction in stress as compared with the normal conditions, as well as antioxidant enzymes which response to different ability for abiotic stresses and proved the favorable conditions to this protein in order to have their a little activity to neutralize the free radicales which are produced under stress of drought.

Finally, using of peroxidase isozyme and protein patterns as a marker for drought tolerance in sorghum and found that the profile of iso peroxidase enzyme was modified during water stress condition, also anew subset of proteins induced by drought conditions compared to the normal conditions and this behavior may be due to its ability to resistance drought stress or due to the effect of drought which may cause some shift in gene expression El-Baz et al., (2003) and Roy and Mandal (2005).

Polyphenol oxidase (ppo) isozymes

Three bands appeared for polyphenol oxidase in sorghum under normal and drought conditions (fig. 3 and table 4). The bands number 2 and 3 were commonly bands detected under all conditions in all parents except the parents number (2 and 4) under normal conditions, the parents (2 and 5) under drought conditions for the second band and the parents number (1 and 3) under drought conditions only for the third band, respectively with different densities and intensities. While, the first band was appeared in the parents (P_1, P_3, P_5) and (P_4) under normal and drought conditions, respectively. It is noted that, the variations occurred in the one level of drought when compared with the normal conditions confirmed that its trigger the induction of compounds that regulate the induction or the activity of the tolerance for drought in sorghum.

The (ppo) activity decreased with increasing the level of drought in the first and third band which showed very little activity because damage the protein which controlled of drought tolerance. These results were in agreement with El-Beltagi et al., (2010) who found that the reason for decreasing in polyphenol oxidase activity after roasting may be due to protein denaturation. Also, Gautam et al, (1998) and Montavon and Bortlik, (2004) reported that roasting treatments decline polyphenol oxidase activity in mushroom and coffee. The previous results are similar to the results of Lee et al., (2007) who reported that antioxidant enzymes were upregulated under salinity stress in rice leaves, and also the enzymes related to metabolic pathway were differently accumulated for the ability for drought tolerance in sorghum.

On the other hand, the highest activity from the two isozymes were found in the parents (P_1, P_3, P_5) and (P_2, P_3, P_4, P_5) under normal and drought conditions respectively. These results were in agreement with Nagesh and Devaraj (2008) who confirmed that quantitative and qualitative alteration in antioxidant enzyme system are often related to level of resistance to stress, with quantitative changes in the enzyme level alterations were also observed in intensities and number of isozyme bands during applied stress like the present results the decrease of isozyme activity indicates gradual degradation of these enzymes on their structural modification under increased drought tolerance in sorghum, whereas the banding pattern expressing differential intensity shows the varying status of an enzyme affected by the stress of drought. Magda A.M. EL-Enany et al (2013).

Table.3 The protein banding patterns of SDS-PAGE of some genotypes of sorghum under normal and drought conditions

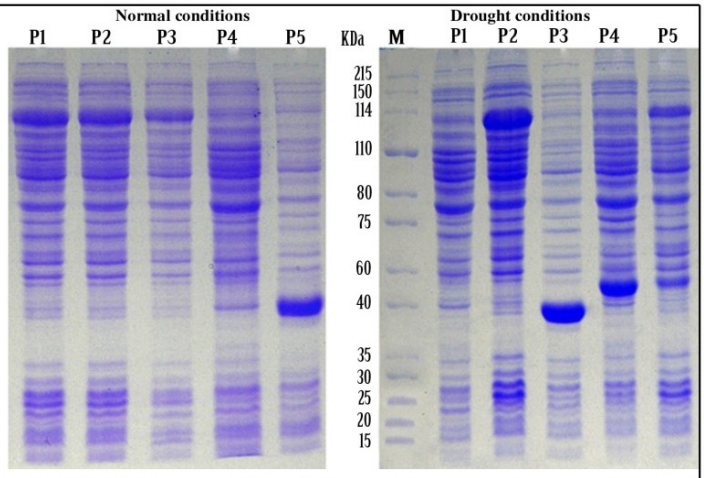
Band No	MW (KDa)	Normal conditions					Drought conditions				
		P_1	P_2	P3	P_4	P_5	P_1	P_2	P_3	P_4	P_5
1	215	+	+	+	+	+	+	++	+	+	+
2	150	+++	++	++	++	+	++	+++	+	+	+
3	114	+++	+++	+++	++	+	+	+++	+	++	+++
4	110	++	+	+	+	+	++	+++	+	++	+
5	80	+	+	+++	+	+	+++	+++	+	+++	++
6	75	+	+	+	+	+	++	++	+	++	++
7	60	+	+	+	+	+	+	+	-	+++	+
8	40	+	+	-	+	+++	+	+	+++	+	+
9	35	+	+	+	+	-	+	+	+	+	+
10	30	+	+	+	+	+	+	+	+	+	+
11	25	+	+	+	+	+	+	+	+	+	+
12	20	+	+	-	+	+	+	+	+	+	+
13	15	+	+	+	+	-	+	+	+	+	+

(+): very faint, (++): faint, (+++): very dark, (-): absence of bands

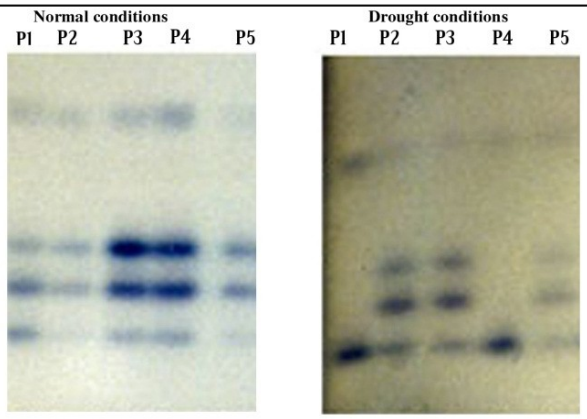
Table.4 Effects of different levels of drought on peroxidase and polyphenol isozymes in leaves of sorghum

Band No	Normal conditions					Drought conditions				
	P_1	P_2	P_3	P_4	P_5	P_1	P_2	P_3	P_4	P_5
Peroxidase isozymes										
1	+	+	+	+	+	+	+	+	+	+
2	+	+	+++	+++	++	-	+++	++	-	+
3	++	+	+++	+++	++	-	+++	+++	-	+
4	++	+	+	+	+	+++	+	+	+++	+
Total	4	4	4	4	4	2	4	4	2	4
Polyphenol oxidase isozymes										
1	+++	-	+	-	+++	-	-	-	++	-
2	+++	-	++	-	+++	+++	-	++	++++	-
3	+++	+++	+	+++	++++	-	+++	-	++	+++
Total	3	1	3	1	3	1	1	1	3	1
Total	7	5	7	5	7	3	5	5	5	5

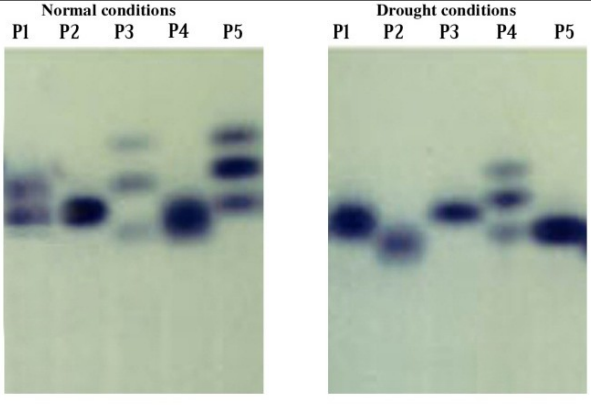
(+): very faint
(++): faint
(+++): dark
(++++): very dark



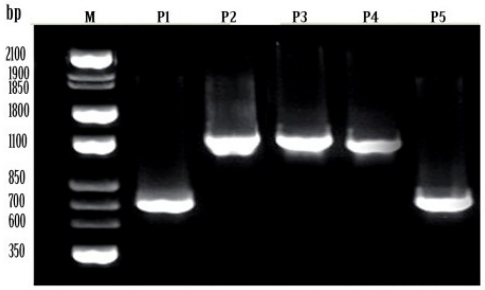
Fig(1):-SDS-PAGE of water soluble protein fraction of the genotypes of sorghum under normal and drought conditions.



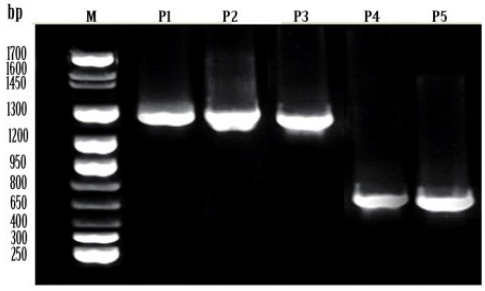
Fig(2):-Electrophoretic patterns In sorghum using peroxidase Isozymes under normal and drought conditions.



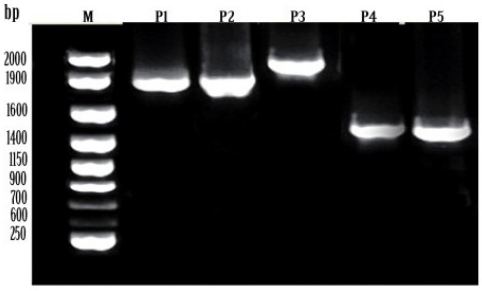
Fig(3):- Electrophoretic patterns In sorghum using polyphenol oxidase Isozymes under normal and drought conditions.



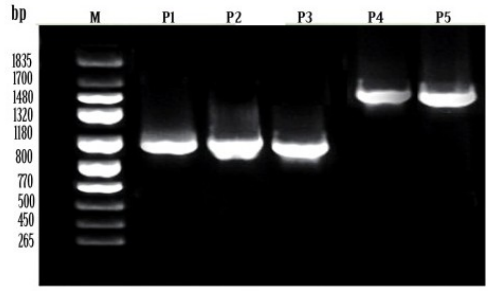
Fig(4):-The densitometric analysis of specific –PCR for the genotypes of sorghum using (RTGIS 5) primer as index for high level of maximum root length in cells .



Fig(5):- The densitometric analysis of specific –PCR for the genotypes of sorghum using (GTLO 14) primer as index for hygroscopic cells in sorghum



Fig(6):- The densitometric analysis of specific –PCR for the genotypes of sorghum using (AMR 14)Primer as index for high level of waxy layers in leaves .



Fig(7):- The densitometric analysis of specific –PCR for the genotypes of sorghum using (HSC12) primer as index for high level of Glycine betaine content .

Specific-PCR-markers

The results in fig. (4); revealed that the band number 5 with molecular weight of 1100 bp was appeared in the parents (P_2, P_3, P_4), while the band number 7 with molecular weight of 700 bp was appeared in the parents (P_1, P_5) of sorghum, which indicated that these bands were specific marker in sorghum using RTGIS 5 primer as index for high level of maximum root lenght in cells and the ability for drought tolerance.

Using GTLO14 primer as index for drought tolerance in sorghum by hygroscopic cells in sorghum, the results in fig. (5), showed that the band number 4 with molecular weight of 1300 bp was observed in the parents (P_1, P_2, P_3), while, the band number 8 with molecular weight of 650 bp was appeared in the parents (P_4, P_5) of sorghum respectively, which indicated that these two bands were specific marker for drought tolerance by compilation or accumulation the high level of hygroscopic cells in sorghum.

The bands number (1, 2 and 3) with molecular weights of (2000, 1900, 1600) were showed in the parents (P_3), (P_1, P_2) and (P_4, P_5), respectively using AMR14 primer as index for drought tolerance by high level of waxy layers in leaves of sorghum in fig. (6)., which indicated that these bands were specific marker for drought tolerance in sorghum. In fig. (7), the bands number (2 and 5) with molecular weight of (1700 and 1180) bp were appeared in the parents (P_4, P_5) and (P_1, P_2, P_3), respectively, which indicated that these bands were marker for drought tolerance in sorghum by increasing the level of Glycine betaine using HSC12 primer. These results were in agreement with those reported by Gautam et al., (1998), Montavon and El-Beltagi et al., (2010).

Finally, Drought tolerance of sorghum plants increasing by high maximum root length to Absorption of water from the distant depths in the soil,hygroscopic cells in the leaves to store water and keep it during the process of transpiration and

used during the lack of water in the soil and high level of waxy layers to prevent the water out of stomata and stored during drought.

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