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# Molecular and Biochemical Markers Associated with Salt Tolerance in Some Sorghum Genotypes

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**Abstract:** Genetic variability among seven sorghum cultivars under salt stress were investigated using ISSR markers, SDS-PAGE of soluble protein and isozyme variations. At molecular level, eleven preselected ISSR primers were used to identify seven samples of sorghum and indicating the genetic diversity among sorghum genotypes with 65% polymorphism. The ISSR-PCR product using degenerate primer of BADH and indicated the appearance of one fragment at molecular size 1650 bp that was detected in seven tolerant sorghum genotypes. At biochemical level, analysis of soluble protein by SDS-PAGE revealed low polymorphism 38.46%. Five isozyme systems including α *Est* (esterases), *Acp (*acid phosphatase), *Adh* (alcohol dehydrogenase), Malic acid, *Ao* (Alkaline phosphate) were used to study the genetic variability among seven sorghum cultivars with 72% polymorphism. The identified molecular and biochemical markers could introduce a great benefit for breeding programs to select the salt tolerant individuals.The detection of salt tolerance genes like BADH could be adding a molecular base add great value to the application of BADH gene in plant salinity tolerance in different sorghum genotypes.

**Key words:** *Sorghum* · ISSR-PCR · SDS-PAGE · Isozymes · BADH gene

## INTRODUCTION

Sorghum is an important staple food throughout the semi-arid Asian and African regions [1]. Compared to maize, sorghum breeding has been neglected in recent decades and the availability of high yielding maize varieties has led to the displacement of sorghum. However, maize is less adapted to drought conditions and thus had lower yield stability [2]. Instead, sorghum may bear advantageous genes that are especially useful in conferring resistance to biotic as well as abiotic stresses. ISSR markers allow simple and cost-effective method to assess genetic variability and similarity within and among cultivars and amplify different regions of genomes. The best molecular markers for genome mapping, marker assisted selection, phylogenetic studied and crop conservation has low cost and labour requirements and high reliability is an inter simple sequence repeat (ISSR) [3]. Microsatellites are very short (usually 10-20 base pair) stretches of DNA random and widely distributed along the genome and they can be analyzed efficiently by the polymerase chain reaction (PCR), using specific primers (forward and reverse) to their flanking regions. The variation in (PCR) product length is a function of the number of ISSR units. Primers based on a repeat sequence, such as (CA) n, can be made with a degenerate 3'-anchor, such as (CA) 8 RG or (AGC) 6 TY [4]. ISSR markers successfully used for estimating of genetic diversity in main crops, for instance barley [5] and canola [6, 7]. When plants are exposed to environmental stress, such as high concentration of salt, plants have several mechanisms to suppress the production of these active molecules. One of these mechanisms is glycine betaine (GB) accumulating. Glycine betaine (GB) is accumulated BADH is involved in abiotic stress tolerance in many crops by catalyzing the formation of GB. Plant abiotic stress response is commonly mediated by increasing *BADH* gene activity [8, 9].

Biochemical markers have received more attention in recent years as the data reflect more truly the genetic variability because they are the direct products of genes, (SDS-protein and isozymes) are proving increasingly valuable in providing input for genetic differentiation and conservation discussions, where there is a basic need to assess some measures of genetic variability in and among cultivars [10]. Isozyme analysis has several advantages as

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compared not only with morphological and physiological characters, but also with other genetic markers; especially isozymes are mostly co-dominant with a simple Mendelian inheritance in most loci, so that the frequency of individual alleles is directly counted. Besides, isozymes can be resolved for most plant species regardless of habitat, size or longevity. The use of SDS-PAGE and isozymes were the cheapest and simplest methods that offer sufficient information and serve as a starting point for DNA-based studies [11, 12].

In this study, ISSR markers, seed storage protein and isozymes variations were carried out on seven sorghum genotypes to assess the genetic variation and relationship among different sorghum cultivars and detection of salt tolerance genes like BADH.

## MATERIALS AND METHODS

A field experiment was carried out at Ras Sudr Experimental Station, South Sinai sandy loam, saline calcareous soil irrigated with saline water. Seven sorghum genotypes were used as shown in Table 1. Some physical and chemical properties of the soil site are presented in Table 2. Chemical properties of irrigation water in Ras Sudr Experimental Station are also presented in Table 3.

**DNA Extraction:** Genomic DNA was extracted from fresh young leaves of ten plants chosen randomly for each population by CTAB method of Doyle and Doyle [13]. Leaves of seven plants from a single population were bulked prior to extraction. DNA was quantitated by spectrophotometer and gel electrophoresis.

**ISSR for DNA Amplification:** ISSR-PCR reactions were conducted using twelve arbitrary 10-mer primers according to Williams *et al.* [14]. The reaction conditions were optimized and mixtures were prepared (25 µl total volumes) consisting of the following: dNTPs (8mM) 1.0µl, Taq DNA polymerase (5U/µl), 0.2µl 10X buffer with 15mM $MgC1_2$, 2.5µl Primer, Template DNA (10-50ng/µl) 1.0 µl, $H_2O$ (dd) 19.3µl. The most reproducible primers were OPB1, OPB2, OPB5, OPB6, OPD1 OPA3.The nucleotide sequences of these primers are presented in Table 4. Amplification was carried out in Strategene Robocycler Gradient 96 which was programmed for 40 cycles as follows: Denaturation (one cycle) 94°C for 4 minutes, (40 cycles) of the following sequence 94°C for 1 minute and 30 second, 36°C for 1 minute and 30 second, 72°C for 2 minutes and 30 second then extension (one cycle) 72°C for 7 minutes. Amplification products were resolved in 1.5% GTG agarose gel electrophoresis with 1x TAE running buffer. The run was performed for one hour at 180 volt for 1 hour and the gel was stained with ethidium bromide. A molecular marker DNA Ladder was used. Bands were detected on UV-transilluminator and photographed by Gel documentation system.

Table 1: Taxa of *sorghum* samples.

| | |
|---|---|
| 1 | S35 |
| 2 | ICSV93046 |
| 3 | ICSV745 |
| 4 | ICSR93034 |
| 5 | CSV15 |
| 6 | JJ1041 |
| 7 | Hybrid 102 |

**Primer Design:** Specific degenerate primers were constructed according to its accession number as shown in Table 5 to recover partial-length of interesting genes. The primers were synthesized at Metabion, Germany. Primer sequences were checked for accuracy using the oligonucleotide software Oligo 4.1 (National Biosciences Inc., Plymouth, MN, USA).

**Polymerase Chain Reaction (PCR):** PCR reaction was accomplished by adding 5µl 10X Taq DNA polymerase buffer, 1µl dNTPs (10mM each) (Bioron, Germany), 2.5 units TaqDNA polymerase (Bioron, Germany), 10 pmol of each primer (forward and reverse), 2 µl DNA and RNase free water up to 50 µl. All PCR reactions were performed in a thermal cycler (GeneAmp PCR System2400) (Perkin Elmer), by pre-heating at 94°C for 5 min followed by 30 cycles of 1 min at 94°C, 1min at annealing temperature 50°C are shown in Table 5 and 2 min for extension at 72°C. Finally, the amplified DNA was incubated at 72°C for 7 min to accomplish a final extension. 20 µl of PCR reaction were subjected to electrophoresis in 1.5 % agarose gel containing ethidium bromide (0.01%), subjected to 100 volts for 1hr and then photographed using UVP gel documentation system, UVP corporation-UK.

**Extraction of Total Protein:** Bulked leaf sample (0.25g) of each sample was ground with liquid nitrogen and mixed with extraction buffer pH 7.5 (50 mM tris, 5% glycerol and 14 mMB- mercapto ethanol) in a mortar with pestle, left overnight then vortexed for 15 sec and centrifuged at 10.000 rpm at 4°C for 10 min. The supernatants were transferred to new eppendorf tubes and kept at -20°C until use for electrophoresis analysis according to Laemmli [15].

Table 2: Chemical analysis of the experimental soil

| | Cations (meq/L) | | | | Anions (meq/L) | | | |
|---|---|---|---|---|---|---|---|---|
| pH | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{--}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ |
| 7.51 | 32.15 | 15.21 | 57.98 | 0.897 | -- | 2.01 | 56.20 | 48.02 |

Table 3: Chemical analysis of the irrigated water

| | | Cations (meq/L) | | | | Anions (meq/L) | | | |
|---|---|---|---|---|---|---|---|---|---|
| EC dS/m | pH | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{--}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ |
| 6.27 | 7.34 | 13.12 | 7.89 | 40.57 | 0.310 | -- | 1.88 | 34.14 | 25.87 |

Table 4: Number of monomorphic bands, number of polymorphic bands, total bands and polymorphism percentages of ISSR primers among sorghum seven cultivars*

| | | | | Polymorphic bands | | | |
|---|---|---|---|---|---|---|---|
| No | ISSR Primer | Primer Sequences | Monomorphic bands | Non-unique bands | Unique bands | Total bands | % Polymorphism |
| 1 | UBC-823 | (TC)8CC | 1 | 7 | 0 | 8 | 87.50 |
| 2 | UBC-825 | (AC)7 T | 2 | 3 | 1 | 6 | 66.66 |
| 3 | UBC-855 | (AC)8 TT | 0 | 10 | 0 | 10 | 100.00 |
| 4 | UBC-857 | (AC)8 GG | 1 | 6 | 2 | 9 | 88.88 |
| 5 | HB9 | (TG)8 GA | 3 | 2 | 0 | 5 | 40.00 |
| 6 | HB10 | (CA)6 AC | 3 | 2 | 0 | 5 | 40.00 |
| 7 | HB12 | (CA)6 GG | 5 | 0 | 1 | 6 | 16.66 |
| 8 | A17899 | (CA)6 AG | 1 | 3 | 0 | 4 | 75.00 |
| 9 | B17899 | (CT)8 TG | 3 | 3 | 1 | 7 | 57.14 |
| 10 | B17898 | (GA)6 CC | 3 | 1 | 1 | 5 | 40.00 |
| 11 | HB20 | (GT)8 CC | 2 | 1 | 1 | 4 | 50.00 |
| Total | -- | -- | 24 | 38 | 7 | 69 | 65.21 |

Table 5: Sequence and annealing temperature of PCR primers used in amplification of (BADH) gene

| Specific Primer | Primer Nucleotide Sequence | Annealing temperature | Product size |
|---|---|---|---|
| BADH | F 5-TCCTCTCGTCTCCAGTCCAC-3 | 50 | 1650 |
| | R-5- AATGCAGACTAACAACCCATGA-3 | | |

**Isozyme Detection:** The Isozymes used were: α-esteras (Est.), Acid phosphatase (Acph.), alcohol dehydrogenase (Adh.), aldehyde oxidase (Ao.), Malic enzyme (Mal). Isozymes were separated in 10 % Native-polyacrylamide gel electrophoresis as described by Stegman [16]. For isozyme extraction, 1 g of fresh leaves was homogenized in 3 ml extraction buffer using a mortar and pestle; centrifuged at 5 000 rpm for five minutes; the supernatant was kept at -20°C until use. For electrophoresis, 50 µl of extract was mixed with 25µl of treatment buffer and 50 µl of this mixture was applied to the well. In gels staining, protocols of Scandalios [17] were used for α and β-Est [18] for Ao, Acph and Adh [19] for Malic and Mdh [20] for Px. Gels were washed two or three times with tap water; fixed in ethanol: 20% glacial acetic acid (9:11 v/v) for 24 hours; and photographed.

## RESULTS AND DISCUSSION

**ISSR Analysis:** Eleven preselected ISSR primers were used in the present study to identify seven samples of sorghum as shown in Tables 4, 6 and Fig. 1. Twenty four monomorphic and thirty eight polymorphic distinct fragments (65% of polymorphism) were revealed in the seven tested cultivars with these eleven primers. The results showed that primers UBC-855 and UBC-857 were highly polymorphic (100% and 88.8% polymorphism). Moreover, primers UBC-823, UBC-825, B17899 and HB20 were moderate polymorphic from 87% to 50% polymorphism. On the other hand, primers HB12, A17899, HB9, HB10 and B17899 showed the lowest polymorphism ranging from 16.6% to 40%. The overall results of eleven primers were illustrated in Table 4. Primers B17899, UBC-825 and HB12 gave the highest total number of bands from 7 to 6 bands. While, primers HB20, UBC-823,

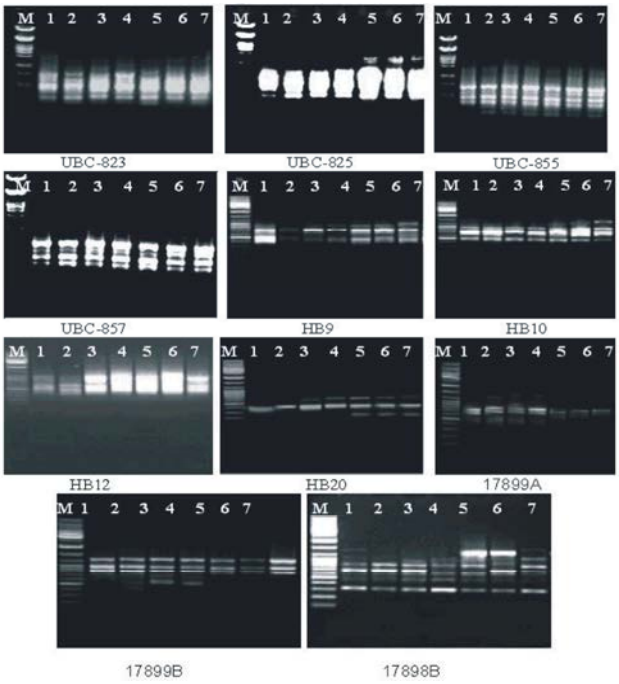


Fig. 1: ISSR primers of UBC-823, UBC-825, UBC-855, UBC-857, HB9, HB12, HB20, 17899A, 17898B and 17899B for sorghum seven cultivars *
*(M) Maker; (S35) cultivar 1; (ICSV93046) cultivar 2, (ICSV745) cultivar 3, (ICSR93034) cultivar 4, (CSV15) cultivar 5, (JJ1041) cultivar 6 and (Hybrid 102) cultivar 7.

Table 6: ISSR primers for seven sorghum genotypes* species - specific marker

| No. of specific primers | ISSR Primer | Genotypes | Unique bands |
|---|---|---|---|
| 1 | UBC-825 | JJ1041 | 1 |
| 2 | UBC-857 | S35 ICSV93046 | 2 |
| 3 | HB12 | ICSV745 | 1 |
| 4 | B17899 | Hybrid 102 | 1 |
| 5 | B17898 | ICSV93046 | 1 |
| 6 | HB20 | ICSV745 | 1 |
| 6 | Total | -- | 7 |

* (S35) cultivar 1; (ICSV93046) cultivar 2, (ICSV745) cultivar 3, (ICSR93034) cultivar 4, cultivar 4, (CSV15) cultivar 5, (JJ1041) cultivar 6 and (Hybrid 102) cultivar 7.

UBC-825, UBC-857, HB9 HB10, B17898, A17899 and HB20 gave the lowest total number of bands from 5 to 4 bands. In general, the result indicated that ISSR markers gave adequate distinguish among sorghum cultivars, poly (AC)-anchored primers were more polymorphic and reproducible than other di-nucleotides and tri-nucleotides motifs. These results are in agreement with those obtained by Yang *et al.* [21], who used the inter simple sequence repeats (ISSR) and microsatellites in soybean cultivars discrimination and reported that microsatellites represent an excellent technique to study the genetic polymorphism. It is evident from the aforementioned results that the high number of molecular markers related to salt tolerance reflects the complexity in this trait besides being affected by the environment. However, the use of marker-assisted selection could enhance the identification of sorghum genotypes tolerant to environmental stress. This approach would enable the molecular plant breeder to detect the promising lines with more confidence in their merits as line selection based on genetic rather than phenotypic basis, with the elimination of the environmental factors as much as possible. Moreover, this process is fast, reliable and cost-effective, reducing the required time for sorghum breeding programs in Egypt.

**Cultivars -Specific -Markers:** Some specific markers for some sorghum cultivars across ISSR analysis are listed in Table 6. Six out of eleven ISSR markers were found to be

species specific. These markers were scored for the presence of unique bands for a given cultivars. ICSR93034 sorghum cultivar had two unique bands could be scored by B17898 and HB12. Then, the remaining cultivars could be distinguished by one band for each marker for ICSV93046, JJ1041, S35 and Hybrid 102. Six from eleven primers gave specific markers. The results are in agreement with those obtained by Pharmawati *et al.* [22] who reported that 17 of 584 ISSR markers were found to be cultivar–specific; Hassan [23] found that 20 out of 110 ISSR markers were specific markers among ten genotypes of *Moringa* species using 11 ISSR primers, Abdel-Tawab *et al.* [24] who showed that 48 out of 164 ISSR markers were specific markers among seven genotypes of *Mentha* and *Ocimum* species using 10 ISSR primers, Rania *et al.* [25] evaluate two inbred lines of grain sorghum for their environmental stress tolerance (salinity) at the field. This analysis revealed some genetic markers (ISSR & RAPD) associated with salt tolerance in grain sorghum that can be utilized during breeding programs via marker-assisted selection and finally. Gezahegn *et al.* [26] confirmed the existence of higher diversity in wild rice populations than cultivated species using ISSR primers producing a total of 93 clear and reproducible bands were generated using four dinucleotides and two tetra nucleotide primers.

**Gene Detection:** In higher plants, GB is synthesized from choline (Cho) via betaine aldehyde (BA). The first and second steps in the biosynthesis of GB are catalyzed by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), respectively [27].The PCR product using specific primer of BADH drought tolerant gene indicated that the appearance of one band with fragment size 1650 bp as shown in Fig. 2 and Table 5.

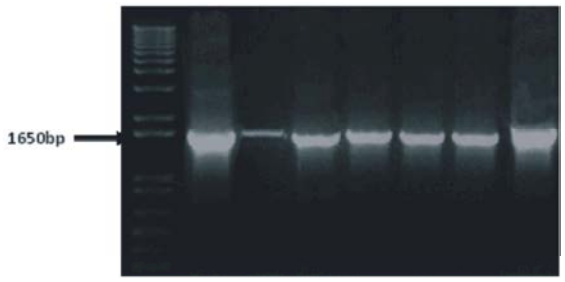


Fig. 2: The PCR product using specific primer of BADH drought tolerant gene indicated that appearance of one band with fragment size 1650bp.
*(M) Maker; (S35) cultivar 1; (ICSV93046) cultivar 2, (ICSV745) cultivar 3, (ICSR93034) cultivar 4, (CSV15) cultivar 5, (JJ1041) cultivar 6 and (Hybrid 102) cultivar 7.

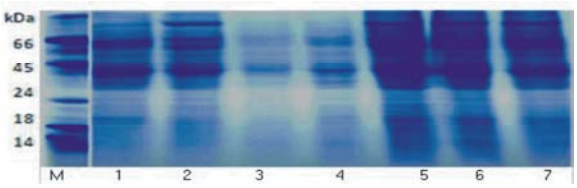


Fig. 3: SDS-PAGE profiles of soluble proteins extracted from some sorghum genotypes* under Ras Sudr conditions
*(M) Maker; (S35) cultivar 1; (ICSV93046) cultivar 2, (ICSV745) cultivar 3, (ICSR93034) cultivar 4, (CSV15) cultivar 5, (JJ1041) cultivar 6 and (Hybrid 102) cultivar 7.

**SDS-PAGE Leaf Proteins:** Data presented in Tables 7 and 8 and Fig. 3 summarized all the results of SDS-PAGE of soluble proteins for different samples of sorghum genotypes. The major variations are expressed as changes in band intensity, appearance or disappearance of some bands. Analysis of gel revealed that the molecular weight of protein subunits ranged between 11.94 to 86.56 kDa. Data showed that CSV15, JJ1041 and Hybrid 102 were resolved into 19 bands, while ICSR93034 was resolved into 20 bands. Also, S35, ICSV93046 and ICSV745 were resolved in 25, 21 and 17 bands, respectively under saline conditions at Ras Sudr. In this respect, the bands of M. wt 67.42, 69.02, 70.67, 73.56 and 84.74 kDa were disappeared in ICSV745, ICSR93034, CSV15, JJ1041 and Hybrid 102. Also, protein band having molecular weight 67.42, 69.02 and 73.56 kDa for ICSV112 revealed the same trend. In this regard, Bands at molecular weight 86.56, 57.97, 44.10 and 11.94 were absent in samples of (ICSV93046, ICSV745 and ICSR93034), (CSV15, JJ1041 and Hybrid 102) and (S35, ICSV93046 and ICSV745), respectively. Also, protein band having molecular weight 53.13 and 57.97 kDa for ICSV745 were not presented. Regarding polymorphism, there were 16 monomorphic bands (common bands), 7 polymorphic bands and 3 unique bands.

Concerning band intensity, there was a detectable change in band intensity for all sorghum genotypes grown under saline conditions at Ras Sudr. It is quite clear from the data that bands of molecular weight 11.94, 15.01, 15.92, 17.79, 19.50, 21.43, 25.22, 26.88, 30.38, 32.23, 33.15, 49.85, 53.13, 78.03 and 86.56kDa in samples of three sorghum genotypes (CSV15, JJ1041 and Hybrid 102) recorded the highest values of band intensity as compared with the same bands of other sorghum genotypes. This was true for sorghum genotypes (S35 and ICSV745) but ranked the 2nd order. In contrast, the same bands of genotypes (ICSV745 and ICSR93034)

Table 7: SDS-PAGE patterns of soluble proteins extracted from some sorghum genotypes under Ras Sudr conditions

| | | Band intensity | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sorghum genotypes | | | | | | | |
| Band number | Molecular weight (kDa) | S35 | ICSV93046 | ICSV745 | ICSR93034 | CSV15 | JJ1041 | Hybrid 102 | Polymorphism |
| 1 | 86.56 | 1.63 | 00 | 00 | 00 | 3.61 | 3.56 | 2.95 | Polymorphic |
| 2 | 84.74 | 2.41 | 3.75 | 00 | 00 | 00 | 00 | 00 | Polymorphic |
| 3 | 78.03 | 2.31 | 2.33 | 1.00 | 1.21 | 4.53 | 4.54 | 4.06 | Monomorphic |
| 4 | 73.56 | 2.43 | 00 | 00 | 00 | 00 | 00 | 00 | Unique |
| 5 | 70.67 | 3.67 | 3.69 | 00 | 00 | 00 | 00 | 00 | Polymorphic |
| 6 | 69.02 | 3.76 | 00 | 00 | 00 | 00 | 00 | 00 | Unique |
| 7 | 67.42 | 3.31 | 00 | 00 | 00 | 00 | 00 | 00 | Unique |
| 8 | 62.52 | 2.57 | 3.75 | 1.14 | 1.25 | 3.99 | 3.56 | 3.24 | Monomorphic |
| 9 | 57.97 | 2.67 | 2.97 | 00 | 1.98 | 00 | 00 | 00 | Polymorphic |
| 10 | 53.13 | 2.84 | 2.82 | 00 | 2.10 | 4.42 | 4.00 | 3.88 | Polymorphic |
| 11 | 49.85 | 3.66 | 3.62 | 1.43 | 1.60 | 4.60 | 3.94 | 3.81 | Monomorphic |
| 12 | 44.10 | 4.78 | 4.71 | 1.38 | 2.23 | 00 | 00 | 00 | Polymorphic |
| 13 | 37.92 | 3.86 | 1.43 | 1.43 | 3.53 | 3.20 | 3.53 | 3.20 | Monomorphic |
| 14 | 35.58 | 3.69 | 3.68 | 1.51 | 3.35 | 4.05 | 4.66 | 3.21 | Monomorphic |
| 15 | 33.15 | 3.27 | 3.08 | 1.85 | 2.43 | 4.00 | 4.08 | 4.61 | Monomorphic |
| 16 | 32.23 | 2.09 | 3.01 | 2.41 | 2.38 | 5.37 | 3.93 | 3.59 | Monomorphic |
| 17 | 30.38 | 2.27 | 1.94 | 1.80 | 2.03 | 4.39 | 3.76 | 3.67 | Monomorphic |
| 18 | 26.88 | 2.26 | 1.92 | 1.42 | 1.36 | 3.07 | 2.71 | 2.37 | Monomorphic |
| 19 | 25.22 | 2.25 | 2.23 | 1.39 | 1.51 | 3.27 | 2.90 | 2.43 | Monomorphic |
| 20 | 23.11 | 2.88 | 2.13 | 1.04 | 1.60 | 3.45 | 2.81 | 2.55 | Monomorphic |
| 21 | 21.43 | 2.54 | 2.25 | 1.18 | 1.70 | 3.46 | 2.69 | 2.72 | Monomorphic |
| 22 | 19.50 | 2.07 | 2.32 | 1.12 | 1.82 | 3.71 | 3.27 | 3.07 | Monomorphic |
| 23 | 17.79 | 1.81 | 2.39 | 1.20 | 1.74 | 3.40 | 3.03 | 2.99 | Monomorphic |
| 24 | 15.92 | 1.72 | 2.04 | 1.21 | 1.44 | 3.29 | 2.71 | 2.55 | Monomorphic |
| 25 | 15.01 | 1.55 | 1.93 | 1.23 | 1.27 | 2.77 | 2.49 | 2.42 | Monomorphic |
| 26 | 11.94 | 00 | 00 | 00 | 1.34 | 2.52 | 2.23 | 1.97 | Polymorphic |
| Total | -- | 25 | 21 | 17 | 20 | 19 | 19 | 19 | -- |

where; 0= no bands, 1.0 = refers to the lowest intensity value, 5.37 = refers to the highest intensity

Table 8: Number, types and percentage of protein polymorphism

| | Polymorphic bands | | | |
|---|---|---|---|---|
| Monomorphic Bands | Non-unique bands | Unique bands | Total bands | Polymorphism % |
| 16 | 7 | 3 | 26 | 38.46 |

recoded the lowest values of band intensity and ranked the 3rd order as compared with the same bands of other sorghum genotypes. This result is in agreement with those reported by Pereya and Thongngam [28] who studied sorghum protein by using SDS-PAGE, consisted of α, β and ∞ forms of kafirin. α-kafirin had the highest proportion among group and the protein concentrate containing 77.48-83.13% protein. Moreover, the results are in agreement with those reported by Ivana *et al*. [29] who evaluated 12 oats and 6 sorghum species and varieties using SDS-PAGE and showed the percentage of the most active protein components - prolamins and glutelins- between 53.43 and 74.96 % of the total storage proteins, in the sorghum collection between 32.25 and 41.36 % of the total storage proteins.

**Isozyme Analysis:** Five isozyme systems including α *Est (*esterases), *Acp (*acid phosphatase), *Adh* (alcohol dehydrogenase), Malic acid, *Ao* (Alkaline phosphate) were used to study the genetic variability among sorghum seven cultivars and their salinity tolerance (Fig. 4). As seen from the results presented in Table 9, total of 36 bands were detected using the five isozyme systems, with 72% polymorphism. The electrophoretically separable variant of isozymes system, are widely used as genetic markers, therefore their analysis can provide a

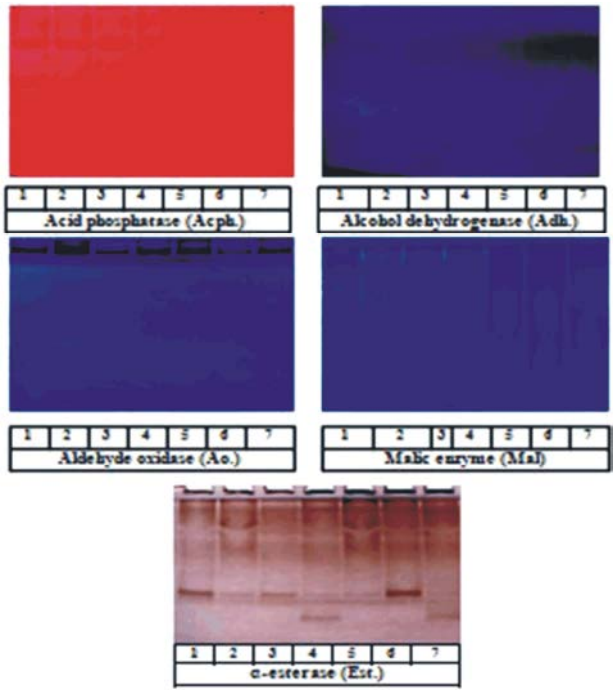


Fig. 4: Zymogram of Acph, Adh, Acph, Malic and AO banding patterns among some sorghum genotypes *under Ras Sudr conditions

* (S35) cultivar 1; (ICSV93046) cultivar 2, (ICSV745) cultivar 3, (ICSR93034) cultivar 4, (CSV15) cultivar 5, (JJ1041) cultivar 6 and (Hybrid 102) cultivar 7

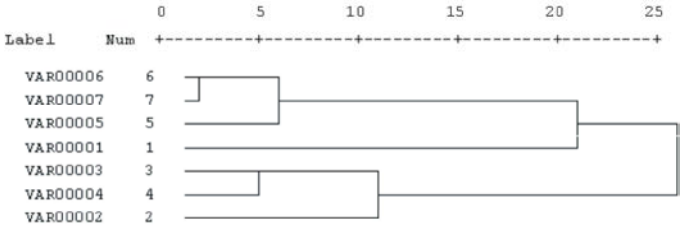


Fig. 5: Dendrogram based on total analysis (protein, isozymes and ISSR) for seven sorghum genotypes* under Ras Sudr conditions

* (S35) cultivar 1; (ICSV93046) cultivar 2, (ICSV745) cultivar 3, (ICSR93034) cultivar 4, (CSV15) cultivar 5, (JJ1041) cultivar 6 and (Hybrid 102) cultivar

precise tool to discriminate wild plant species under saline stress conditions. The identification of isozymes patterns and measuring the band intensity of each one is very important to investigate each isoform activity. The hydrolysis of ATP by acid phosphatase (Acph) is a critical process in the energy metabolism and metabolic regulation of plant cells. The requirement of acid phosphatase activity has also varied according to plant species, developmental stage and type of tissue. Data presented in Table 9 and illustrated in Fig. 5 indicated that acid phosphatase electrophoretic patterns include a total of three bands, one was polymorphic (No.3) and two bands were monomorphic (No.1 and 2). The band (No.3) was detected in sorghum genotypes (S35, CSV15, JJ1041 and Hybrid 102), but being missed in other genotypes. These findings indicated that four sorghum genotypes namely; S35, CSV15, JJ1041 and Hybrid 102 have the same gene expression, while ICSV93046, ICSV745 and ICSR93034 has different genetic behaviour which can be used to distinguish it from the other genotypes. Concerning band intensity, the highest value was 1.94 (No.2) in samples of JJ1041 and Hybrid 102, while the lowest was 1.00 (No.1and 3) in sample of S35. Also, acid phosphatase showed 33.33% polymorphism among other isozyme systems. In this concern, Tso and Chen [30] and Regla *et al.* [31] agreed with the obtained results and

Table 9: Polymorphism percentages generated by ten isozymes systems in seven sorghum genotypes under Ras Sudr conditions.

| | | Polymorphic bands | | | |
|---|---|---|---|---|---|
| Isozymes systems | Monomorphic bands | Unique bands | Non- unique bands | Total bands | % Polymorphism |
| Acid phosphatase (Acph.) | 2 | 0 | 1 | 3 | 33.33 |
| Alcohol dehydrogenase (Adh.) | 1 | 1 | 0 | 2 | 50 |
| α-esterase (Est.) | 1 | 1 | 5 | 7 | 85.71 |
| Aldehyde oxidase (Ao.) | 2 | 0 | 1 | 3 | 33.33 |
| Malic enzyme (Mal) | 1 | 0 | 1 | 2 | 50 |
| Total | 10 | 6 | 20 | 36 | 72.22 |

indicated that acid phosphatase is dimeric isozyme universally occur in the leaves, stems and roots of most plants.

Alcohol dehydrogenase (Adh) removes two hydrogen atoms from the substrate and passes them to a suitable coenzyme acceptor like NAD+ or NADP+ in reverse reactions. Data presented in Table 9 and illustrated in Fig. 4 indicated that Adh banding patterns revealed a total of two bands with different intensities, one was monomorphic (No.1), thus can be considered as a common band for different genotypes. The other band (No.2) was detected in sorghum genotype Hybrid 102 (unique band) while disappeared in other genotypes. Regarding band intensity, the band (No.1) in sample of ICSV745 recorded the maximum value, while the band (No.1) in sample of ICSV93046 revealed the reverse effect. In addition, alcohol dehydrogenase (Adh) showed 33.33% polymorphism among other isozyme systems. The results are in agreement with the findings of Mukhlesur and Hirata [32] who concluded that Adh gene family has two or three loci in angiosperm species.

Esterases (EST) are a genetic family hydrolyse ester bond in lipid to produce plant energy for biochemical reactions, to alleviate the energy level for the other metabolisms especially under stress conditions [33]. Data presented in Table 9 and illustrated in Fig. 4 indicated that α-esterase (Est.) pattern revealed the presence of about 7 bands for the seven sorghum genotypes, five polymorphic (No.2, 3, 4,5 and 6) and one monomorphic band (No.1). Band (No.1) is presented with all sorghum samples. In this respect, the band (No.2) was disappeared in S35, ICSV745 and ICSR93034 as compared with the other genotypes. Also, the band (No.5) revealed the same trend in samples of Hybrid 102. However, the bands (No.3) and (No.4) are detected in samples of sorghum genotypes (S35 and ICSV745) and (ICSV15 and JJ1041), respectively. In addition, there were detectable changes in band intensity for sorghum genotypes grown under saline conditions. Band No.5 in sample of S35 and JJ1041 recorded the maximum value of band intensity, while band No.1 in sample of ICSV15 recorded the lowest value as compared with the other bands of all sorghum genotypes. Moreover, α esterase exerted 85% polymorphism among the studied genotypes. However, Kumar and Gupta [34] ascertained that these patterns and can be successfully used to characterize different Indian mustard genotypes. The obtained results are in agreement with those reported by Ahmed *et al.* [35], who pointed that detected genetic diversity for esterases in *Brassica* members and *Acacia*.

Aldehyde oxidases (AOs) may participate in stress responses, because it catalyses the oxidation of abscisic aldehyde to ABA, in the last step of ABA synthesis [36]. Data presented in Table 9 and illustrated in Fig. 4 indicated that aldehyde oxidase (Ao) has 3 bands, one was polymorphic (No.1) and two bands were monomorphic (No.2 and 3). The band (No.1) was disappeared in ICSV745 as compared the other sorghum genotypes. In addition, aldehyde oxidase recorded 33.33% polymorphism among other isozymes systems. In this connection, sorghum genotype (ICSV93046) gave the highest value of band intensity (No.2), while the lowest value was obtained by CSV15 (band No.1). The result is in accordance with the findings of Edit *et al.*[36] who separated two isoenzymes of aldehyde oxidase can be separated from potato tubers (Solanum tuberosum) by polyacrylamide gel electrophoresis and with Mitsunori *et al.* [37] who measured Aldehyde oxidase activity in seedlings of wild type or an auxin-overproducing mutant, superroot1 (sur1), of Arabidopsis thaliana. Activity staining for aldehyde oxidase after native polyacrylamide gel electrophoresis separation of seedling extracts revealed that there were three major bands with aldehyde oxidase activity in wild-type and mutant seedlings.

As shown in Table 9 and Fig. 4, it is obvious that Malic acid (Ma) banding patterns comprise two bands with different intensities, one was monomorphic (No.1) and the other band was polymorphic (No.2). In this regard, band number 2 is presented in all sorghum

Table 10: Similarity matrix of total analysis (protein, isozymes and ISSR) markers for seven sorghum genotypes

| Items | S35 | ICSV112 | ICSV745 | ICSR93034 | ICSV15 | JJ1041 | Hybrid 102 |
|---|---|---|---|---|---|---|---|
| S35 | 100 | | | | | | |
| ICSV112 | 44.1 | 100 | | | | | |
| ICSV745 | 30.5 | 79.8 | 100 | | | | |
| ICSR93034 | 37.4 | 85.1 | 1.000 | 100 | | | |
| ICSV15 | 53.7 | 0.340 | 0.196 | 0.340 | 100 | | |
| JJ1041 | 62.8 | 0.158 | 0.090 | 0.081 | 0.824 | 100 | |
| Hybrid 102 | 62.8 | 0.233 | 0.091 | 0.158 | 0.824 | 0.952 | 100 |

genotypes except three genotypes (ICSV112, ICSV745 and ICSR93034) under saline conditions. Concerning band intensity, genotype S35 showed the maximum value recording 1.38 (No.2) followed by genotype Hybrid 102 which recorded 1.35. However, genotype CSV15 recorded the lowest value of band intensity (No.1). Malic acid (Ma) exerted 50% polymorphism among the studied genotypes. These results are in agreement with those obtained by Sang *et al.* [38] who concluded that significant increase in the activity of Ma in the NaCl-stressed barley root was highly correlated with the increased expression of the constitutive isoforms as well as the induced ones. In addition, the results suggest the significance of temporal and spatial regulation of each antioxidant isoform in determining the competence of the antioxidant capacity under saline stress.

**Combined Analysis Based on (Protein, Isozymes and ISSR):** Based on total analysis (protein, isozymes and ISSR), similarity matrix was developed by SPSS computer package system (Table 10) and shown in Fig. 4. The closest relationship was scored between ICSR93034 cultivar and ICSV745 cultivar with similarity 95.2% and JJ1041 cultivar and Hybrid 102 cultivar with similarity %100. While JJ1041 cultivar and ICSR93034cultivar gave the lowest similarity of 8 % which were considered distantly related and not closely related cultivars. The dendrogram based on (protein, isozymes and ISSR), separated the seven sorghum cultivars into two main clusters. Moreover, ICSV112, ICSV745 and ICSR93034 cultivars were separated into two main subclusters together and S35, ICSV15, ICSV745, JJ1041 and Hybrid 102 were clustered together into two main subcluster.

## CONCLUSION

In this study we successfully used the molecular marker ISSR with to identify seven sorghum cultivars and indicating the genetic diversity among sorghum genotypes, detecting salt tolerance genes like BADH which have a great value to the application of BADH gene in plant salinity tolerance in different sorghum genotypes and we successfully used biochemical markers (isozymes and protein) to identify successfully polymorphism among seven sorghum cultivars that could introduce a great benefit for breeding programs to select the salt tolerant individuals.

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