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Isolation of glyoxalase II (*gly II*) and salt overly sensitive (*SOS2*) alleles from Egyptian sorghum and enhancing salt stress tolerance in yeast

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Two salt stress related alleles namely, glyoxalase II (*gly II*) and salt overly sensitive (*SOS2*) were isolated by RT-PCR from Egyptian sorghum cv. R3. The sequencing results confirmed the isolation of *Sbgly II and SbSOS2* alleles, respectively. Both alleles were cloned separately into the pYES2 expression vector for expression in *Saccharomyces cerevisia*. Yeast growth under different NaCl stress concentration, i.e. 0, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2 and 2.3 M. the results revealed significant growth differences between the transgenic yeast and control. proline accumulation increased significantly in all transgenic yeasts with *gly II* and *SOS2*. While, these values were significantly decreased in the controls by increasing NaCl concentration. The present results suggested that the *Sbgly II* and *SbSOS2* could be mediated the improving the sat tolerance of different eukaryotic systems

Keywords: Salt stress, *glyoxalase II*, *SOS2*, proline content, *Sorghum bicolor*.

INTRODUCTION

Overcoming food shortage through self-reliance and sustainable agriculture is the main goal all over the world. Abiotic stresses as water deficit, increased salinity of soil, and extreme temperatures, cause 50 % reduction in crop yield worldwide (Bray et al, 2000 and Tuberosa and Salvi, 2006). The glyoxalase pathway system has been recognized as one of the key of detoxification mechanisms for salt stress tolerance. The glyoxalase system comprises glyoxalase I, an enzyme that catalyzes the formation of S-D-lactoylglutathione from the hemithioacetal formed non-enzymatically from methylglyoxal and glutathione. While, glyoxalase II catalyzes the hydrolysis of S-D-lactoylglutathione to regenerate glutathione and liberate D-lactate.Methylglyoxal is a potent mutagenic and cytotoxic compound known to arrest growth in G1 phase of the cell cycle, reacts with the major macromolecules like DNA, RNA and protein, and increases sister chromatid exchange. Methylglyoxal induces oxidative stress in cells either directly through increased generation of ROS or indirectly by forming AGEs (advanced glycation end-products) with protein and nucleotide moieties (Wienstroer et al. 2012 and Hasanuzzaman et al. 2017). The Salt Overly Sensitive (SOS) pathway comprising SOS1, SOS2 and SOS3 genes has been recognized as the key mechanism controlling ion homeostasis under salinity stress. SOS2 component of this pathway encodes a serine/threonine protein kinase that together with SOS3 activates downstream Na^+/H^+ anti-porter SOS1, reestablishing cellular ion homeostasis under salinity stress (Kaur et al. 2015). Glyoxalase II has been isolated and characterized from a number of

sources, including humans(Cordell *et al.* 2004); yeast (Bito*et al.* 1997); rice (Yadav*et al.* 2007), and *Brassica juncea*(Saxena *et al.* 2005). Also, *SOS2* has been isolated and characterized from *Brassica juncea* (Kaur*et al.* 2015) and *Arabidopsis thaliana* (Batelli et al. 2007). In this study, we report the isolation of the *SbglyII and SbSOS2* allel for salinity tolerance from salt treated Egyptian sorghum plants with 200 mm NaCl and the expression of the isolated alleles in yeast as model eukaryotic system under different concentrations of salt stress.

MATERIALS AND METHODS

Plant material, growth conditions and stress induction of *Sb-glyII and SbSOS2*.

Elite sorghum (*Sorghum bicolor* (L.) Moench) cv. R3 were kindly provided by the Sorghum Department, Field Crops Research Institute, ARC, Giza, Egypt. Seeds sterilization and germination according to Shireen et al. (2014). Stress inducibility of the *gly II* and *SOS2* transcript Four-day-old seedlings of sorghum were Four days later the seedlings were transferred to ½ MS medium supplemented with 200 mMNaCl for 6 days in light.

Isolation of the Sbgly II and SbSOS2 alleles and sequencing analysis from Egyptian sorghum.

Total RNA was extracted from total fresh sorghum plantlets using of the RNeasy Plant Mini Kit (Qiagen, cat. # 74104). The gly II and SOS2 alleles were amplified from the cDNA of *Sorghum bicolor* using RT-PCR using the Access Quick T-RT-PCR System. The full length *glyII and SOS2* cDNA was PCR amplified using forward primer with *Sb-gly II*-F (5´ATGAGGATGCTGTCGAAGGC´3 and *Sb-glyII*-R5´TCAGAAGTTATCTTTTGCTCGACGG´3 and for *SbSOS2SOS2*-F5´AAGCTTATGGCGGGCGCGGCGGGGCGGAA´3and*SOS2*R5´CTAGCATGTGGTGTCCTCAGCACGC´3. These primers were designed based on the nucleotide sequences of Sorghum bicolor hypothetical protein mRNA accession number XM_002462620.1 for *Sbgly II* and Sorghum bicolor hypothetical protein mRNA accession number XM_002438609.1 *SbSOS2* as shown by National Center for Biotechnology Information (NCBI) and the PCR temperature profile for *Sbgly II* was comprised of 1 cycle at 45^{o}C/45 min, 1 cycle at 94^{o}C / 2 min 30 cycles of 94˚C /30 sec, 60˚C /45 sec and 72˚C/1 min and 1 cycle at 72˚C/10min. while, for the *SbSOS2*, the amplification temperature profile was as follows: 1 cycle at 94^{o}C/2 min ,30 cycles of 94˚C /45 sec, 58˚C /30 sec and 72˚C for 1.30 min and 1 cycle 10 min at 72˚C /10min *SbSOS2* . Moreover, to facilitate the cloning of the amplified alleles, the recognition sites of the restriction enzymes *EcoR I* and Hind III for *Sbgly II* and *SbSOS2*, respectively. For homology analysis, gly II and SOS2 PCR products were cloned in the pGEM-T vector and selected on IPTG, X-gal, and ampicillin plates. The clones were confirmed with restriction enzyems using the above-mentioned. The BLAST search program was used for sequence comparisons of DNA and amino acid sequences in GenBank NCBI.

Construction of the yeast expression vector and transformation.

After sequencing, the recombinant pGEM-T vectors harboring the *Sbgly II* and the *SbSOS2* alleles in pGEM-T vector were digested with *EcoRI*and *Not1* and *Hind III* and *Sac I* to liberate the inserts, respectively. The inserts were then ligated to the pYES2 yeast expression vector cut with the same restriction. The *Sbgly II* or *SbSOS2*/pYES2 plasmids were transformed into *S. cerevisiae* competent INVSc1 yeast cells by the Li-acetate method (Gietzet al. 1992) as a model eukaryotic system. Transformation of yeast was verified by colony PCR as a rapid and sensitive method for DNA amplification in yeast to detect the integration of the two isolated alleles in yeast genome (Awaly et al. 2015).

Expression analysis of the *Sb-glyII* and *Sb-SOS2* allele in yeast under different salinity conditions.

a- Measurement of yeast growth

Transgenic yeasts were grown on synthetic minimal defined medium (SC) (Awaly et al. 2015).

Sb-glyII and SbSOS2 alleles Expression analysis in transgenic yeast by RT-PCR

The transgenic *S. cerevisiae* INVSc1 cells harboring the expression plasmid SbglyII/pYES2 and SbSOS2/pYES2 were grown on minimal medium YPD according to awaly et al. (2003). Total RNA was extracted from transgenic yeast and non-transgenic yeast using sv total RNA isolation kit promega^{TM} (cat. No. Z3100). The RT-PCR reaction mix was set up as mentioned in above mention.

Determination of intracellular proline content in transgenic yeast

Proline content was determined according to (Bates et al. 1973; Sasano et al. 2012)

RESULTS

Isolation of Sb *gly II* and *SbSOS2* alleles and sequencing analysis.

The full-length cDNA of *SbglyII*and *SbSOS2* were isolated by RT-PCR from Egyptian sorghum cv. R3 plants under stress of 200 mMNaCl. This was performed using specific primers for each allele designed based on the nucleotide sequence of sorghum bicolor hypothetical protein mRNA. Bands indicating a fragment size of 1011bp and 1350bp for *Sbgly II* and *SbSOS2* respectively were shown by agarose gel electrophoresis (Fig. 1). The *Sbgly II* and SbSOS2 open reading frame (ORF) encodes for a protein of 336 and 449 amino acids respectively. *SbgLy*II-2 showed significant similarities with other reported GLY II from diverse species such as *Sorghum bicolor*, *Zea mays L., Oryza sativa,Brassica juncea, Triticumaestivum*and *Hordeumvulgare*, respectively. While, SOS2 showed significant similarities with other reported SOS2 from diverse species such as *Sorghum bicolor,Zea mays, Oryzacoarctata,Hordeumvulgare, Oryza sativa* and *Triticumaestivum*, respectively. These results agreement with Wang et al. (2004); Saxena et al. (2005) and Yadav et al. (2007)

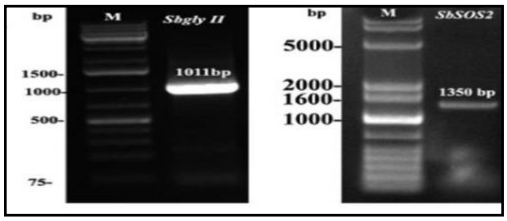
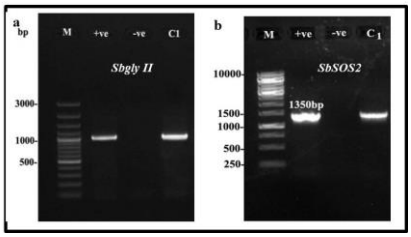


Figure. 1.Isolation of *Sbgly II* and *SbSOS2* alleles by RT-PCR.

Cloning and transformation of isolated alleles into yeast

The*Sb-glyII* and *Sb-SOS2* were cloned in the yeast expression vector pYES2 under the control of a galactose inducible and glucose repressible *GAL1* promoter to develop the pYES2-Sbgly II and pYES2-SbSOS2. The pYES2Sb-*gly II* and pYES2Sb-*SOS2* plasmids were isolated and confirmed by restriction digestion using the restriction enzymes *E.coR1* and *Not1* for *Sbgly II* and *Hind III* and *Sac I* for *SbSOS2*. The yeast *Saccharomyces cerevisiae* is an excellent model organism to produce heterologous proteins, and is suitable for investigating the mechanisms underlying stress tolerance (Serrano and Montesinos, 2003). The transformed colonies were selected on synthetic media without uracil (SC-U) and verified by colony PCR. PCR analysis revealed the presence of both transalleles*(Sbgly II* and *SbSOS2)* in yeast as illustrated in (Fig. 2).



Figure(2). Verification of *Sbgly II* (a) and *SbSOS2* (b) allele expression into transgenic yeast *via* colony PCR analysis.

Accordance of these results there are many authors have already described the transformation of yeast using pYES2 vector with different abiotic stress genes Daio et al.(2010); Ghosh et al. (2014); Li et al. (2014) and Kaouthar et al. (2016)

Evaluating transgenic yeast harboring *Sbgly II* and *SbSOS2* to salinity stress

Effect of salt stress on the growth of yeast

Yeast has played a major role in identifying functionality of plant stress related proteins. It is already known that, at the cellular level, the mechanisms of stress tolerance are similar in yeast and plants and therefore by over expression in yeast and functional selection approaches, plant stress alleles can be identified (Mulet et al, 2004). Results in Table (1) showed the evaluation of yeast growth on various concentrations of the NaCl showed the yeast negative control without pYES2 did not grow on SC lacking uracil. While, the growth of positive control yeast cells with PYES2 was significantly lower than those harboring Sbgly II-PYES2 or the SbSOS2-PYES2 under different concentration of NaCl. In this context, these results are in agreement with these reported by Zhang et al. (2011); Awaly et al. (2015) and Jogawat et al. (2016).

RT-PCR

The results presented in Fig. (3) Showed

amplification of the trans-alleles in transgenic yeast at the expected size for *Sbgly II* and *SbSOS2* ~1011 bp and ~1350bp, respectively. While, no band was detected in the negative control. Therefore, the RT-PCR results confirmed the expression of *Sbgly II* and *SbSOS2* in yeast. The efficiency of the reverse transcriptase-PCR (RT-PCR) as indicator for the presence of the transgene transcript in the transcriptome of transgenic yeast previously reported by Guo et al. (2010); Awaly et al. (2015) and Nakahara *et al.* (2015).

Determination of intracellular proline content in transgenic yeast under salt stress

Data presented in Table (2) showed that the mean values of proline accumulation increased significantly in all transgenic yeasts. While, these values were significantly decreased in the controls by raising the NaCl. These results suggested that the increase in proline content may contribute to improve the salt stress tolerance as well as maintenance of the membrane integrity of the transgenic yeast cells compared to the controls under stress conditions. These results agreement with reported by Sasano, et al. (2012) and Awaly et al. (2015).

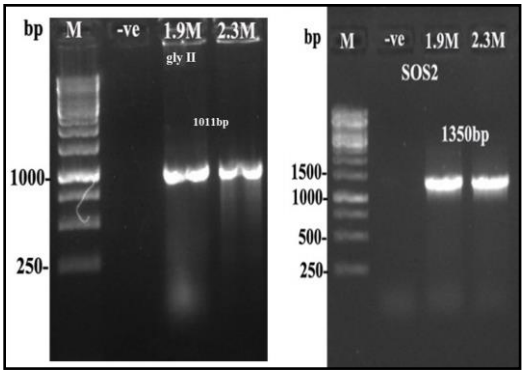


Figure. (3) Verification of *Sbgly II and SbSOS2* allele expression into transgenic yeast *via* RT-PCR analysis.

Table 1. Mean values of the growth of the transgenic yeast harboring Sbgly II and SbSOS2 and positive and negative controls under different concentration of salt stress at OD_{600}.

Salt Conc. (M)	Negative control (pYES2-)	Positive control (pYES2+)	*Sbgly II*	Negative control (pYES2-)	Positive control (pYES2+)	*SbSOS2*
0.0	0.0170_{aB}	3.2010_{aA}	3.2330_{aA}	0.015_{aB}	3.605_{aA}	3.621_{aA}
1.7	0.0170_{aC}	1.8300_{bB}	3.1420_{aA}	0.016_{aC}	2.124_{bB}	3.564_{aA}
1.8	0.0160_{aC}	1.0320_{cB}	1.7940_{bA}	0.017_{aB}	1.202_{cB}	2.456_{cA}
1.9	0.0180_{aB}	$0.\ 6450_{bA}$	1.2020_{cA}	0.015_{aB}	0.534_{cdB}	1.427_{bA}
2.0	0.0150_{aB}	0.163_{deB}	0.9250_{cA}	0.013_{aB}	0.211_{dB}	0.972_{dA}
2.1	0.016_{aB}	0.041_{deB}	0.5830_{dA}	0.019_{aB}	0.082_{dA}	0.651_{eA}
2.2	0.014_{aB}	0.0320_{dA}	0.3710_{dA}	0.012_{aA}	0.037_{dA}	0.461_{fA}
2.3	0.0160_{aA}	0.0180_{eA}	0.1090_{eA}	0.015_{aA}	0.023_{dA}	0.139_{gA}

Table 2. Mean values of intracellular proline content in the transgenic yeast harboring Sbgly II and *SbSOS2* and positive and negative controls under different concentration of salt stress.

Salt Conc. (M)	Negative control (pYES2-)	Positive control (pYES2+)	*Sbgly II*	Negative control (pYES2-)	Positive control (pYES2+)	*SbSOS2*
0.0	14.680_{aA}	14.750_{aA}	14.90_{bcA}	12.59_{bA}	12.6_{bA}	12.63_{eA}
1.7	14.560_{aA}	14.62_{aA}	16.31_{bcA}	14.4_{aA}	14.5_{aA}	14.7_{dA}
1.8	12.56_{abB}	12.69_{abAB}	18.56_{abA}	11.3_{cB}	11.4_{bB}	16.9_{cA}
1.9	10.76_{abB}	10.78_{abB}	20.90_{aA}	9.18_{dB}	9.3_{cB}	18.3_{cA}
2.0	6.8870_{bcB}	6.81_{bcB}	21.38_{aA}	4.75_{eB}	4.7_{dB}	19.5_{bA}
2.1	4.0600_{cB}	4.1000_{cB}	21.375_{aA}	2.74_{fB}	2.8_{eB}	21.04_{aA}
2.2	2.6250_{cB}	2.6230_{cB}	21.312_{aA}	1.04_{gB}	1.1_{fB}	21.35_{aA}
2.3	1.3120_{cB}	1.440_{cB}	21.250_{aA}	0.81_{gB}	0.8_{fB}	21.22_{aA}

Numbers with the same letters are not significantly different at 5%

CONCLUSION

In the present investigation, the two salt stress tolerance alleles (*Sb-gly II* and *Sb-SOS2*) were isolated from Egyptian sorghum using RT-PCR. The tolerance of transgenic yeast to salt stress indicates the protective effect of two alleles via a common cellular pathway that activated by salt stress. Therefore, the present results suggested that the *Sbgly II* and *Sb-SOS2* alleles could be used for genetically improve economical important crops like maize, rice sorghum, wheat, *etc*. to salt stress.

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CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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