DIFFERENTIAL EXPRESSION PROFILES OF APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) GENE FAMILY IN EGYPTIAN SUGARCANE (SACCHARUM SPONTANEUM L.) IN RESPONSE TO PHYTOHORMONES AND A BIOTIC STRESS

Mohamed Ewas
E-mail: mohamed_ewas82@yahoo.com

The APETALA2/Ethylene-Responsive Factor (AP2/ERF) gene family is a transcription factors that is particular to plants. AP2/ERFs play essential roles in growth and development regulation, biotic and abiotic stressors tolerance, and responding to plant hormones. However, information on the roles of AP2/ERF gene family in Egyptian sugarcane (Saccharum spontaneum) is lacking. In the current study, a genome-wide analysis was performed to identify the AP2/ERF genes in S. spontaneum. Using bioinformatics techniques, 288 SsAP2/ERF have been acquired in total. They were classified into five sub-families based on phylogenetic study, with 14 AP2, 105 DREB, 4 RAV, 143 ERF, 1 Soloist, and 21 ANT. Gene structure, chromosome localization and conserved domain were investigated for SsAP2/ERF genes. As opposed to its homologue genes, which more often express in nodes and buds, tissue-specific expression analysis demonstrated that SsAP2/ERF genes were numerous and expressed ubiquitously in all examined tissues, with slightly higher levels in roots followed by leaves, and flowers. Interestingly, 6 SsAP2/ERF genes that contains two AP2 domains exhibited diverse expression patterns in response to phytohormones including abscisic acid, gibberellic acid, salicylic acid, and methyl jasmonate along with various abiotic stresses such as drought, high salinity, high or low temperature. Concisely, results of this research provide a deep insight towards further functional exploration of the SsAP2/ERFs in response to phytohormone and abiotic stress with the eventual goal of developing crop production.

Keywords: expression profiles, AP2/ERF family, sugarcane, phytohormones, abiotic stress
INTRODUCTION

Egyptian sugarcane *Saccharum spontaneum* L. is regarded as one of the world's earliest cultivated crops. Egypt is known to be one of the oldest native ranges of this subspecies (Gaber et al., 2009). Its habitat extends on the border of water streams within the Egyptian deserts and Nile delta (Abd El-Gawad and El-Amier, 2017). Commercially grown in tropical and subtropical areas. When favorable environmental conditions for the crop are present, it is the most formative crop (Gaber et al., 2009 and FAO and UN-Water, 2021). Almost 80% of sugar and 40% of ethanol are produced globally using sugarcane (*Saccharum spp*.), a traditional C₄ crop with the highest photosynthetic rates of any crop (Lam et al., 2009; Zhang et al., 2013 and Li et al., 2020). The three major species of the genus *Saccharum* are *S. spontaneum*, *S. officinarum*, and *S. robustum*, according to standard classification (Irvine, 1999). A hybrid between *S. spontaneum* and *S. officinarum* led to the development of the recent sugarcane cultivar. The original species for research on sugarcane are thought to be *S. spontaneum* and *S. officinarum*. However, the only autopolyploid with allele-defined genome data accessible in *Saccharum* is *S. spontaneum* AP85-441, which was developed from the anther of octoploid SES208 (Moore et al., 1989 and Zhang et al., 2018). According to assumptions, *S. officinarum* contributed to the genetic basis of the *Saccharum* hybrid's high sugar content, and *S. spontaneum* to its environmental stresses including biotic and abiotic stresses (Roach, 1972). In Egypt, *S. spontaneum* spread as wild plants in Egyptian deserts, which are characterized by various harsh environmental stresses, including drought, heat, salinity, and other stressors (Zahran et al., 2016). It is extremely harsh to correctly identify all members of some gene families based solely on sequence consideration, particularly for the massive gene families, such as AP2/ERF, because of the possible collapsing of homologous sequences that happened during the arrangement of the tetraploid *S. spontaneum* genome (Li et al., 2020). Inclusive understanding a gene family's molecular architecture and evolutionary history in a plant species is the first step in the direction of comprehending the physiological functions and metabolic processes involved in various growth phases (Zhang et al., 2016). Recent research has shown that most plant species included at least five members of the largest gene family known as AP2/ERF.

One of the biggest gene families, the APETALA2/Ethylene-Responsive Factor (AP2/ERF) gene family encodes transcription factors (TFs) unique to plants. The AP2/ERF domain, which has between 60 and 70 amino acids and participates in DNA binding, is what distinguishes the AP2/ERF superfamily (Guo et al., 2016). The AP2/ERF superfamily is further classified into the AP2 (APETALA2), ERF (Ethylene Responsive Factors), Soloist, RAV (Related to...
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ABI3/VP), and DREB (Dehydration Responsive Element Binding) sub families (Sakuma et al., 2002; Wessler, 2005; Nakano et al., 2006 and Liu et al., 2013). Members of the AP2 subfamily contain several AP2/ERF domains or absence conserved WLG motifs inside the AP2/ERF domains. TFs of the RAV subfamily contain individual AP2/ERF and B3 domains, while DREB and ERF subfamilies have single AP2/ERF domain, the rest of TFs are assigned as soloist (Sakuma et al., 2002). The members of the AP2/ERF gene family are crucial in controlling how plants develop and how well they can withstand biotic and abiotic stressors (Liu et al., 2013; Schmidt et al., 2013 and Jiang et al., 2014). The AP2/ERF gene family has been discovered in many diverse plants, including Arabidopsis and rice (Nakano et al., 2006), maize (Liu et al., 2013), sorghum (Yan et al., 2013), soybean (Zhang et al., 2008), and foxtail millet (Lata et al., 2014). However, no study has been carried out to identify or characterize the members of gene family AP2/ERF in S. spontaneum. In the current study, bioinformatic techniques were used to identify SsAP2/ERF genes in Egyptian sugarcane S. spontaneum and created a phylogenetic tree. Using drawing tools, most of the SsAP2/ERF genes were localized to chromosomes, and duplication processes were also examined. Via published RNA sequencing, the expression patterns of AP2/ERF genes were revealed. Finally, quantitative real-time polymerase chain reaction (qRT-PCR) was applied to measure the expression level of the only 6 AP2/ERF genes contain two AP2 domains in response to a biotic stress including drought, high-salt stress, heat shock and cold along with hormone stresses including exogenous abscisic acid (ABA), gibberellic acid (GA3), salicylic acid (SA), and methyl jasmonate (MeJA). These findings will be beneficial for prospective research on the AP2/ERF family in various plant species.

MATERIALS AND METHODS

1. Plant Material and Sequence Database Searches

Samples of Egyptian sugarcane S. spontaneum were taken from the Nile delta habitat where it grows along the edge of streams (Location data 31°39′16.9″E). Following Boulos (2005), the samples were identified. To gather every S. spontaneum SsAP2/ERF gene member, numerous database searches were conducted. The sugarcane genome hub. Tripal database infrastructure (https://sugarcane-genome.cirad.fr/), TAIR (The Arabidopsis Information Resource) (http://www.arabidopsis.org/), and the plant TF database (http://planttfdb.cbi.pku.edu.cn/) were used to acquire the AP2/ERF sequences of S. officinarum, Arabidopsis thaliana, S. spontaneum, (Jin et al., 2017). The genomic information for S. bicolor as well as additional species were gathered from NCBI (https://www.ncbi.nlm.nih.gov/) and Phytozome.
Zhang lab produced the *S. spontaneum* genome data; its GenBank accession number is QVOL0000000. (Zhang et al., 2018).

2. Identification of AP2/ERF in *S. spontaneum*

Using the BLASTP program and default parameters, an HMM profile of the AP2/ERF (PF03106) was derived from the Pfam protein family database (http://pfam.xfam.org/) (Finn et al., 2016). This profile was used to recognize probable AP2/ERF from the *S. bicolor* genome sequence (Ling et al., 2011). The chosen sorghum AP2/ERFs were then utilized as query sequences in BLASTP searches against the *S. spontaneum* predicted sequences. The HMM profile of AP2/ERFs domains was used as the query to search all possible proteins, and the sequences with *E*-values lower than 1e-10 were chosen for additional study. Subsequently using the online ExPASy-ProtParam programme (http://web.expasy.org/protparam/), both the chemical and physical features of the putative SsAP2/ERFs were determined. The genes that were wrongly predicted were manually annotated.

3. Multiple Sequence Alignment, Phylogenetic Analysis and Classification of Sugarcane AP2/ERFs

DNAMAN was used to perform numerous sequence alignments on 288 putative SsAP2/ERF proteins. Using MUSCLE in MEGA 7.0’s default parameters, the domain sequences of SsAP2/ERFs and AtAP2/ERFs were aligned (Kumar et al., 2016). Using MEGA 7.0, the NJ technique, the Poisson model, pairwise deletion, and the bootstrap test replicated 1,000 times, the phylogenetic tree based on the alignments was created. The previously reported categorization of SsAP2/ERFs and the multiple sequence alignment were used to categorize SsAP2/ERFs into several groups and subgroups. (Ross et al., 2007; Rushton et al. 2010 and Xu et al., 2016).

4. Exon-Intron Structure of SsAP2/ERF Genes

Diagrams were collected via the online tool Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/), and the exon-intron structures of the sugarcane AP2/ERF genes were established depending on their coding sequence alignments and corresponding genomic sequences (Hu et al., 2015).

5. Chromosomal Locations and Collinearity Analysis for All SsAP2/ERFs

The database of the *S. spontaneum* genome was used to determine the precise position of SsAP2/ERFs on the chromosomes. The Multiple Collinearity Scan toolbox (MCScanX) and the BLASTP program (*E*-value < 1e-5) were utilized to examine the duplication pattern for each SsAP2/ERF gene (Wang et al., 2012).

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6. Plant Growth and Stress Treatments

For gene expression profiling investigation, rhizomes of Egyptian sugarcane plants (S. spontaneum) were planted at Tanta University’s experimental greenhouse in Gharbia, Egypt, during the 2021/2022 season. Ammonium nitrate was added in the amount of 40 kilogram per feddan. Irrigation practices were administered at 10–15-day intervals throughout the growing season, except for August and September, which were at 3–5-day intervals. Two-month-old uniformly grown S. spontaneum plants were exposed to various stressors or hormone treatments. Salt stress was generated by watering the plants with a 200 mM NaCl solution. Drought stress was reproduced by placing detached leaves on filter paper in 70% relative humidity at 25°C. Cold or heat stress conditions were induced by transplanting the plants to a growth chamber and keeping them at 4°C or 40°C, respectively. The wounding was done by pinching the leaves with forceps. To address hormones and oxidative stress, 100 µM ABA, 100 µM GA₃, 100 µM SA, and 100 µM MeJA were sprayed directly onto S. spontaneum plants. Following each treatment, leaves from three distinct plants (three biological replicates) were collected and promptly frozen in liquid nitrogen before being held at 80°C until RNA was extracted. Salt stress was generated by watering the plants with a 200 mM NaCl solution. Drought stress was reproduced by placing detached leaves on large filter paper in 70% relative humidity at 25°C. Cold or heat stress conditions were induced by transplanting the plants to a growth chamber and keeping them at 4°C or 40°C, respectively. To address hormones and oxidative stress, 100 µM ABA, 100 µM GA₃, 100 µM SA, and 100 µM MeJA were sprayed directly onto S. spontaneum plants. Following each treatment, leaves from three distinct plants (three biological replicates) were collected and promptly frozen in liquid nitrogen before being held at 80°C until RNA was extracted.

7. Expression Analyses of SsAP2/ERFs

The first-strand complementary DNA (cDNA) was generated using 3 µg of total RNA and 200 U of M-MLV reverse transcriptase (from Invitrogen), following the manufacturer’s instructions. Total RNA was isolated using Trizol solution (Invitrogen). Using first-strand cDNA as a template, RT-PCR was performed to amplify a 400 bp fragment of each SsAP2/ERF gene with 31 cycles. As an additional internal control, the actin was amplified for 24 cycles. Using the SYBR Premix Kit F-415 and an AB StepOnePlus PCR equipment from Applied Biosystems, real-time PCR was carried out on an optical 96-well plate. (Thermo Scientific). A relative quantification method50 was used to determine relative gene expression. The list of all primers used in this investigation can be found in Table (1).
Table (1). Forward and reverse primers used in the qRT-PCR genes expression studies.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sequence (5'–3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>SsAP2/ERF-1 - F</td>
<td>ATTTCCGCAACCACCATTTCCC</td>
</tr>
<tr>
<td>SsAP2/ERF-1 - R</td>
<td>CTGCCAACACACCACCTTAC</td>
</tr>
<tr>
<td>SsAP2/ERF-2 - F</td>
<td>GCGGAGGGGAGAGTAATTGA</td>
</tr>
<tr>
<td>SsAP2/ERF-2 - R</td>
<td>ACCGGGACTTTTCCAGCAGA</td>
</tr>
<tr>
<td>SsAP2/ERF-3 - F</td>
<td>TCCACCAGATCCAAACCCAA</td>
</tr>
<tr>
<td>SsAP2/ERF-3 - R</td>
<td>GCTTCTTGCTGTCTAAA</td>
</tr>
<tr>
<td>SsAP2/ERF-4 - F</td>
<td>GATCCCCAAGCCACAAATC</td>
</tr>
<tr>
<td>SsAP2/ERF-4 - R</td>
<td>AAATTAAGCCGAGCGATGG</td>
</tr>
<tr>
<td>SsAP2/ERF-5 - F</td>
<td>TTTCAATGCGTGCTCAA</td>
</tr>
<tr>
<td>SsAP2/ERF-5 - R</td>
<td>GCACGCGTTATGTTGAGG</td>
</tr>
<tr>
<td>SsAP2/ERF-6 - F</td>
<td>GGATAAGAGTCGAGTG</td>
</tr>
<tr>
<td>SsAP2/ERF-6 - R</td>
<td>AAGTTCAACAGGCATCAC</td>
</tr>
<tr>
<td>Actin - F</td>
<td>TGGAATGGAAAGCTGCGT</td>
</tr>
<tr>
<td>Actin - R</td>
<td>TTGTCTTCATGCTG</td>
</tr>
</tbody>
</table>

RESULTS

1. Identification and Characterization of AP2/ERF Gene Family in S. spontaneum

In general, 288 genes were identified as agreeable AP2/ERF genes in S. spontaneum. The expected SsAP2/ERF genes (public name and locus ID has been presented in Supplementary Table S1 in specifics) were subsequently chosen depending on the position of chromosome and their family categorization (Table S1). According to the classification, they were divided into 14 AP2, 105 DREB, 4 RAV, 143 ERF, 1 Soloist and 21 ANT. Thirty five AP2 TFs with a single or two AP2 domains that were identical with AP2 domains in double domain groups were categorized into the APETALA2/Ethylene Responsive Factor gene family (AP2/ERF), while 4 genes include B3 type domain were categorized as RAV gene family. Only one domain from the ERF subfamily, which was further subdivided into the ERF and DREB subgroups, was present in 248 genes. Also, a particular gene known as SsAP2/ERF-288 resembled other relatives that belonged to the Soloist subgroup (Table S1). In the 20 chromosomes of the S. spontaneum, the distribution of SsAP2/ERF genes was shown to be uneven (Fig. 1). The chromosomes with the greatest and smallest number of AP2/ERFs were 2 and 16 (30 genes) and 5 (9 genes), respectively.

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2. Phylogenetic Relationships and Gene Structure Analysis of SsAP2/ERF

Based on the numerous correlations of all SsAP2/ERF with Arabidopsis AP2/ERF genes, phylogenetic analysis was carried out to evaluate the evolutionary links of the SsAP2/ERF genes. SsAP2/ERF family proteins were used to create an unrooted phylogenetic tree (Fig. 2). ERF clades were also split up into 10 groups. The ERF families could be further separated into a subgroup of DREB and the ERF families, much like the Arabidopsis assortment criteria (Guo et al., 2016).

The following six groups (V-X) were the ERF subgroups, and the remaining four groups (I-IV) belonged to DREB (Fig. 3). Among 35 AP2 TFs only 6 AP2/ERF genes contain two AP2 domains (Fig. 4). The analysis of phylogenetic tree rearranged the six AP2/ERF genes according to the degree of their structure similarity or divergence with each other. The examination of SsAP2/ERF proteins for conserved motifs revealed that each of the proteins has two AP2 structural domains (Motif 1 and Motif 2, or AP1 and AP2) that comprise AP2/ERF domain sequences. The SsAP2/ERF gene motifs on the same branch are comparable in number, type, and arrangement, and the functional variations in...
tomato SsAP2/ERF genes may result from variations in the distribution of conserved motifs (Fig. 4).

**Fig. (2).** An unrooted phylogenetic tree of AP2/ERF family proteins in *Saccharum spontaneum*. The complete sequences of 288 AP2/ERF family proteins identified in this study were aligned by ClustalX2.1 and the phylogenetic tree was constructed using the neighbor-joining method with MEGA7.0 software.
3. Synteny Analysis of SsAP2/ERF Genes

To evaluate the molecular evolutionary links between species, synteny analysis is a crucial analytical method in comparative genomics (Zhao and Schranz, 2017). The AP2/ERF gene was more homologous on Egyptian sugarcane S. spontaneum and S. officinarum, likely due to their close kindred, according to a homology analysis of the SsAP2/ERFs between S. spontaneum and other species, as shown in Fig. (5). Interestingly, SsAP2/ERF2 and SsAP2/ERF5 correspond to two TF pairs found in the respective organisms Arabidopsis thaliana and S. officinarum. Even in the presence of gene duplications or
chromosomal rearrangements, SsAP2/ERF synteny analysis revealed substantial collinearity.

**Fig. (5).** Syntenic relationships between homologous SsAP2/ERFs of Saccharum spontaneum and other species including Arabidopsis thaliana and Saccharum officinarum.

4. **Tissue-Specific Expression Analysis of SsAP2/ERF Genes in S. spontaneum**

RNA was extracted from roots, nodes, internodes, buds, leaves and flowers. Using qRT-PCR, the expression of all SsAP2/ERF genes in the Egyptian sugarcane *S. spontaneum* was identified. The SsAP2/ERF gene expression profile showed that there were differences in the transcription level of the six SsAP2/ERF genes in different tissues of *S. spontaneum*. The expression of SsAP2/ERF4 was higher than other SsAP2/ERF genes, as shown in Fig. (6). As opposed to its homologue genes, which more often express in nodes and buds, tissue-specific expression analysis demonstrated that SsAP2/ERF genes were numerous and expressed ubiquitously in all examined tissues, with slightly higher levels in roots followed by leaves, and flowers (Fig. 6), suggesting their fullest potential function outside of nodes and buds. The tissue-specific expression analysis of SsAP2/ERF genes in *S. spontaneum* revealed that each of SsAP2/ERF1, SsAP2/ERF2 and SsAP2/ERF4 were expressed in roots followed by leaves, and flowers higher than the remain tissues including buds, nodes and internodes. On the other hand, the transcripts of SsAP2/ERF3, SsAP2/ERF5 and SsAP2/ERF6 varied from one tissue to another with different levels (Fig. 6).
Fig. (6). Tissue-specific expression analysis of SsAP2/ERF genes in Saccharum spontaneum. a. Expression patterns of SsAP2/ERF1; b. Expression patterns of SsAP2/ERF2; c. Expression patterns of SsAP2/ERF3; d. Expression patterns of SsAP2/ERF4; e. Expression patterns of SsAP2/ERF5; f. Expression patterns of SsAP2/ERF6 using qRT-PCR (relative to actin).
5. Differential Expression Profiles of SsAP2/ERF Genes in Response to Abiotic Stress

To better understand and validate the expression of these identified AP2/ERF genes, the six SsAP2/ERF genes that contain two AP2 domains were picked to assist scientists in detecting their transcription levels in the leaves of 2-months-old S. spontaneum in response to drought, high salt, cold and heat stresses using qPCR (Fig. 7). The SsAP2/ERF genes were significantly induced by some of the stress conditions. Under drought stress, the transcripts of all SsAP2/ERF genes were induced varyingly (Fig. 7a). Under drought stress conditions, the gene SsAP2/ERF4 had the highest expression, followed by SsAP2/ERF1, then SsAP2/ERF2, and the gene SsAP2/ERF5 had the lowest expression compared with their expression levels under normal conditions (Fig. 7a). Under high salt stress (200 mM NaCl), the expression of SsAP2/ERF1, SsAP2/ERF2, SsAP2/ERF3, SsAP2/ERF4, SsAP2/ERF5 and SsAP2/ERF6 increased approximately up to 2, 2.5, 3, 4, 2.5 and 6-fold, respectively (Fig. 7b). Interestingly, subjecting S. spontaneum plants to cold stress (4°C) resulted in the accumulation of SsAP2/ERF transcripts as follow, SsAP2/ERF1, SsAP2/ERF3, SsAP2/ERF4, SsAP2/ERF5 and SsAP2/ERF6 up to 20, 18, 16, 16-fold, while transcripts of both SsAP2/ERF2 and SsAP2/ERF5 genes did not show any significant response to cold stress (Fig. 7c). On the other hand, heat stress (40°C) gradually up-regulated the expression of SsAP2/ERF1, SsAP2/ERF2, SsAP2/ERF3, SsAP2/ERF4, SsAP2/ERF5 and SsAP2/ERF6 by approximately 4, 3, 5, 4, 2 and 1.5-fold, respectively (Fig. 7d). In general, among the SsAP2/ERF gene family, the genes most responsive to abiotic stress are SsAP2/ERF1, SsAP2/ERF2, and SsAP2/ERF4.

6. Differential Expression Profiles of SsAP2/ERF Genes in Response to Phytohormones

To investigate the hormonal response of SsAP2/ERFs, four major hormones were chosen for this study including ABA, GA3, SA, and MeJA. The current study’s findings revealed, in general, a rapid response of SsAP2/ERFs genes to the applied hormones. As compared to other applied hormones, the highest transcription levels of SsAP2/ERF genes were associated with the ABA treatment. Furthermore, the transcripts of SsAP2/ERF1, SsAP2/ERF2, SsAP2/ERF3, SsAP2/ERF4, SsAP2/ERF5 and SsAP2/ERF6 rapidly increased up to 12, 8, 6, 10, 5 and 6-fold, respectively within 12 hours in response to ABA (100 μM) (Fig. 8a). A similar pattern was seen for transcript induction by SA (100 M), which resulted in 2, 1.5, 2, 2.5, 2.5, and 1.5-fold increases in SsAP2/ERF1, SsAP2/ERF2, SsAP2/ERF3, SsAP2/ERF4, SsAP2/ERF5, and SsAP2/ERF6 mRNA accumulation (Fig. 8b). Regarding GA3 treatment, the response of
SsAP2/ERF genes varied significantly from one another, with SsAP2/ERF3, SsAP2/ERF5, and SsAP2/ERF6 being markedly elevated while SsAP2/ERF1, SsAP2/ERF2, and SsAP2/ERF4 being notably reduced (Fig. 8c). MeJA (100 M) caused up-regulation of SsAP2/ERF1, SsAP2/ERF4, and SsAP2/ERF6 by approximately 3, 2, and 4-fold, respectively, and down-regulation of SsAP2/ERF2 and SsAP2/ERF5, with no significant difference in SsAP2/ERF3 transcript levels (Fig. 8d). Overall, ABA and SA hormones were more effective than the other hormones (Fig. 8).

**Fig. (7).** Expression profiling of SsAP2/ERF gene family in response to abiotic stress compared to control using qRT-PCR (relative to actin). a. Expression profiling of SsAP2/ERF gene family in response to drought stress; b. Expression profiling of SsAP2/ERF gene family in response to salt stress; c. Expression profiling of SsAP2/ERF gene family in response to heat stress; d. Expression profiling of SsAP2/ERF gene family in response to cold stress.

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Fig. (8). Expression profiling of SsAP2/ERF gene family in response to phytohormones compare to control using qRT-PCR (relative to actin). 

a. Expression profiling of SsAP2/ERF gene family in response to abscisic acid (ABA); 
b. Expression profiling of SsAP2/ERF gene family in response to gibberellic acid (GA$_3$); 
c. Expression profiling of SsAP2/ERF gene family in response to salicylic acid (SA); 
d. Expression profiling of SsAP2/ERF gene family in response to and Methyl jasmonate (MeJA).

**DISCUSSION**

1. **Identification of the Egyptian Sugarcane S. spontaneum AP2/ERF superfamily**

The AP2/ERF superfamily is one of the most extensive collections of TF families in crops. It additionally plays a vital role in the transcriptional control of

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environmental stresses including tolerance to heat shock, cold, wounding, drought and salt stressors along with pathogen resistance and intricate developmental processes such as fruit ripening, seed germination, flowering, and leaf senescence (Klücher et al., 1996; Dubouzet et al., 2003; Yang et al., 2011; Schmidt et al., 2013 and Zhu et al., 2014). The AP2/ERF gene family in plants has been extensively studied depend on plant genome sequencing (Nakano et al., 2006; Zhang et al., 2008; Sharma et al., 2010; Liu et al., 2013; Yan et al., 2013; Lata et al., 2014; Sun et al., 2014 and Thamilarasan et al., 2014). However, knowledge of the sugarcane *SsAP2/ERF* genes is currently limited. 288 *SsAP2/ERF* genes were discovered from the 87289 (up-regulated and down-regulated genes) identified genes and genomic DNA database (Li et al., 2020) to further study the *AP2/ERF* family in Egyptian sugarcane *S. spontaneum*. Each of them possesses distinguishing characteristics, including at least one conserved AP2/ERF domain. surprisingly, *S. spontaneum* had more *SsAP2/ERF* genes than *Arabidopsis thaliana* (147 genes), *Oryza sativa* (164 genes) and *Zea mays* (210 genes) (Nakano et al., 2006 and Liu et al., 2013). Furthermore, the numbers of certain subfamilies were comparable. The number of *AP2* subfamily members in *S. spontaneum* and *Arabidopsis thaliana*, for example, was 14 and 18, respectively, which was half of the number in rice and maize. *RAV* subfamily counts in *S. spontaneum*, *Hordeum vulgare*, *Arabidopsis thaliana*, *Oryza sativa* and *Setaria italica* were 4, 6, 6, 7, and 5, respectively. The numbers of other subfamilies, on the other hand, were dramatically different. In the *DREB/ERF* subfamily of *S. spontaneum*, for example, 105 genes have been discovered. *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, and *Setaria italica* have 122, 131, 163, and 138 genes, respectively. The present study showed that *S. spontaneum* has only 6 *AP2/ERF* genes that contains 2 AP2 domains. The fewer number of *AP2/ERF* family genes in *S. spontaneum* proposed that there may be more additional *SsAP2/ERF* genes present in the undiscovered genomic locales or that the evolutionarily constrained chromosomal duplication occurred in barley (Guo et al., 2016). The function of genes is significantly influenced by the conserved motifs found in TFs (Sakuma et al., 2002). In *Arabidopsis*, fifty conserved domains were found that were not part of the AP2/ERF motif (Nakano et al., 2006). In the current study, Five AP2/ERF protein domains were examined; domain 1 (a portion of the AP2/ERF domain) was found in all gene members, whereas the other domains were found outside the AP2/ERF motif. The importance of these conserved amino acid residues for the *AP2/ERF* subfamily genes involved in various types of physical contact with DNA is likely indicated by their presence (Sakuma et al., 2002).
2. Expression Analysis Suggested SsAP2/ERF Genes May Play Essential Roles During Plant Growth and Development Along With Abiotic stress and Hormone Response

Tissue-specific expressions that result at a specific growth stage can be used to discover genes that play a role in determining the exact characteristics of individual tissues. The expression pattern of 288 SsAP2/ERF genes was discovered by RNA sequencing in this work, which helped to analyze the function of the SsAP2/ERF genes in S. spontaneum. Surprisingly, the SsAP2-4 gene was also known as Cleistogamy 1 (Cly1)/SsAPETALA2 (SsAP2), and it was an ortholog of the Arabidopsis thaliana AP2 (AT4G36920.1), TOE3 (AT5G67180.1), and the Oryza sativa AP2-like gene Os04g0649100 (Nair et al., 2010 and Houston et al., 2013). Cly1 expression was discovered in the root, leaves and lodicule up to the stamen primordium stage via in situ RNA hybridization (Nair et al., 2010). Another gene, HvDREB2.2, commonly known as Nud (Nudum), regulates covered/naked caryopsis in Hordeum vulgare and exhibits expression in the caryopsis 14-day-post anthesis instead of the hulls or leaves (Taketa et al., 2008). SsAP2/ERF4 showed relatively high transcription levels in roots, leaves, and flowers at two-month-old plants in the current study. Consequently, more complex specificity expression analysis is useful to decipher the role of SsAP2/ERF genes.

In their native surroundings, plants experienced negative environmental pressures. They have developed a wide range of molecular strategies to deal with them. In plants, 2 ABA dependent and 2 ABA independent signal transduction pathways are implicated in the genes’ responses to drought, high-salt, and high or low temperature stress (Shinozaki and Yamaguchi-Shinozaki, 1997 and Nakashima et al., 2000). The expression profiling of SsAP2/ERF genes under various hormones stress unrevealed the vital roles of phytohormones including ABA, SA, MeJA and GA3 in impacting environmental stress. The fact that Egyptian sugarcane S. spontaneum is one of the plant species that grows wild in desert environments and is characterized by a variety of adverse environmental conditions such as drought, salinity, and high or low temperature, it requires a distinctive genetic stock that enables it to resist these a biotic stress, including SsAP2/ERF gene family. Considering the ongoing climatic challenges, using crop wild relatives to increase genetic diversity and enhance crop adaptation appears to be a promising and sustainable strategy for agricultural development (Kapazoglou et al., 2023). Crop wild close relatives are earlier generations or progenitors of cultivated crop species, as well as other nearby relatives via evolutionary history which can naturally cross with cultivated species, occasionally using supporting methods (Choudhary et al., 2017). To investigate the molecular mechanisms underlying plant environment adaptation under abiotic Egyptian J. Desert Res., 73, No. 1, 109-130 (2023)
stress and hormone response, gene expression analysis of SsAP2/ERFs should be helpful. The function of the differentially expressed genes between S. spontaneum and S. officinarum needs to be further examined in relation to abiotic stress and hormone response.

**CONCLUSION**

The present study sought to discover and describe the S. spontaneum AP2/ERF TFs and 288 SsAP2/ERF genes were identified after a thorough genomic search. All their information was verified by looking at entire cDNA or EST sequences. Analysis and comparisons were made of the chromosomal locations, exon-intron structures, conserved motif combinations, and phonological relationships of SsAP2/ERFs. Based on the amount of AP2 domains and probabilistic functions, SsAP2/ERFs may be divided into five subgroups. In addition to being evaluated in relation to heat, cold, salt, and drought stress, the gene expression of the six SsAP2/ERF genes contains two AP2 domains in various tissues (including roots, nodes, internodes, buds, leaves and flowers) was also examined. The role of many SsAP2/ERF genes in plant development and stress response was discovered, and they might be investigated further. This study unveils for the first time the regulation, structure, evolution, and transcription levels of the SsAP2/ERF family, which simplifies the process of the SsAP2/ERF gene function analysis and develops a basis for a deeper comprehension of the molecular mechanisms underlying plant development and physiological stress processes in S. spontaneum.

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ملامح التعبير التفاضلي للعائلة الجينية APETALA2/ethylene-responsive factor (AP2/ERF) 
في قصب السكر المصري (Saccharum spontaneum L.) 
للهرمونات النباتية والإجهاد اللاحيائي

محمد عويس
وحدة الوراثة والسيتولوجي، قسم الأصول الوراثية، مركز بحوث الصحراء، القاهرة، مصر.
قسم الوراثة الجينية، كلية علوم الحياة، جامعة هاوزينج الزراعية، ووهان، الصين.

عائلة الجينات APETALA2/ethylene-responsive factor (AP2/ERF) هي عوامل نسخ خاصة بالنباتات، وتلعب أدواراً جوهرية في تنظيم النمو والتطور، وتتحمل الضغوطات الحيوية وغير الحيوية، والاستجابة للهرمونات النباتية. ومع ذلك، لا توجد معلومات عن أدور العائلة الجينية في قصب السكر المصري (Saccharum spontaneum). في الدراسة الحالية، تم إجراء تحليل على مستوى الجينوم لتحديد جينات AP2/ERF في S. spontaneum باستخدام تقنيات المعلوماتية. تم تصنيفهم إلى خمس عائلات فرعية بناءً على دراسة النشوء والتطور، وهي 41 AP2 و 50 DREB و 4 RAV و 341 ERF و 1 Soloist و 12 ANT.

تم فحص بنية الجينات ومواقع الكروموسومات والمناطق المحفوظة لجينات SsAP2/ERF على S. spontaneum و S. officinarum. من المثير للاهتمام أن الستة جينات SsAP2/ERF التي تحتوي على نطاقين AP2 قد أظهرت أنماط تعبير متنوعة استجابة للهرمونات النباتية، كما قدمت نتائج هذا البحث نظرة عميقة نحو المزيد من الاستكشاف الوظيفي لـ SsAP2/ERF المحاصل.

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