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MicroRNA expression profiles in response to drought stress in *Sorghum bicolor*

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ABSTRACT

The regulatory role of small non-coding RNAs that are 20–24 nucleotides in length has become the foremost area of research for biologists. A major class of small RNAs represented by the microRNAs (miRNAs), has been implicated in various aspects of plant development including leaf pattering, meristem function, root patterning etc. Recent findings support that miRNAs are regulated by drought and other abiotic stresses in various plant species. In this study, were report the expression profiling of 8 known abiotic stress deregulated miRNAs in 11 elite sorghum genotypes, under watered and drought conditions. Significant deregulation was observed with miR396, miR393, miR397-5p, miR166, miR167 and miR168. Among these, the expression levels of sbi-miR396 and sbi-miR398 were the highest in all the genotypes. The expression of sbi-miR396 was maximum in the grain sorghum HSD3226 under well-watered conditions and the profile shifted towards HSD3221 under drought stress. Forage accessions, N98 and Atlas, showed an opposite behavior in expression patterns of miR397-5p in drought physiologies. Such dynamic expression patterns could be indicative of prevailing drought tolerant mechanisms present in these sorghum accessions. This data provides insights into sorghum miRNAs which may have potential use in improving drought tolerance in sorghum and other cereal crops.

1. Introduction

Sorghum is an important cereal crop in dryland agriculture because of its use as food and livestock feed. Sorghum grain production ranks fifth globally and it feeds 5 million people, mostly from the poor and under-developed areas of Africa and Asia (Haussmann et al., 2002; Mace et al., 2013; Paterson, 2008). Its importance has increased with increasing global pressure for food and aberrant climatic changes that has forced researchers to focus on other crops to find an answer to food security challenges, especially for poor people. The crop is known for its hardiness and stability in marginal land farming with extreme tolerance to low input levels of water and fertilizers (Paterson et al., 2009). It is a close relative to sugarcane with the sweet sorghum being tall and producing high biomass in addition to sugar. It has thus attracted attention as an emerging bioenergy crop due to high sugar content in its stems (Bowers et al., 2003; Dillon et al., 2007; Vermerris et al., 2007).

The origin and early domestication of sorghum took place in north-east Africa and the earliest known record of sorghum is near the Egyptian-Sudanese border, which dates back to 8000 B.C (Kimber et al., 2013). Spread of sorghum resulted in disruptive selection on the basis of agronomic advantages suitable for different locations, but this radial movement across different areas lead to emergence of immense diversity in the sorghum crop. Sorghum landraces and wild relatives of cultivated sorghum from these centers of diversity are therefore rich sources of resistance to diseases, insect pests and other stresses such as high temperature and drought (Rosenow and Dahlberg, 2000). Moreover, its relatively small diploid genome (735 Mbp) makes it an excellent grass model to study C4 plant physiology (Paterson, 2008). Preliminary exploration of genomic diversity based on conventional approaches such as RAPD, RFLP, SSR, SNP genotyping have clearly revealed considerable polymorphism between cultivated and wild type sorghum (Aggarwal et al., 1999; Agrama and Tuinstra, 2004; Ng'uni et al., 2011; Smith et al., 2000; Williams et al., 1990; Yoshida, 2004). But these studies are limited in number and do not pertain to changes

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in developmental stages or environmental stress (Winter and Kahl, 1995). For example, it is important to capture the differential behavior of various accessions to drought conditions, even though sorghum is a dry land crop.

Sudanese region has the largest collection of wild and cultivated sorghum varieties due to early domestication of sorghum in this area. Genetic diversity analysis with the help of ISSR markers has identified 97% polymorphism among 50 sorghum accessions from Sudan (El-Amin and Hamza 2014). There is however very little information about the genetic relationships within the sweet sorghums and grain sorghum genotypes. The molecular characterization of these accessions holds great value for exploiting the genetic pool to generate hardy cultivars tolerant to changing climatic conditions. Such studies are important for better crop improvement strategies.

Whole genome sequencing of sorghum has re-emphasized the correlation of phenotypic differences among varieties with respect to their genotypic diversity (Mace et al., 2013). This has led to identification of several microRNAs (miRNAs) by different groups (Du et al., 2010a; Katiyar et al., 2012; Zhang et al., 2011). The deciphering of miRNA expression patterns in different cultivars of sorghum will provide a better understanding of their genetic responses. This will help in selecting abiotic stress resistant varieties that can be used for further crop improvement program. In this study, we investigated the effect of drought stress on the expression levels of selected miRNAs across 11 elite accessions of African sorghum. A functional correlation has been drawn with their predicted targets to obtain meaningful insights into the differential response of the miRNAs in the varied genotypes.

2. Materials and methods

2.1. Plant materials and stress treatment

11 Sorghum genotypes were used in the study. Seeds of eight grain sorghum genotypes were provided by the Germplasm Bank of the Plant Genetic Resource Unit (The Agriculture Research Corporation, Wad Medani), they were collected from three different regions in Sudan, namely, West Darfur (Western Sudan), Red Sea (Eastern Sudan), and Bahr El Jabel (South Sudan). The released variety ArfaGadamak is also a grain sorghum genotype. The other two genotypes include N98 and Atlas, which are breeder lines for sweet sorghum from University of Nebraska.

Sorghum seeds were sown in Shambat Experimental Field, College of Agriculture, University of Khartoum. A randomized block experiment with two replicates was done. Control plants received regular irrigation every 10 days whereas drought stress was imposed on the plants by increasing the watering interval to 21 days. Leaves were taken from all controlled and stressed plants and stored in RNase free tubes containing RNA Shield™ (Zymo Research) until RNA extraction was performed.

2.2. RNA isolation and stem-loop RT PCR

Total RNA was extracted from 0.5 g of leaf tissues using the Guanidium isothiocynate extraction method of Chomczynski and Sacchi (2006) with slight modifications. 200 ng total RNA was used to synthesize cDNA using miRNA-specific stem-loop primer with Superscript reverse transcriptase III (Invitrogen) as per manufacturer's specifications. Primers used in this study are listed in Table 1. A pulsed RT reaction was performed in a thermal cycler as follows: 30 min at 16 °C, 60 cycles at 30 °C for 30 s, 42 °C for 30 s and 50 °C for 1 s. RT enzyme was inactivated by incubating the reaction at 85 °C for 5 mins. 1 μl of direct cDNA was used for PCR using miRNA specific forward primer and universal reverse primer to get a 63 bp amplification product. 18S rRNA was used as an endogenous control. Relative abundance was calculated as Integrated Density Values (%IDV) by normalizing the obtained values with 18S rRNA. The PCR based expression analysis was performed using three replicates for each set and the calculated standard deviation for each set is shown as error bars on the graphs.

2.3. Target prediction and GO annotation

Putative targets for selected miRNAs were predicted by psRNAtarget webserver (Dai and Zhao, 2011) using transcripts from SbGDB (http://plantgdb.org/SbGDB/) (Duvick et al., 2008). Default parameters were employed and mRNA sequences with a score ≤ 3 were considered as potential targets. All the predicted mRNAs were searched in Plant Transcription factor database, PlantTFDB 3.0 to search possible Transcription factors (Jin et al., 2014). Interrelationships between miRNA and their predicted targets were studied by creating a visual interaction map using Cytoscape 2.8.3 (Shannon et al., 2003). For 7 miRNAs, target prediction was done using hsp size (length of complementary scoring) of 20 but for miR398, the hsp size was modified to 19. GO annotation and enrichment analysis was carried out by AgriGO with default parameters (p-value ≤ 0.05) (Du et al., 2010b). GO enrichment was done using Singular Enrichment Analysis by employing Plant GO slim. Pathway enrichment analysis for predicted target mRNAs was done using Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology database (Kanehisa and Goto, 2000).

3. Results and discussions

3.1. Selection of sorghum genotypes and the miRNAs

Sorghum from Sudan has been used worldwide as a source for improving germplasm, for drought tolerance, nutritional quality, stalk strength, insect and disease resistance (Rosenow and Dahlberg, 2000). However, no research has been done on these Sudanese genotypes to reveal the role of miRNAs that may be governing these traits. Sorghum is a dry land plant that can avoid stress either by conserving water through an efficient leaf and stomata characteristic (by early closure of stomata, increasing photosynthetic efficiency, reduction of cuticular transpiration, lipid deposition on leaves, leaf area reduction and morphology of leaf surface) or improving water uptake by root or through osmotic adjustment to lower the osmotic potential (Acevedo and Fereres, 1993; Blum, 1988). Efficient water uptake is an important determinant of drought tolerance, as it depends on root size (length or mass), activity and spatial distribution (Blum and Arkin, 1984).

An exhaustive study on 40 Sudanese sorghum genotypes under water stress conditions (Assar et al., 2009) identified Arfa Gadamak as a high yielding grain sorghum variety under both normal and drought stress conditions. It was thus, recommended to be used as parent in breeding programs for drier areas. The comparative analysis also revealed higher concentrations of K and Fe in the seeds of tolerant genotypes than the susceptible ones. The concentration of Fe was found to decrease with maturity in the tolerant group but it increased with maturity in the susceptible group. The seeds of Arfa Gadamak variety also contained high level of Zn in its young seedlings (Assar et al., 2002).

For this study, 11 elite accessions of sorghum genotypes (Fig. 1) including the Arfa Gadamak, a released variety were selected. The details of these accessions are listed in Table 2. Further, eight conserved miRNAs (miRBase release 20) that are known to be deregulated under abiotic stress were chosen for profiling. These include sbi-miR160, sbi-miR166, sbi-miR16, sbi-miR168, sbi-miR393, sbi-miR396, sbi-miR397-5p and sbi-miR398. These stress-