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IDENTIFICATION OF GENES EXPRESSED DURING WATER STRESS IN *FOUQUIERIA SPLENDENS* SSP. *BREVIFLORA*

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ABSTRACT: We identified genes in *Fouquieria splendens* ssp. *breviflora* (Fouquieriaceae) that were expressed due to water deficits. RNA was extracted from dehydrated stem samples that were collected directly from Camargo, Querétaro following hydration for two weeks. The plants were grown in two states, drought and hydrated. We then performed subtractive hybridization. The obtained sequences were inserted into a plasmid and sequenced. Analyses were performed using the Blastx and Expasy ScanProsite database. These results revealed the expression of genes that corresponded to the RNA polymerase II β-subunit of chloroplast, rpoC2; the cell binding site known as tripeptide RGD; catalase; and chitinase class I and III. These expressed genes are important because they are involved in the response to oxidative stress and the defense response and development in *Fouquieria splendens* ssp. *Breviflora*.

INTRODUCTION: The main cellular damage in plants is related to water stress [1]. Physiological studies have shown that sugars (e.g., raffinose, sucrose, trehalose, and sorbitol), alditols (e.g., mannitol), amino acids (e.g., proline) and amines (e.g., glycine betaine, and polyamines) accumulate under stress conditions in different plant species. It has been suggested that the changes in these metabolites at the cellular level are associated with a protective function or the maintenance of cellular structural components.

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One plant response to water stress is stomatal closure, which prevents water loss by limiting the rate of transpiration; however, the internal CO_2 concentration is diminished as a side effect and reduces photosynthetic rates. Approximately one-half of all terrestrial plant communities are routinely exposed to extended periods of water deficit, and drought conditions are the leading cause of yield losses to economically important crops, pasture deterioration, and livestock deaths worldwide. Given these circumstances, genetically improved plants with increased tolerance to water stress must be developed.

Thus, it is vital that studies are conducted to elucidate the plant physiological response to drought stress and to identify and select drought responsive genes in species with an evolutionary history of high tolerance to these specific abiotic stress conditions to meet the technological demands

of local cultivation [2]. *Fouquieria splendens ssp. breviflora* is a species that is well-adapted to drought conditions due to its native distribution in arid regions of northern Mexico and the deserts of the southwestern United States. The main objective of this study was to identify specific gene sequences expressed during water stress in *F. splendens ssp. breviflora* using PCR-based subtractive hybridization. The generated sequences were compared with genes obtained from samples under hydration to identify potential candidate genes expressed under natural stress conditions.

MATERIALS AND METHODS:

Stems of *Fouquieria splendens ssp. breviflora* were collected in the town of Camargo, Peña miller municipality, state of Queretaro, Mexico, which is located 1760 m.s.l. (Lat 21.0763, Long 99.7333). Ten stems were collected in February during the dry season. Tissue was collected from five stems and stored at -70 °C prior to use. Five stems were subjected to outdoor hydration for two weeks and subsequently produced leaf material. Total RNA was extracted from the two stem tissue types (-70 °C stored dry season tissue and hydrated tissue) using the SV Total RNA Isolation System (Promega).

RNA was used at a concentration of 10 µg in a total volume of 4 µl for each reaction. Subtractive hybridization was performed using the reagent kit SelectTM cDNA PCR kit, Clontech subtraction (Cat 637401), according to the manufacturer's instructions. The cDNAs obtained were amplified via PCR using primers provided by the manufacturer. The amplicons were purified with the Wizard SV Gel and PCR Clean-Up System (Promega), and electrophoresed on a 1.5% (m/v) agarose gel. The DNAs were ligated into the pCR ® 2.1-TOPO ® plasmid, and Transformed into E.coli Top10 (Invitrogen).

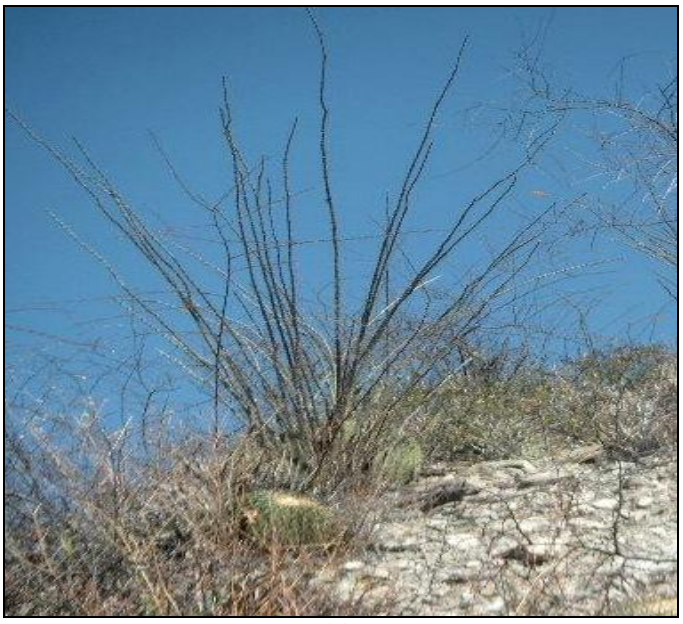
White colonies were obtained from Luria- Bertani (LB) plates selected with kanamycin (50 mg / L), X-Gal (40 mg / mL) and were stored in 80% glycerol at -70 °C. Double-stranded DNA from individual clones were isolated by plasmid DNA extraction with alkaline lysis using the reagent kit Rapid Plasmid Purification Systems (Marligen Bioscience, Inc.). Plasmids were digested with *EcoRI* (Promega) and observed on a 1% agarose gel. Following digestion, the plasmids were sequenced using the M13 forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers in an Applied Biosystems sequencer. Plasmids with inserts were sequenced using the dideoxynucleotide chain terminator method (Big Dye) in an ABIPRISM model 310, version 3.4 ABI-EC 1A. Two subsequent sequence analyses were performed.

The first sequence analysis was performed using the Staden program package Pregap4 (http://staden.sourceforge.net/). The second analysis was performed using the NCBI VecScreen tool(http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html).We searched for potential open reading frames (ORFs) (Redding Open Frames) using the ORF Finder program from NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html).BLAST was applied to search for similarities among sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the blastx and tblastx tools [3].

RESULTS:

F. splendens ssp. *breviflora* was obtained from Camargo, Queretaro; the first stem collection was obtained from the hill, and the rods with leaf buds were obtained after they were left in water for one week (**Figure 1**). The sequencing results were analyzed to assess the sequence quality, and search segment vector pollutants were assessed using VecScreen (NCBI) and the Staden Package (Trev Pregap4 and 1.9).

Only three sequences were important for the bioinformatics analysis. Sequence results were the following: sequence number one encoded the β subunit of RNA polymerase and rpoC2, sequence number two encoded the cell binding site known as the tripeptide RGD and sequence number three encoded chitinase class I, Chitinase class III and Catalase (**Table 1**). Amino acid sequences closely related to the translated *F. splendens ssp. breviflora* were found in plants reported in the NCBI database using the blastx and tblastxtools. Clone analysis using ScanProsite (Swiss Institute of Bioinformatics) was performed to analyze protein families and domains (**Table 2**).



(a)

(b)

FIG. 1: a) *FOUQUIERIA SPLENDENS SSP. BREVIFLORA* STEM COLLECTION IN THE TOWN OF CAMARGO, PEÑA MILLER MUNICIPALITY, STATE OF QUERETARO. b) *FOUQUIERIA SPLENDENS SSP. BREVIFLORA* RODS WITH LEAF BUDS AFTER HAVING BEEN LEFT IN WATER FOR ONE WEEK.

TABLE 1: RESULTS OF THE BIOINFORMATIC ANALYSIS

Clone	Protein sequence	Species	Similarity (%)	E-value
1	• β subunit RNA polymerase	• *Zea mays, Oryza sativa, Chlamydomonas reinhardtii*	66-75	6e-30 until
		• *Populus trichocarpa*	78-91	1e-37
	• Gene: rpoC2			3e-35
2	Cell binding site known as tripeptide RGD*	• Arabidopsis lyrata	87	6e-22
3	• Chitinase class I	• Elaeis guineensis	60-100	6e-08 until
	• Chitinase class III	• Sphenostylis stenocarpa		0.0039
	• Catalase (EC1.11.1.6)	• Zea mays		

*****Arg-Gly-Asp

TABLE 2: INFORMATION OF PROTEINS FOUND IN THE PROSITE OF EXPASY DATABASE.

Sequence	Amino acid sequence	ProSite code
1	GRGRGISVSPQNGMMPERIFIQTLIGRVLADDIYMGPRCIATRNQDIGIGLINRFVTFPA	PS00007
1	VSPQNGMMPERIFIQTLIGRVLADDIYMGPRCIATRNQDIGIGLINRFVTFPAQPISIRT	PS00007
		PS00005
1	GRGRGISVSPQNGMMPERIFIQTLIGRVLADDIYMGPRCIATRNQDIGIGLINRFVTFPA	PS00007
1	GRGRGISVSPQNGMMPERIFIQTLIGRVLADDIYMGPRCIATRNQDIGIGLINRFVTFPA	PS00007
2	MAQLRQGKE*KKHLTPSCILHLARGDIAQLVVFRSCNWVVAITGWMSNCPGGNDS	PS00016
3	KYLGRDHAKGEFQHTGGRY*WIRARYQAWRNHGHSCFLCEIVIRSQFHTTYEPEA	PS00006
3	MLPARMLCGIVSG*QFHTGNSYDHDYAKL	PS00342
3	MITPSLVPSSDPLVTAASVLEFALSVVAAE	PS00006

DISCUSSION: We identified RNA polymerase II β subunit of chloroplast gene in *F. splendens* ssp. *breviflora* as an important gene for drought tolerance. Plastid RNA polymerase was present in higher plants, in PEP (plastid-encoded plastid RNA polymerase), and NEP (nucleus-encoded plastid RNA polymerase) multi-subunit complexes. Similar to the rpoC2 gene, which is located in the chloroplast genome, this gene encoded PEP transcription promoters.[4]

Several studies have shown that plastid genes are transcribed by at least two plastid-encoded RNA polymerases, and these results demonstrated that the chloroplast RNA polymerase sigma factor was associated with antimicrobial defense proteins in higher plants and algae.[5] We found a short microbody C-terminal sequence in *F. splendens* ssp. *Breviflora* was similar to chitinase class I and III in Elaeis guineensis and Sphenostylis stenocarpa, respectively. In plants, chitinases function in defense and development. We detected a catalase in *F. splendens* ssp. *breviflora* with a 60% similarity to a catalase (EC.1.11.1.6) from Zea mays, which has been shown to play a role in defense against oxidative stress. This catalase has been shown to not to eliminate superoxide anions and hydrogen peroxide, but it also prevents hydroxyl radical-OH formation and reactive oxygen species (ROS).

These toxic products, which are produced within the chloroplast, can be effectively. Removed to prevent lipid peroxidation, inhibit CO_2 fixation, and the photooxidation of chloroplast pigments. Photosynthetic cells can tolerate elevated oxygen levels in the presence of protective mechanisms involving endogenous glutathione, ascorbate, carotenoids, and enzymes, which effectively remove toxic products prior to the occurrence of cell damage.[6] The main damage to chloroplasts caused by water stress includes structural changes as a result of excessive bloating, deformation of the lamellae, grana intergrana, and the appearance of lipid droplets. Changes in the water regime and temperature can differentially affect chloroplast structural characteristics in various cultures.[7] As a result of chloroplast damage due to water stress, photosynthetic processes are affected by lipase action as well as the addition of specific unsaturated fatty acids and aging, as demonstrated in isolated plastids.

This effect can be particularly observed in reduced glycolate production. Increasing lipase activity can result in glycine decarboxylation. The above hypothesis suggests that water stress in drought-sensitive species results in hydrolytic activity, which degrades not only storage products but also the structural system of organelles, including ribosomes, chloroplasts, and mitochondria.[8] A cell binding site with an Arg-Gly-Asp sequence reported in Arabidopsis lyrata is similar to the sequence found in *F. splendens* ssp. *breviflora*, which has been termed a tripeptide RGD and plays a role in cell adhesion.[9]

Our interpretation of the *F. splendens* ssp. *breviflora* sequence data suggests that the species has adapted at the molecular level in response to water stress in an arid environment. Many plants are regularly exposed to environmental changes and have evolved morphological and physiological mechanisms to survive under extreme or stressful biotic and abiotic stress. *F. splendens* ssp. *breviflora* has clearly adapted to extreme adverse conditions, such as drought stress. In addition, the mechanisms used to repair damage operate once water is available; solutes are accumulated, the cell. wall rehydrates, and protective proteins restore cell metabolic function. Thus, knowledge regarding the physiology of *F. splendens* ssp. *breviflora* may offer potential benefits to agriculture, where transgenic crop effectiveness is related to the commercialization of new products derived from native plants with traits that are desirable to the agricultural industry.

In this study, we contribute key elements to understand the drought tolerance molecular biology of *F. splendens* ssp. *breviflora*; however, further research is required to fully elucidate the mechanisms imparting drought tolerance to this taxon that is well-adapted to arid regions of northern Mexico and the southwestern US.

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