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IDENTIFICATION OF CSD1, SOS4, SAT32, HVA22 AND RCI3 GENES IN *RICINUS COMMUNIS* L.

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ABSTRACT

Ricinus communis is a rapidly growing perennial shrub and is monotypic specie that belongs to the family Euphorbiaceae and possesses numerous medicinal and traditional values for the maintainace of life that is free from diseases. 62,592 ESTs of *Ricinus communis* were explored for five genes CSD1, SOS4, RCI3, HVA22 and SAT32 of model plant *Arbidopsis thaliana* that respond to salt stress. The query coverage that expressed of *Ricinus communis* with model plant genes was 93% (CSD1), 63% (SOS4), 56% (RCI3), 48% (HVA22) and 13% (SAT32). The presence of same conserved domains superoxide dismutase (CSD1), Horseradish peroxidase and related secretory plant peroxidase (RCI3) ,TB2/DP1 HVA22 superfamily (HVA22) and interferon related protein conserved regions (IFRD-C) in *Ricinus communis* along with good query coverage proved the presence of these genes in *Ricinus communis*. Findings are proved to be helpful in understanding the salt stress regulating mechanism in *Ricinus communis* and analyzing this medicinally important plant for stress responsive genes.

INTRODUCTION

Traditional medicines contain many natural crude drugs that can help human beings to treat many disorders and diseases and one of those plant is *Ricinus communis*. It is popular by the name of 'castor plant' and widely distributed throughout the regions of tropics as ornamental plant (Jena & Gupta, 2012). *Ricinus communis* L. (castor bean), is a monotypic species belonging to spurge family (Euphorbiaceae, 2n = 20). Castor is a non-edible oilseed crop that is broadly cultivated in tropical, temperate, sub-tropical countries for its important economic value (Qiu *et al*., 2010). The plant of castor oil is a suckering perennial and fast-growing shrub. Castor is a small a soft wooded (not hardy) tree and is up to 6 meter or more. The stems are varying in pigmentation (Jena & Gupta, 2012). Castor plant was cultivated for the colour of flower and leaf and for the production of oil. The stems contain pigmentation (Chadha, 1972). In Castor oil ricinoleic acid is present in high concentration. More than 85% of which has confer unique industrialized properties to the oil. Castor oil can be used for many industrial applications like varnishes and paints, synthetic polymers of nylon type, lubricants and hydraulic fluids, and cosmetics (Babitaa *et al*., 2010).

Genome sequence of castor bean is an important source for genome evolution study (Chan *et al*., 2010). One of the major abiotic stresses is the salt stress that greatly reduces the growth and development of many plants. But *Ricinus communis* L. is very resistant to the salt stress and no change will occur in *R. communis* under salt stress. However water content relative to leaf and water potential decreased under salt stress. In addition, salt stress is not responsible for increase membrane damage in *R. communis* but it results an increase in Na+ content (Neto *et al*., 2014). Temperature is another type of abiotic stress and an important factor of environment. Temperature can also have a great influence on plant growth especially on roots .In case of *Ricinus communis* cold temperatures of roots causes' leaf growth reduction of *Ricinus communis* (Poire *et al.*, 2010).

Along with temperature and salinity the availability of water also affects plants. In *R. communis* those plant growing either in drought and good watered conditions, during the night the rate of growth peaked and in late afternoon minimal values occurred. Small change in diurnal growth peak amplitude occurs mainly by drought stress (Schurr *et al.*, 2000). In the mustard family a small plant is *Arabidopsis thaliana* which can be used as a model system for plant biology research. (Meinke *et al*., 1998). The size of the Arabidopsis genome is about 100 Mbp (Meyerowitz, 1994) and the chromosomes are much smaller from the chromosomes of higher plants**.** Extensive molecular and genetic information regarding the *Arabidopsis* genome, provided by the novel mutagenic system's production and international collaborations, through which immense array of mutants can be identified (Pyke, 1994). A great amount of biological information has also been generated with the generation of genomic era. A successful tool is the bioinformatics where the genes of interest subjected to various insilico studies in order to recognize the genes, and find out all relevant biological information from those genes of interest. Such an examination of data is utilized and facilitate under field research. (Showalter *et al*., 2001). Bioinformatics is a computational method which is very helpful for understanding and organizing biological macromolecules and their function. In molecular biology, for bioinformatics an information system was produced in 1988 at the national institute of health named NCBI National Centre for Biotechnology Information (Benson *et al*., 2009). There is a system for

integration of the data base that is helpful in searching of new genes. There are 91 million protein and DNA sequences are included in Entrez molecular sequence database system. NCBI has a resource for protein annotation named BLAST. BLAST (Basic Local Alignment Search Tool is a search program for detection of sequence similarity (Altschu *et al.*,1990).

Conserved Domain Database (CDD) is another resource of NCBI that can be used for protein sequences annotation along with the functional sites and location of conserved domain footprints. CDD includes manually curated domain models that utilizes 3D structure of proteins in order to find out the domain model, insight into their function, sequence, structure and relationship. The detection of a conserved domain can act as the main clue towards protein's molecular or cellular function (Marchler-Bauer *et al.*, 2010). Bioinformatics aim in organizing the data in a helpful way, by which the researches can take advantage of existing data and can also submit highly developed entries soon after their formation. Secondly bioinformatics aims in generating tools and resources which are useful in analysis of data. Third aim of bioinformatics is to make use of these generated tools in analysis of data, and illustrate the results in a biologically meaningful way (Pearson & D.J, 1998).

Materials and Methods

Five stress resistant genes were identified in plant *Ricinus communis* L by using NCBI. This work was done by using another plant as a reference which is the model plant called *Arabidopsis thaliana.*

Fetching of Model Plant Genes

The genes of *Arabidopsis thaliana* are CSD1, SOS4, HVA22, RCI3, SAT32, abiotic stress resistant genes and their selection was made via literature surveys. These genes and their coding sequences were obtained through NCBI's home page. The cDNAs of full length were then selected which were required in order to analyze the plant genes. Then in FASTA format these sequences were conserved.

Finding ORF

For the stress resistant genes sequences the open reading frames were find out by using NCBI's another resource called ORF (Open Reading Frame). The finder produces each ORF's range and its protein traslocation. Then refrences gene's FASTA sequence was subjected to ORF. The same procedure was repeated for each and every gene individually. The start and stop point of ORF was marked then by the using this software the sequences of longest frame were selected.

Homology Search

Basic local alignment search tool also called BLAST is a search engine for sequence judgment. To conduct the homology search the reference genes were subjected to BLAST against *Ricinus communis* in order to accomplish the BLAST investigation against *Ricinus communis*. Firstly the name of species *(Ricinus communis*) was selected, then an option "somewhat similarities" was selected and then blast results were searched. The query coverage which was highest noted down. Again the ORF was found out and the start and

stop point were marked. The same procedure was applied for the remaining genes and then best similar genes were conserved.

Finding Conserved Domains

There is another component of NCBI called the CDD conserved domain database which is the NCBI's Entrez query and recovery system. Nucleotide sequence annotation along with conserved domain function and footstep sites are delivered through CDD, Entrez, and interactive search services Pre-computed available annotation, as well as submissions of ORF, from FASTA sequence consuming to rapidly detect putative matches.

RESULTS

Identification of Novel Genes in *Ricinus communis* L.

A computational approach called bioinformatics which is used to understand the function of macromolecules. This method is used for the identification of the novel genes in plant *Ricinus communis* by using the *Arabdiopsis thialiana* like reference system. All the genes CSD1, SAT32, SOS4, HVA22A, RCI3, were identified in *Ricinus communis* with the help of NCBI's home page and its different resources.

ORF

With the help of Open Reading Frame (ORF) full length of micro RNAs along with their ORF length of each gene were identified. SOS4 was with highest mRNA length i-e., 1268 as well as with highest ORF i-e., 1032 in the model plant species *Arabdioposis thialiana*. Whereas the length of mRNA and ORF of SOS4 in *Ricinus communis* was 772 and 106 respectively. After SOS4 the RCI3 genes stands on second number with 1209 mRNA Length and 981 ORF length *in Arabidopsis thialaiana* and have a mRNA with length of 712 and ORF with a length of 291in R*icinus communis.* The 3rd gene SAT32 have a full length of mRNA (1931) and full ORF length (1321) in *Arabdipsis thialaina* whereas in R*icinus communis* has a mRNA with full length of 365 and its ORF was 147. In *Arabidopsis thialiana* the full length of mRNA of CSD1 was 872 and its ORF length was 459, the mRNA length and the ORF length of the same gene i-e CSD1 in *Ricinus communis* was 673 and 459. The length of mRNA was 813 for HVA22 and its ORF was 375 in *Arabdiposis thialaina* whereas HAV22 have mRNA length (605) and ORF length (375) in *Ricinus communis L.*

Table: 4.1. ORF length of CSD1, HVA22, RCI3, SAT32, SOS4.

Genes.	***A.thaliana***		***R.communis***	
	mRNA length	ORF length	mRNA length	ORF length
SOS4	1268	1032	772	106

RCI3	1209	981	712	291
SAT32	1931	1321	365	147
CSD1	872	459	673	459
HVA22	813	375	605	375

Homology Search

With homology search we find out the query coverage and percentage identity of the 5 novel genes. As a result of this homology search CSD1 gene has a highest query coverage and highest percentage identity in *Ricinus communis*. The different query coverage and percentage identity of all the 5 novel genes in *Ricinus communis* were in 4.2.

The query coverage of CSD1 was 93 and its percentage identity was 79%.The query coverage of RCI3 was 73% and its percentage identity was 67%. Query coverage of HVA22 was 48% and its percentage identity was 76%. Query coverage of SOS4 was 63% and its percentage identity was 77%. The lowest query coverage of SAT32 was 135 and its percentage identity was 71% in *Ricinus communis.*

Table 4.2. Homology searches of genes in *R.communis*.

Gene name.	Query coverage	Percentage identity.
CSD1	93%	79%
RCI3	73%	67%
HVA22	48%	76%
SOS4	63%	77%
SAT32	13%	71 %

Table 4.3. Conserved domains of genes in *R.communis*

Gene name	*A. thialiana*	*R. communis*
CSD1	Cu-Zn superoxid e-dismutase super family	Cu-Zn superoxid e-dismutase super family
RC13	Plant_ peroxidase_lik e super family	Plant_ peroxidase_lik e super family
HVA22	TB2_DP1_hV A22 super family	TB2_DP1_hV A22 super family
SOS4	Ribokinase _pf kB_ like super family	Ribokinase _pf kB_ like super family
SAT32	IFRD super family.	IFRD_C

DISCUSSION

A strategy was conducted to detect the presence of stress resistant genes in *Ricinus communis* L. by using a tool of bioinformatics named NCBI and its different resources like BLAST, CDD, FASTA, ORF etc. The study was carried out by using the model Plant *A. thaliana*. A similar work was done in previous years in order to detect proteins of salt tolerance in *A. thaliana* (Horie *et al*., 2009). In addition to these (Breyne & Zabeau, 2001) bioinforamtics approach was applied for large-scale cDNA sequencing and genomic projects for detection of various genes of unknown function. Bioinformatics study indicated much protein's response towards stress of salt that could be collected into various function collections and may contribute in different physiological aspects of *A. thaliana* (Verbruggen *et al*., 1993). Schultz *et al*., (2002) used an approach of bioinformatics approach to detect genes of candidate AGP from *Arabidopsis.*

As the sequencing of genomes takes place, the development of tools of bioinformatics are required

for analyzing such kind of data accurately and efficiently. Through BIO OHIO program 28,952 protein sequences were searched that are encoded by the *Arabidopsis* genome (Showalter *et al*., 2010). The *A. thaliana's* entire proteins, salt response overview and functional analysis together with analysis of domain, molecular functions and biological processes pathways were carried out by utilizing DAVID tools (Guo *et al*., 2012).

Comparing the results of the present study to the results of Guo *et al*., (2012), the conserved domains of stress resistant genes were identified by utilizing the BLAST tool of bioinformatics which is accecceible at the home page of NCBI. Further Guo *et al*.,(2012) worked at *A. thaliana's* entire protein's, salt response overview and functional analysis together with analysis of domain, molecular functions, and biological processes pathways were carried out by utilizing DAVID tools.

The focus of this study was primarily on *Ricinus communis*. Using similar method same results obtained by the work of (Showalter *et al*., 2010; Verbruggen *et al*., 1993; Guo *et al*., 2012).

Due to numerous environmental stresses (ROS) reactive oxygen species are accumulated and this accumulation is an important cause of loss in productivity of crop (Allen *et al*., 1997; Mittler, 2002; Apel & Hirt, 2004; Foyer & Noctor, 2005; Bartels & Sunkar, 2005). The present study identified the conserved domain of RCI3 in *R. communis* which is peroxidase like super family. Peroxidase means oxidoreduction catalyzing enzyme between reductants and hydrogen peroxide ($H_2O_2 + AH_2 \rightarrow 2H_2O + A$). Peroxidases are splitted up into 3 superfamilies on the bases of their catalytic and structural properties (Churin et al., 1999). Another conserved domain is Superoxide dismutase which is conserved domain of CSD1 in *R.communis* and are divided into three subfamilies also involved in many functions of plants like peroxidase. According to Fridovich, (1995) Superoxide dismutases (SODs) are the first line of resistance against extremely toxic superoxide radicals via fast converting superoxide to molecular oxygen and hydrogen peroxide (H_2O_2). Form their subcellular sites; small family of genes encodes SODs enzyme's three classes that make difficult the elucidation of SOD roles in plants. This trouble can only be solved thorough initiation of analysis of SOD genes of *Arabidopsis*. It can now only be possible in order to detect the whole SOD arsenal of *Arabidopsis* because of accessibility of huge numbers of genomic DNA and cDNA sequences in *Arabidopsis* (Newman *et al*., 1994; Rounsley *et al*., 1996; Delseny *et al*., 1997).

The third conserved domain of HVA22 was TP2_DP1 HVA22 like super family was identified in *Ricinus communis*. Through two of the Barley gene analyses of HVA22 and HVA1, it is indicated that response of HVA22 and hva1 to ABA depends upon relationship between two cis- acting elements which are present in their promoters coupling element (CE) and an ABA response element, these two elements were coined together and form a complex reponse promoter of ABA (ABRC) (Ross & Qingxi, 2006). To gain information from barley that whether from barley the regulatory motifs were preserved in *Orayza sativa*, and be utilized to infer inducible genes of ABA from their promoters (Panel B,) were searched inside the genome of rice for finding homologues of the ABA inducible HVA1 of barley (Shen *et al*., 1996).

CONCLUSION

The present study was conducted to identify 5 genes in *Ricinus communis* (CSD1, SAT32 HVA22, RCI3, SOS4) that showed response to different environmental stresses through NCBI data base. The tools of bioinformatics that were used during this research work were BLAST, NCBI, ORF, CDD. It is concluded that the existing genes in *A. thaliana* that are involved in responding to abiotic stress also exist in the *Ricinus communis* via insilico identification. A great insight about response may be provided through the all of the genes that exist in *Ricinus communis.* Genes that were identified can

be established via wet lab with the help of bioinformatics analysis. Other plants with manipulation of detected responsive genes of stress in which these genes does not exist may also be explored in the same way.

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