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# Extraction of *Acacia senegal* MYB transcription factor mRNA by using homology searching approach: An important tree species of arid environment

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***Abstract***
*Acacia senegal one of the most dominant biomass producer of the sandy areas of the desert, belonging to (Fabaceae family and genus Acacia) commonly known as Kummat. These plants has well known to contain high levels and different types of secondary metabolites (gum Arabic), which are often responsible for their therapeutic and pharmacological activities. In-group of transcription factors of plants, MYB (myeloblastosis) gene family most diversified in various aspects. The study was to finding MYB genes sequences by using homological approaches. Applied standardized method for isolation and purification of genomic DNA from Acacia senegal leaves followed by an amplification of MYB genes. Primers has designed using online Genefisher2 and Primar3 software with high reproducibility at an optimal annealing temperature of 54°C. The isolated DNA has successfully amplified with all seven primers designed from homological MYB gene sequences of rice genome. Polymerase chain reaction (PCR) has carried out in a final reaction volume of is 25 µl, lowed concentrations of primer (0.2µM) and Taq polymerase(1U). The amplified products were visualized as single compact bands of expected size of 400-500 base pairs. These sequences has successfully submitted to National Center for Biotechnology Information (NCBI) public database and obtained nucleotide Genbank Accession number (KF364950) and Locus (KF364950) for a public database. After the assigned accession number, there are 3 transcription factor MYB93-like mRNA sequences found from Prosopis alba which is a gum exudation tree species belong to its native ranges. This analysis used to study the genetic diversity of MYB genes in the arid trees. Moreover, it enables us to further understand the genetic diversity of MYB gene in relation to sequence analysis and find conserved molecular markers linked to secondarymetabolite regulatory mechanism.*
***Keyword:****Acacia senegal, mRNA, myeloblastosis and transcription factors*

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## I. Introduction

The potential of modern science to substantially benefit arid areas mainly deflation arid zone of India, which are at vulnerable due to the consequences of aeolian erosion and deliberated activities, leading to the biodiversity loss as well as extending the list of the threatened plants in the IUCN in a particular region. *Acacia senegal* one of the most dominant biomass producer of the sandy areas of the desert, belonging to (Fabaceae family and genus *Acacia*) locally known as Kummat, has remarkable adaptability to the adverse environmental conditions. Kummat, is a medium to large size with multiple utility like bio-fencing, food, fuel and nitrogen fixing tree distributed to arid and semi-arid regions of the country having fuel, metabolites of medicinal value and gums as its powerful implements in various applications. These plants has known to contain high levels and different types of secondary metabolites (gum Arabic), which are often responsible for their therapeutic and pharmacological activities. In modern technology, extraction of theparticular metabolite/pathwaywith the respective enzyme/promoters during the pre-mature stage of the plant definitelyboost up pharmaceutical production, urging the need for the plant conservation (Pirttilä*et. al*,.2001). Earlier studies also defined that *A. senegal*grown in various land forms and its girth class effect gum production (Pareek *et. al*,.2017 a & b).

The myeloblastosis (MYB) transcription factor gene family is found in all eukaryotes. In historical point of view, "Oncogene" v-MYB was the first MYB gene identified in avian myeloblastosis virus (Klempnauer*et. al*,.1982). Three v-MYB-related genes i.e., c- YB, A-MYB and B-MYB has subsequently identified in many vertebrates and implicated in the regulation of cell apoptosis, differentiation, and proliferation (Weston K, 1998). A homolog of mammalian c-MYB gene, i. e. Zea mays C1, who involved inregulation of anthocyanin biosynthesis, was the first MYB gene to be characterized in plants (Paz-Ares J *et. al*,.1987) Moreover, plants encode large number of MYB genes as compared to fungi and animals (Feller A *et.*

*al,*.2011; Yanhui C *et. al,*.2006). According to the number of adjacent MYB repeats, MYB transcription factors has further classified into four major groups, i.e. 1R-MYB, 2R-MYB, 3R-MYB and 4R-MYB. 1R-MYB, 2R-MYB, 3R-MYB and 4R-MYB contain one, two, three and four MYB repeats, respectively.

MYB transcription factors play a key role in plant development, secondary metabolism, hormone signal transduction, disease resistance and abiotic stress tolerance (Allan AC et. al, 2008; Cominelli E *et. al,*. 2009). Several R2R3-MYB genes are involved in regulating responses to environmental stresses such as drought, salt, and cold (Agarwal M. *et. al,*.2006; Yanhui C *et. al,*.2006). Other important functions of MYBs have control of cell cycle regulation, secondary metabolism, meristem formation and thecellular morphogenesis (Ito M *et. al,*. 2001; Haga N *et. al,*.2007; Ouyang *et. al,*.2007).

## II. Material Methods

All the above find fact, homology-modeling search by using "MYB *Acacia senegal*" key word in NCBI public database. However, 1603 clusters of expressed transcripts found in UNI gene database. Out of which, 1320 expressed transcripts found in plants. We are extract the information from NCBI database and finally Rice genome (Riechmann*et. al,*.2000; Zeng *et. al,*.2007) were selected for homolog sequences of MYB transcription factor for *A. senegal* tree.

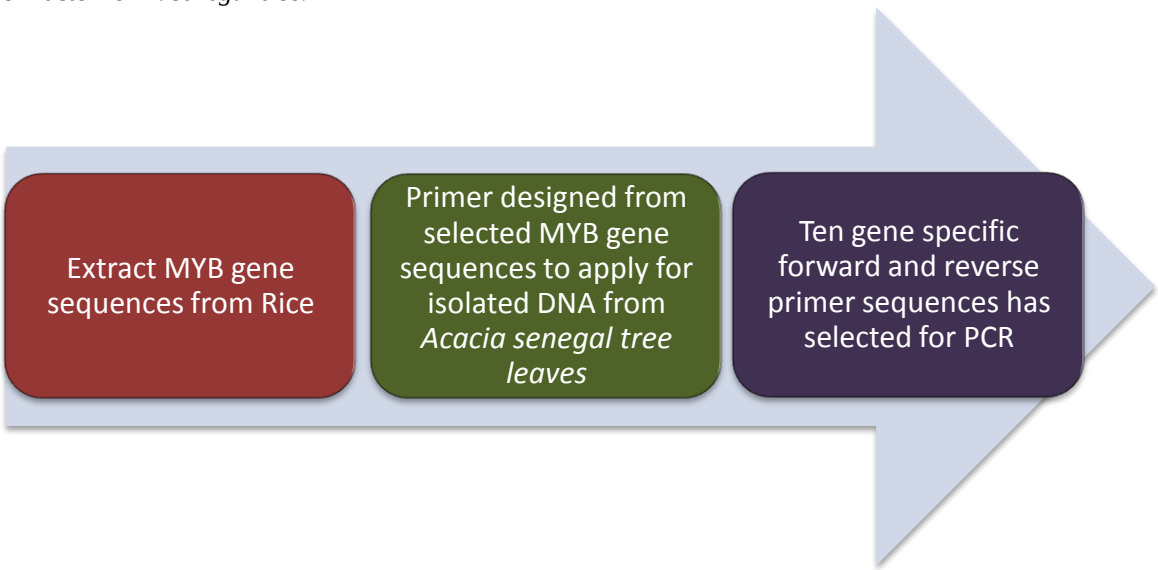


**Fig. 1 Step by step procedure to designed MYB gene specific primers through homology modeling approach for *Acacia Senegal***

I. **Sample fixation and pre-extraction washing:** Leaves of medium size and age has used for isolation of DNA. Leaves has picked in the field, immediately frozen in liquid nitrogen (−196 °C) and ground to a fine powder using a pestle and mortar. Fixed samples were stored in the laboratory at −20 °C for up to an overnight.

II. **DNA isolation using Xcelgen Kit:**Following standard the manufacturer's protocol, The Ultra violet (UV) absorbance has checked at 260 and 280 nm for determination of DNA concentration and purity. Purity of DNA has judged based on optical density ratio at 260:280 nm (Dellatropa, 1983; Saghai-Maroof*et. al,*.1984). The DNA having ratio 0.8 to 2.0 has considered being of good purity. Quality of the extracted DNA has assessed in two ways. Firstly, the A260/A280 absorbance ration was determined using spectrophotometer (UV-Visible Elico spectrophotometer). The level of DNA purity was determined by the A260/A280 absorbance ratio.DNA purity has further determined by agarose gel electrophoresis. Agarose 0.8% (w/v) in 0.5X TAEi.e. a mixture of Tris base, acetic acid and Ethylenediaminetetraacetic acid (pH 8.0) buffer has used for submarine gel electrophoresis. Ethidium bromide (1%) has added @ 10µl /100ml. The wells has charged with 5µl of DNA preparations mixed with 1µl gel loading dye. Electrophoresis has carried out at 80V for 30 min at room temperature. DNA has visualized under UV using UV trans-illuminator. The DNA has used further for PCR (Fig.2).

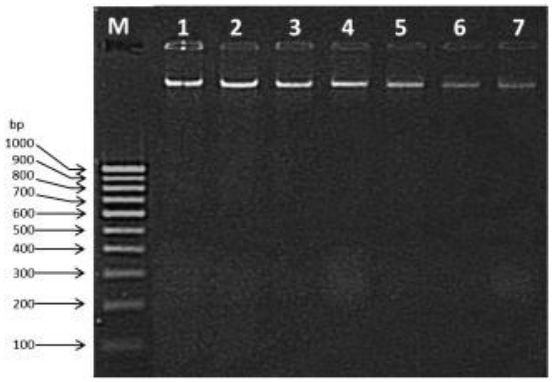


**Fig. 2 Genomic DNA of *Acacia senegal* form 1-7 and (M) ladder, resolved in 0.8% Agarose gel.**

III. **Identification of MYB gene family in Rice:**To identify "MYB transcription factor family genes", we searched and obtained genes annotated as MYB in public database (NCBI). Rice sequences obtain from NCBI server and selected corresponding MYB transcription factor gene location. Download all the relative sequences with referenced loci. Randomly selected query sequences for further primer design.

IV. **Target Primer design:**Gene specific primers were designed using IDT PrimerQuest (http: //www. idtdna.com/scitools/applications/primerquest/default.aspx). We find total number of 10 forward and reverse primers, which are used as a homolog primer in *A. senegal* plant (Table 1).

**Table 1. List of target gene specific primers of rice, which are used as a target primer of *A.senegal* MYB transcription factor sequence.**

| Forward Primer Sequence | Reverse Primer Sequence |
|---|---|
| CCTCATGAACTACATTGCCTGCCA | AGTTCTTGATCTCGTTGTCCGTCCT |
| CGCAAGCTCAAGCTCAAACAGCAA | TTGGTGCTGGTCCTCATGATGTTG |
| TTGGCAACAAGTGGGCAGCAATAG | AGGGTTGTAGTAGTTTGGGCTGGT |
| ACACCAAGCAAACTCCATGCACAG | AGTTCATCGCTTCTTTGCTGCCAC |
| ATCAGGGCTCAATAGGACAGGCAA | TCCTGGGCTTTCTTCCTCATGTGT |
| CAAAGCAACTCCCACCATCAGCAA | ATAGTTCCTTCGGTGGCACCTTCA |
| ATTGGAGAGGTGTGGCAAGAGTTG | GGAGCTTCTTCTTGAGGCACGAGT |
| AACGACGTCAAGAACCACTGGAAC | TGCAGGAAGTCGTCGCAGGTGTA |

V. **Polymer Chain Reaction:** PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thinned wall PCR tube in Eppendorf Thermal Cycler. PCR tubes containing the mixture has tapped gently and spin briefly at 10,000 rpm. The PCR tubes with all the components has transferred to thermal cycler. The primer (forward and reverse) used for an amplification of genomic DNA (Table 2&3).

**Table 2 Composition of reaction mixture for PCR**

| **Components** | **Quantity** | **Final** |
|---|---|---|
| DNase-RNase free water | 7.5µl | -- |
| 2X PCR master mix (MBI Fermenas | 12.50 µl | 1x |
| Forward Primer (10P/ µl | 1.00 µl | 10 Pmole |
| Reverse Primer (10 Pmole/ µl | 1.00 µl | 10 Pmole |
| Diluted DNA (30 ng/ µl) | 3 µl | -- |
| Grand Total | 25.0 µl | -- |

**Table 3 Steps and conditions of thermal cycling for PCR**

| **Steps** | **Temperature** | **Time** | **Cycles** |
|---|---|---|---|
| Initial Denaturation | 95°C | 2 min | 1 |
| Final Denaturation | 94°C | 30 Sec | 30 |
| Annealing | 50-54°C | 30 Sec | |

| | | | |
|---|---|---|---|
| Extension | 72°C | 90 Sec | |
| Final Extension | 72°C | 10 min | 1 |

VI. **Visualization the PCR product:**To confirm the targeted PCR amplification, 5 µl of PCR product from each tube was mixed with 1 µl of 6X gel loading dye and electrophoreses on 1.2 % agarose gel containing ethidium bromide (1 per cent solution @10 µl/100 ml) at constant 5V/cm for 30 min in 0.5 X TAE buffer. The amplified product has visualized as a single compact band of expected size under UV light and documented by gel documentation system (Fig. 3).

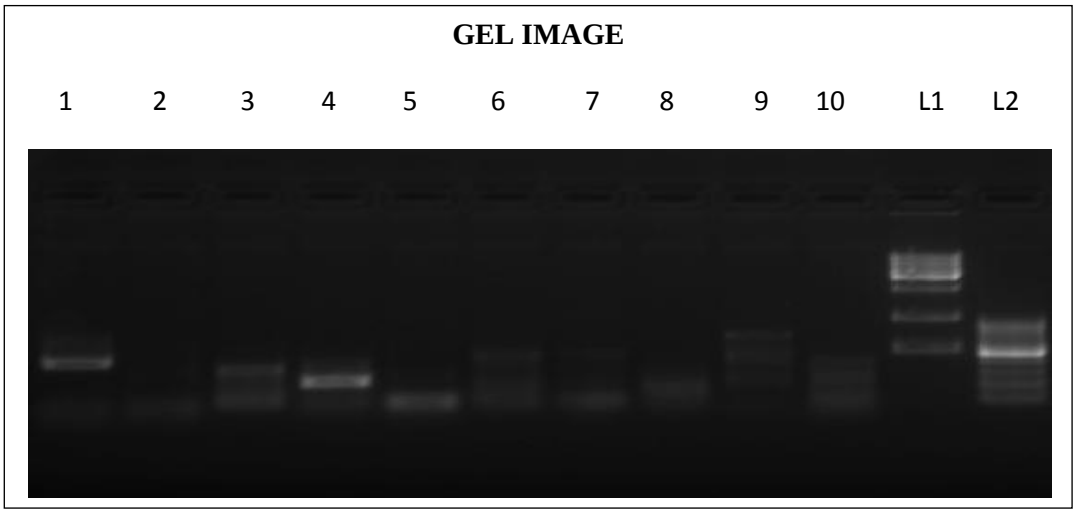


**Fig 3. L1=1 KB Ladder (starting from bottom 500BP, 1KB, 2KB, 3KB, 4KB, 5KB, 6KB, 7KB, 8KB) and ladder L2=100 BP (starting from bottom-100BP, 200BP, 300BP, 400BP, 500BP, 600BP, 700BP, 800BP, 900BP, 1KB) showing amplification of MYB gene from *Acacia senegal*.**

**VII. Sequencing of Purified DNA**

**Sequencing of Gene Segment:** The concentration of the purified DNA has determined and has subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Sequencing has carried out using BigDye® Terminator v3.1 Cycle sequencing kit following manufacturer's instructions.

**Cycle Sequencing:**Cycle sequencing has performed following the instructions supplied along with BigDye® Terminator v3.1 Cycle Sequencing Kit. The reaction has carried out in a final reaction volume of 20µl using 200µl capacity thinned wall PCR tube. The cycling protocol (Table 4) has designed for 25 cycles with the thermal ramp rate of 1$^{o}$C per second.

**Table 4 Cycling protocol for sequencing reaction**

| Step* | Temperature | Time |
|---|---|---|
| Denaturation | 96$^{o}$C | 10 sec |
| Annealing | 53$^{o}$C | 5 sec |
| Extension | 60$^{o}$C | 4 min |

**** Repeat step 1 to 3 for 25 cycles***

The contents hasmixed on a shaker for 30 minutes at 300xg. Eluted PCR products has placed in a sample plate and covered with the septa. Sample plate has heated at 95$^{o}$C for 5 min, snap chilled and loaded into autosampler of the instrument.

**Electrophoresis and Data Analysis:**Electrophoresis and data analysis has carried out on the ABI 3730xl Genetic Analyzer using appropriated Module, Basecaller, yeset/Primer and Matrix files.

**Submission of MYB Transcroptom factor sequence in NCBI public database:**Submission of MYB transcroptom factor sequence in NCBI public database: To find out ORF in candidate sequences obtained from sequencer, sequences has input to ORF finder. Systematically methods apply to find out best sequences. The most important source of new data for GenBank® is direct submissions. The submission wizard helps to upload the sequences in GenBank. After successfully submitted sequences, primary accession no obtained from GenBank. Finally, one sequences has identified and allot accession number i.e. KF364950 (Pareek *et. al*,.2013).

## VIII. Result and discussion

A major problems in isolation of high quality DNA from mature tree leaves especially in arid region trees, however it still an important issuesbecause it will contain high amount of phenolic compounds (Katterman and Shattuck, 1983) and as well as polysaccharides (Barzegari*et. al*,. 2010; Li *et. al*,.2010; Varma *et. al*,.2007). During experiments, we noticed that leaves size and age, and locality are very important traits of isolation of PCR usable DNA (Kim CS *et. al*,.1999; Khanuja*et. al*,.1999; Sablok*et. al*,.2009). Medium size and age of leaves are suitable for isolation high quality DNA. It is remarkably that no any sequences in NCBI data base related to *A. senegal* MYB gene. It very difficult to compare homologue sequences from model plant (Rice), withthe help of various tools.Conserved domains of MYB gene related sequence selected for designing primers. In this study, we find out seven MYB gene specific novel sequences. Out of which, only one sequence were successfully submittedto GenBank and published with GeneBank identifier ID (KF364950); Locus (KF364950) and rest six sequences were submitting to ESTs database because these sequences were very short in length (range less than 200). The identify nucleotide sequences similar to the KF364950, a blast results revealed that 3 sequences of *Prosopis alba* tree species were identical in relation to its taxonomical approaches. Out of 101 identical organisms, in which 3 sequences of*Prosopis alba* transcription factor MYB93-like mRNA which identical locus are LOC114719063,LOC114716567 and LOC114729076 found with query cover range 48% to 54% identical. This study indicated that more precisely study will need for arid tree species.It is interestingly that only one sequence was found till date related to *A. senegal*MYB gene partial sequence in NCBI public database.

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**Competing interests**

The authors declare that they have no competing interests.

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