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ISOLATION AND SEQUENCING OF RESISTANCE GENE ANALOGS FROM THE NBS-LRR REGION OF *COFFEA ARABICA* GENOME

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ABSTRACT Coffee is one of the most economically important crops in India and also across the world. Apart from the abiotic stress, a USD 495.5 billion coffee industry suffers from the outbreak of various diseases caused by pathogenic fungus, bacteria and viruses. The presence of Resistance Gene Analogs (RGAs) in coffee plant is the most prominent marker of resistance against the pathogens. The current study aims to isolate resistance gene analogs from the nucleotide binding site – Leucine rich repeats (NBS-LRR) region of the *Coffea arabica* chromosome.

KEYWORDS : Coffea arabica, disease resistance, resistance gene analogs, agriculture

INTRODUCTION:

Coffee industry is one of the most valuable industries worldwide. Coffee is among the top ten most traded commodities across the world. There are more than 100 botanical species of Coffea (Davis *et al*., 2006), with *Coffea arabica* and *Coffea canephora* being the most cultivated. Coffee cultivation form the major industry and a source if high revenue for many coffee growing countries. Vietnam, Colombia, Brazil, India and Indonesia are among the major coffee producing states.

The major threats to the coffee industry are the diseases that affect productivity and ultimately revenue. Currently, coffee industry suffers mainly due to diseases caused by bacteria, fungus and pests. Coffee plant is mostly infected by pathogens that include Cercosporiosis (caused by *Cercospora coffeicola*), bacterial blight (caused by *Pseudomonas syringae* pv. *Garcae*), anthracnose (caused by *Colletotrichum coffeanum*), root-knot nematodes (caused by *Meloidogyne spp.*), coffee berry disease – CBD (caused by *Colletotrichum kahawae*), and coffee leaf rust – CLR (caused by *Hemileia vastatrix*) (Cabral *et al*., 2016; Krishnan, 2017). Coffee Leaf Rust is one of the deadliest diseases of coffee and has presence across the world (McCook and Vandermeer, 2015; Cabral *et al*., 2016).

Plants, in response to any kind of threat, either biotic of abiotic, have evolved resistance mechanisms. A wide range of plant resistance genes have been isolated from wide range of plant species recently that confer resistance against pathogenic attack (Richard and Ronald, 2000).The resistance genes are grouped into 5 classes based on the protein that they encode (Dangl and Jones, 2001). The largest classes of discovered resistance genes (collectively called as R genes) are those that code for the nucleotide binding site (NBS) and the Leucine-rich repeat (LRR) domain. Each part of the domain of NBS-LRR protein is anticipated to have a specific function. The Nucleotide Binding Site (NBS) domain is suggested to have nucleotide triphosphate hydrolyzing activity (ATPase or GTPase, etc) and they regulate the signal transduction by conformational changes (Martin *et al*., 2003; Leipe *et al*., 2004). The two major N-terminal amino acid sequences are the intracellular signaling domains that are similar to the Drosophila toll/mammalian interleukin-1 receptor (TNL, Toll-NBS-LRR) and the coiled-coil (CNL, CC-NBS-LRR). They precede the NBS domain involved in specific signal transduction (Jones and Dang, 2006); DeYoung and Innes, 2006). The carboxyl-terminal region has the LRR that mediate specific protein interaction that help to recognize the pathogen effectors (Van der Hoorn *et al*., 2001; Kushalappa et al., 2016). The nucleotide polymorphism and variable regions in the LRR region allows them to detect a specific pathogen effector molecule (McHale *et al*., 2006; Ellis *et al*., 2000). Due to continuous response to diverse pathogen races, the extreme variability in the inter and intra-specific regions of the NBS-LRR have been responsible for gene duplication, recombination, unequal crossing over, deletion, point mutation and selection pressure (Ribas *et al*., 2011).

Keeping in view, the ever evolving nature of the pathogenic microbes, it becomes crucial to investigate the presence of the resistance genes in the coffee varieties available at different places. The current study aims to isolate the resistance gene analogs (RGAs) from the NBS-LRR region of *Coffea arabica* plant.

MATERIALS AND METHODS:

Genomic DNA Isolation:

Fresh leaves (100 mg) were used to isolate genomic DNA from Coffee plant using Plant Genomic DNA isolation kit from Bunshi Bioscience. The genomic DNA was quantified by Agarose gel methods (Tweedie and Stowell, 2005; Dash *et al*., 2020).

Primers used for study:

Degenerate primers to match the P-loop motif within the sense direction and GLPL in the opposite direction during the identification of RGAs that are analogs of resistance genes (RGAs) within Coffea arabica were used (Deepak *et al*., 2012).

PCR Amplification and Sequencing:

PCR reaction of set using 30ng of genomic DNA as template with 1 mM dNTP, 3U Taq Polymerase, primer concentration was kept at 10 pmol and nuclease free water was used to maintain the volume to 20µl. The PCR program was set as follows: initial denaturation at 94℃ for five minutes, followed by a cycle of denaturing at the 94℃ for 30 seconds, then annealing at the 58℃ for 45 seconds, elongation at 72℃ for 1 minute 30 seconds before final extension at 72℃ for 10 minutes.

Gel extraction of Amplified Product:

PCR products were electrophoresed in 1.5% Agarose gel using 100bp DNA ladder. Precise size of the amplified product extracted from the agarose gel by incubating with Agarase enzyme at 55℃ for 1hr. Further DNA was purified by solvent purification method.

Cloning:

The purified amplicon was cloned in TA cloning vector (Biobharti Life Science Pvt. Ltd.).The insert was ligated directly to the thymine (T)-tailed Plasmid vector using T4 DNA Ligase enzyme As the product of PCR have adenine (A) overhangs. The PCR product is directly used as insert for cloning with T- vector. The positive clones were screened via selection based on antibiotic resistivity.

Sequencing:

Sanger sequencing method was used for sequencing. ABI Prism Platform was used.

RESULTS:

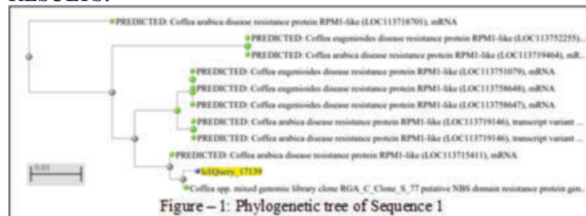

Figure – 1: Phylogenetic tree of Sequence 1

Sequence analysis of the obtained sequence was compared with the existing sequences in NCBI database. BLOSSOM62 alignment method was used to align the sequence with existing RGA from coffee and other crops. The alignment results showed percentage of identity considered E-value was used to consider the result and generate which confirms the closeness of RGA identified with Coffea arabica. Hence, further more addition of RGA is required to understand the role of

resistance genes in Coffea arabica against disease.

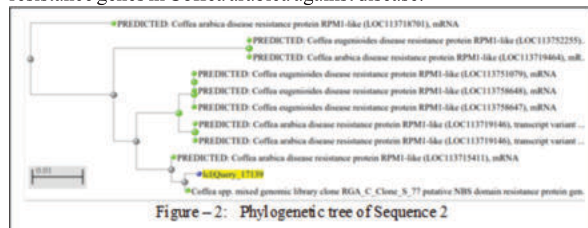

Figure – 2: Phylogenetic tree of Sequence 2

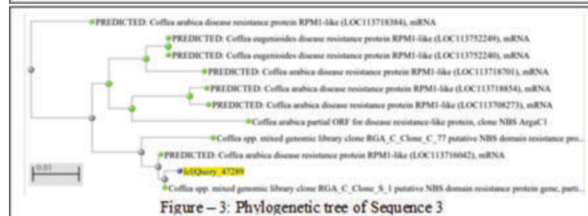

Figure – 3: Phylogenetic tree of Sequence 3

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