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COMPARATIVE STUDY OF DIFFERENT APPROACHES TO ISOLATE DNA FROM COFFEA ARABICA LEAVES

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ABSTRACT Genomic DNA isolation from high phenolic content plant has been a tough task for the molecular biologists. The polyphenols, polysaccharides that act as self defense for the plant, hinders with the reactions of DNA isolation procedures. Coffee plant is one such plant of high phenolic content. Being of high economical value, coffee plant needs to be studies at genomic level to counter the diseases and other abiotic factors that hamper the coffee production in India as well as worldwide. The current study aims to compare different methods to isolate genomic DNA from coffee plant.

KEYWORDS : Coffea arabica, disease resistance, agriculture, DNA isolation, etc.

INTRODUCTION:

Coffee is one of the most common beverages that can be found in almost every household around the world. It comes under the top ten most traded commodities in the world. Coffee is mostly cultivated in countries like Brazil, Colombia, Vietnam, Indonesia, India etc. Coffee forms the source of livelihood to millions of people around the world. There two varieties of coffee that are traded around the world, *Coffea arabica* and *Coffea canephora*. Among the two, arabica is market leader in the coffee industry. It accounts for 70% of the world coffee production. The total world coffee production stood at 10.88 million bags (60 kg bags) in the year 2022 till December (International Coffee Organization, n. d). In the financial year 2022-23, total coffee production from India was recorded to be 360,500 metric tons. In financial year 2022-23, coffee export from India stood at 1.02 billion USD. This is 32.54% higher as compared to the previous year (India Brand Equity Foundation, 2022). India exports 70% of its produce as coffee is a prominent source of foreign exchange. In India, coffee is grown in Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, Orissa and North-Eastern parts of India.

Coffee industry faces many challenges apart from market and other abiotic factors. Coffee cultivation is affected by a number of diseases that drastically hamper the productivity which results in economic loss to thousands of coffee farmers and also to the government (Cerda *et al*., 2017). Major diseases that impact the coffee industry are caused mainly by bacteria, fungus and pests. Bacterial diseases of coffee include Bacterial halo blight, bacterial leaf spot, bacterial leaf blight etc. (Badel and Zambolim, 2018; Korobko and Wondimagegne, 1997). Coffee Leaf Rust, Coffee Berry Disease, Coffee Wilt Disease, Brown Eye Spot, and root rot disease (Hindorf *et al*., 2011). These diseases can cause a serious loss of yield of up to 75% (Gichuru *et al*., 2021). Fungal diseases of coffee include Coffee Leaf Rust, Coffee Berry Disease, Coffee Wilt Disease, root rot disease etc. (Muller et al., 2009; Hailu, 2020; Hindorf and Omondi, 2011))Apart from bacterial and fungal infections, coffee plants also face threat from various pests like Coffee Berry Borer or *Hypothenemus hamperi causes berry borer disease. Stem borers like Monochamus leuconotus* (Pascoe), *Bixadus sierricola* (White), *Plagiohammus sp*, *Neoclytus cacicus* (Chevrolat) and *Xylotrechus quadripes* (Chevrolat). Coffee research mostly involves developing coffee varieties that are disease resistant and pest resistant. To counter the pathogens, it becomes important to develop disease resistant coffee cultivars. Till date, a total of nine resistant genes have been identified in coffee cultivars. The resistant genes are denoted by SH. In *C. arabica*, resistance genes SH1, SH2, SH4 and SH5 are found, in *C. canephora*, SH6, SH7, SH8 and SH9 are found and in *C. liberica* SH3 is found. The SH6, SH7, SH8, and SH9 genes were also found in Hibrido de Timor variety (Bettencourt and Noronha-Wagner, 1971). These resistance genes can be identified by the presence of N-terminal Nucleotide binding sites - Leucin Rich Repeats (NBS-LRR) (Kumar and Sreenath, 2012).

Genetic research forms the best available strategy in the field of plant research to develop a resistant variety of any crop. Identification of the mechanism of action of the pathogen under study provides all the necessary information that may be required to make the genetic changes in the plant crop. The most basic requirement for any type of genetic study is the technology to isolate high quality nucleic acids, DNA or RNA. The advanced technologies of today's time like Next Generation Sequencing demand a very high quality and quantity of DNA. Quality refers o the purity of the DNA sample. It must be ideally free from all kind of proteins, RNA and any other biomolecule contaminations. Certain plant species accumulate a high concentration of polysaccharides and phenolic compounds that hinder with the isolation of quality DNA (Healey *et al*., 2014). All the polysaccharides and phenolic compounds bind strongly to the nucleic acid during the isolation procedure and interfere with the reaction steps (Angeles et al., 2005; Hanania et al., 2004; Puchooa and Khoyratty, 2004). High molecular weight DNA isolation (long DNA fragment, 30-50kb) are necessary analysis like Next Generation Sequencing and is also useful in studies like large scale genomic variations, structural variations etc. Therefore, it is crucial to obtain high quality, high purity and high quantity genomic DNA of the plants for further high level molecular analyses such as Inter Simple Sequence Repeats and Random Amplification of Polymorphic DNA PCR based on molecular marker and marker-assisted selection (Tanaka and Ikeda, 2002). A variety of commercial isolation kits are available in the market to isolate the genomic DNA from plants. However not every kit is effective with every sample.

Plant DNA extraction and purification from Coffea arabica is a lengthy and laborious process compared to other tree plant species because coffee contains high amounts of polysachharides, polyphenols and various secondary metabolites such as alkaloids, flavonoids and phenols which usually interfere during DNA isolation. Even co-isolation of highly viscous polysaccharides along with DNA was the major problem encountered during coffee DNA isolation (Mishra et al., 2008). Several methods have been reported for minimizing the DNA extraction step and cost (Berthoumieu and Meyer, 1991; Edwards et al., 1991). Instead of that none of the protocols found suitable for Isolating DNA from Coffee. In this study, different available isolation kits were compared for their efficiency to isolate high molecular weight, high quantity genomic DNA form leaf sample of *Coffea arabica* plant.

MATERIALS AND METHODS:

Four different approaches were tried for isolating genomic DNA from coffee plant. 1. Sundried powdered leaves were taken for DNA isolation using Mix alkyltrimethyl ammonium bromide (MTAB) in the lysis buffer (2% w/v CTAB, 100 mM Tris HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4M NaCl). 2. Fresh middle aged leaves using cetyltrimethylammonium bromide (CTAB) (2% w/v CTAB, 100 mM Tris HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl). 3. Using column based Qiagen kit. 4. Using column based kit from Bunshi Bioscience. Protocol was followed as the manual provided in the kit.

RESULTS AND DISCUSSIONS:

The best quality DNA was isolated using the kit from Bunshi Bioscience (Fig – 1). DNA from the Qiagen kit was low quality and low quantity (Fig. – 2). Sundried leaves processed with MTAB lysis buffer yielded sheared DNA that cannot be used for genomics studies Fig. – 3). Fresh leaves processed with CTAB based lysis buffer gave better DNA yield (Fig. – 4).

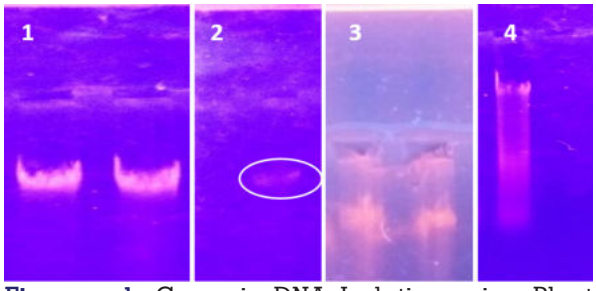

Figure – 1: Genomic DNA Isolation using Plant genomic DNA Isolation kit (Bunshi Bioscience),

Figure – 2: DNA using Qiagen Kit,

Figure – 3: DNA using MTAB Lysis Buffer,

Figure – 4: DNA isolation using CTAB based Lysis Buffer.

CONCLUSION:

The comparative study of different approach to isolate DNA from coffee leaves concluded that the approach to obtain good quality genomic DNA can be achieved by using kit method. Among the two kits used in this study, the genomic DNA isolation from Coffea arabica leaf using Plant genomic DNA Isolation Kit (Bunshi Bioscience) proved to be a better choice in comparison to the Qiagen kit.

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