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Extraction of Genomic DNA from *Gossypium sps.* Without Detergent Suitable for PCR

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Abstract Extraction of DNA is the prerequisite for many biotechnological works like marker assisted selection particularly in commercial crops such as cotton. Here we report a simple, rapid and inexpensive procedure for the isolation of DNA from cotton seeds for laboratory purpose. This procedure avoids the usage of detergents like CTAB/SDS which are inconvenient in preparation and time consuming. In addition, this procedure involves the usage of various reagents in different combinations for the effective removal of polyphenols, proteins, salts and polysaccharides making DNA suitable for PCR. Therefore, this method can be employed for various applications even in low equipped laboratories.
Keywords *Cotton, DNA, SDS, Microsatellites*

1. Introduction

The cotton, the white gold, a variety of plants of the genus *Gossypium*, includes 50 species, four of which are cultivated, 44 are wild diploids and two are wild tetraploids [1] and is the major commercial crop in many developing countries. With the advent of molecular biology techniques, there is a large need of rapid DNA extraction procedures for cotton. Isolation of plant nucleic acids for use for southern blot analysis, polymerase chain reaction (PCR) amplifications, restriction fragment length polymorphisms (RFLPs), arbitrary primed DNA amplifications (RAPD, APPCR, DAF), and genomic library construction, is one of the most important and time-consuming steps. Even though many commercial plant genomic extraction kits available, but their availability to certain developing countries and high cost can be limiting, especially when handing a large number of samples and considering experiments with limited financial resources [2].

Traditional methods for cotton genomic DNA involves the prior preparation of detergents like sodium dodecyl sulphate which is time consuming and inconvenient. In addition, the extracted DNA contains many contaminants like polyphenols, proteins and polysaccharides which may interfere with the PCR amplification. Hence, our objective is to develop a simple, rapid and inexpensive method for the

isolation of high quality genomic DNA from cotton seeds even in low equipped laboratory. An array of DNA isolation protocols have been optimized and were used in various combinations to isolate quality DNA from cotton seeds as well in leafs for analyses [2, 3, 4, 5, 6, 7]. This modified method avoids the usage of detergents and also the use of reagents like 2–mercaptoethanol, Phenol: chloroform: Isoamylalcohol can effectively remove contaminants of extracted DNA solution making it suitable for PCR.

2. Materials and Methods

Different commercially available segregating F1 hybrid cotton seeds and their respective parents were taken and incubated overnight in dark individually with distilled water. After soaking seed coat was removed and 30mg of inner mass was crushed gently using mortar and pestle. Total DNA from seeds of cotton was extracted with extraction buffer (1M Tris HCl, 0.5M EDTA, 5.0M NaCl and 1% beta mercaptoethanol) and kept for incubation at 67^0C for 1 hour and cooled on ice. In this study all the centrifugation steps were done at 13,000 rpm using microfuge (Eppendorf Inc.) at 4^0C. Then the clear supernatant was precipitated with equal amount of isopropanol and 10M ammonium acetate mixture and again centrifuged at 13,000g to get nuclei precipitate. This was dissolved in 100ul of TE and purified with RNase A, Phenol:Chloroform:Isoamylalcohol (25:24:1), Chloroform:Isoamylalcohol (24:1), 5M sodium acetate, absolute alcohol respectively to remove RNA, proteins and salts. High quality DNA obtained was dissolved in autoclaved 50ul TE buffer. The yield of DNA extracted was measured using a UV-VIS Spectronic Genesys 5 (Biorad Inc.) spectrophotometer at 260nm. The quantity and purity of the extracted DNA was given in the Table 1.

Table 1: *Quantity and Purity of DNA extracted from Gossypium sps*

VARIETY	A 260		Purity of DNA		QUANTITY (ug/ml)	
	Without SDS	With SDS	A 280	A260/A280	Without SDS	With SDS
TCHH 144	0.025	0.016	0.022	1.136364	250	160
TCHH 252	0.011	0.010	0.006	1.833333	110	100
TCHH 004	0.019	0.018	0.010	1.9	190	180
TCS 226	0.005	0.004	0.004	1.25	50	40
TCHH 009	0.025	0.017	0.023	1.086957	250	170
TCS 173	0.023	0.017	0.016	1.4375	230	170
TCS 2	0.015	0.019	0.011	1.363636	150	190
TCHH 118	0.027	0.024	0.022	1.227273	270	240
TCS 160	0.006	0.008	0.004	1.5	60	80
TCS 33	0.031	0.026	0.022	1.409091	310	260

The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. Concentration of DNA obtained with or without detergent was compared for novelty and reliability of the procedure on a 0.8% agarose gel by comparing band intensity with that of standard amounts of uncut lambda DNA. The DNA was checked for PCR amplification using various microsatellite primers pairs taken from cotton marker database and were obtained from Bioserve Inc. The details of primer pairs used for the study were given in Table 2. PCR reactions were performed with the Eppendorf PCR System 2400. The PCR conditions must be optimized for other thermo-cyclers and annealing temperatures must be optimized for each primer set. Each 15ul reaction volume contains 2X PCR buffer, 200uM of each dNTPs, 1U Taq polymerase, 15pm/ul each of forward and reverse primers for Microsatellites. (all reagents were obtained from Qiagen Inc.) and 30ng genomic DNA. PCR consists of one cycle of 94°C, 7 min, which was followed by 27 cycles of 94°C, 45 secs; 55°C, 1 min; 72°C, 2 min, and finally one cycle of 72°C, 10 min. The PCR products were analyzed by electrophoresis using

a 3% agarose gel in TBE buffer. DNA was stained by soaking the gel in a 10 mg/ml ethidium bromide solution.

Table 2: *Details of Microsatellite Primer Pairs Used for the Study*

Primer pair	Repeat type	Forward Primer	Reverse Primer
JESPR7	$(GAA)_{14}$	GCTGACGGAAGTGACAGGACCCT	GTCTCCTTCCCCTTCCTCTTCTTC
JESPR10	$(GAA)_{20}$	GAGGCAATGTCGGATGTGGGC	GCAAGTAGGTGGTGGCCGGAG
JESPR14	$(CTT)_{17}$	GGGAGGGGGTGAATAAACGGTG	GGTCAGGTAAACTTGCCATAGTGGG
JESPR21	$(GAA)_{15}$	GAGGGGGTGAATAAACGGTGAGG	CGGCTTCTCTTGCTTAGATCTGGAC
JESPR34	$(CTT)_{11}$	TGGTACCGGGGGATTCAGTGTGCCAC	ATGTGGCGCATCAGATCCGGTC
JESPR43	$(CAA)_{15}$	CGGCTTACAACAACAACAAC	GCTTCTCTTGCTTAGATCTGGAC
JESPR52	$(GAA)_{12}$	GCCGTACAATCACAGATTGGGAC	GCGCTTCTCATTGAGTCATCCTG
JESPR58	$(CTT)_{10}$	CCGCCCTTCTCTTGCTTAGATCTGG	GGAGCCAATTGAGAAGTGAATCCAA
JESPR72	$(CTT)_{10}$	CGCCCTTCTCTTGCTTAGATCTGG	GGGCAAGCTGACGATGAGGAATG
JESPR84	$(CTT)_{20}$	GACTCCCGGAGGCAATCAGAG	CCAGGGCTCATACTATCGCTGC
JESPR289	$(GAA)_{8}$	CATTGCATTTTGCCCC	AATCTAGCGCACAAGGGC
JESPR101	$(TA)_{8}(GT)_{15}$	CCAAGTCAAGGTGAGTTATATG	GCTCTTTGTTACTGAAATGGG

3. Results and Discussion

The physical characteristics of the final DNA pellet were white with no visible discoloration. The genomic DNA isolated with the given protocol yielded 50-250ng/ul of DNA which could be useful for minimum of 1500 PCR reactions. The quantity and purity of the extracted DNA was given in the Table 1. In general, the quality and quantity of extracted DNA depends on reagents used for extraction, precipitation temperature and duration. Particularly, a detergent is the essential component of DNA extraction buffer for breaking down cell and nuclear membrane to make extraction possible. Though, the usage of detergent like SDS yields good quantity DNA, simple extraction procedures yielding high quality DNA without SDS were not reported yet in cotton. The successful extraction of useful DNA from plants is associated with all extraction steps for molecular techniques used in the next steps such as PCR amplification, digestion and DNA sequencing. Particularly, the phenolic contents of the plants as well as other substances such as polysaccharides and proteins make difficult DNA extraction result in low quality and low quantity DNA [8, 9]. Current methods produce degraded and denatured DNA or give extremely poor yields. However, ccommercial kits are available from many biotech companies yielding good quality DNA, but they are expensive when hundreds or thousands of samples are extracted for DNA. To overcome all these difficulties, we modified the available genomic DNA extraction methods to get good quantity DNA with high quality. Representative photograph of the extracted DNA compared with that of DNA extracted with SDS was given in Figure 1.

The suitability of the DNA isolated as a template in PCR amplification reactions such as RAPD and Microsatellite was analyzed. The SSR-PCR amplification product profiles using DNA as template from F_1 plants and their corresponding parents was analyzed. The DNA isolated from all the plants yielded consistently amplified products. As an example the amplified products obtained from the parents and some F_1 seeds after PCR using primer pairs JESPR101 and JESPR289 are shown in Figure 2. The use of Phenol: Chloroform: Isoamylalcohol removes the proteins and lipids present in the DNA solution. Moreover, Sodium chloride and β-mercaptoethanol were added in the extraction buffer to take care of the polysaccharides and the polyphenols associated with DNA which is the compounds which could contribute to the inhibition of the DNA amplification during PCR reactions. Hence there were no additional steps needed for the removal of these compounds [10, 11, 12, 13].

Hence, this method is an attractive alternative for the extraction of plant DNA because of its efficiency and the speed of this method together with the use of inexpensive facilities and the absence of chemicals like CTAB and SDS. These results show that the DNA extracted by this simple, low cost

and safe protocol can be used in PCR-based applications, and in laboratories lacking state-of-the-art equipment and technology.

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Figures

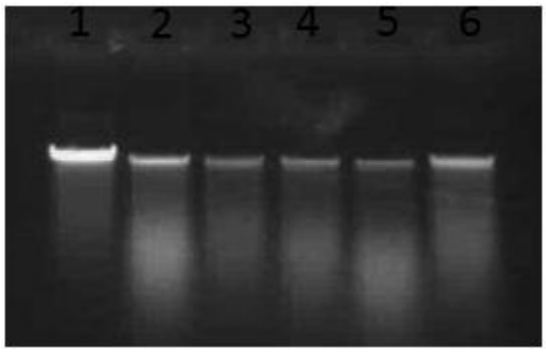

Figure 1: *DNA An Extracted from Single Cotton Seeds: Lambda DAN 150g Lanes 2-4: DNA Isolated Using the Modified Protocol Lanes 5-6: DNA Isolated with SDS.*

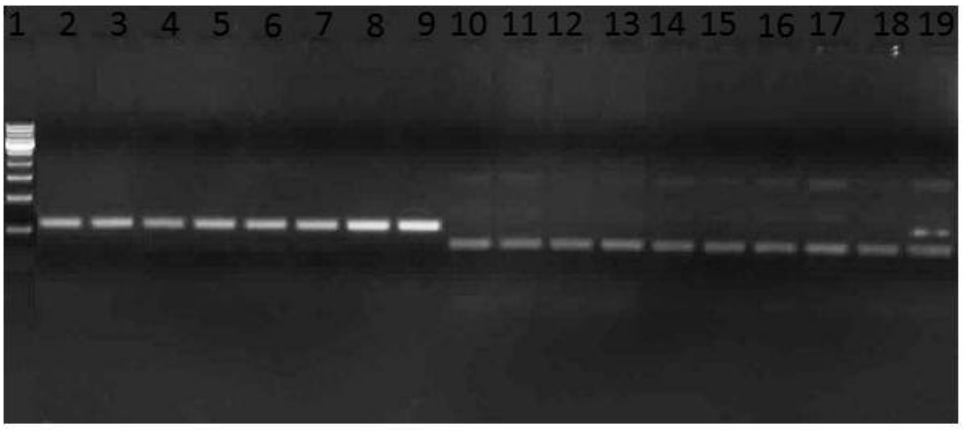

Figure 2: *Amplified PCR Product Profiles with Microsatellite Primer Pairs: 500bp Ladder, Lane 2-9: Amplified DNA Extracted with the Given Modified Protocol with Primer Pair JESPR 289. Lane 10-19: Amplified Pattern of DNA with Microsatellite Primer Pair JESPR101.*

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Research Article | Open Access

Decision Support and Database Management System "AroMed" on Commercially Exploited Medicinal and Aromatic Plants of India

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Abstract Identification of plants and commercial promotion of plant species can be achieved by utilizing information technology based database development; new technology can help to improve quality and cataloging procedures to organize the botanical species. Further, we can share the same information with any other interested parties via the Internet. Now personal computers with large memory-storage-systems have brought the computing and database maintenance procedures to the desktop of users. In recent years, the Internet has literally tapped into all desktop computers. The "Information Technology Network Highways" opened the doors of many remotely located libraries or other information repositories to data seekers by simply logging on to a web site. Development of database on electronic media and development of a web site on medicinal plants will provide the necessary exposure the world regarding the plant species of commercially exploited Aromatic and Medicinal Plants of India. Substances derived from plant material have been used in perfumes, most of cosmetic items, flavor, very useful Ayurvedic, Unani, Homeopathy Medicine etc. Since time in memorial for medicinal purposes. For example, plants such as Ginger, Tulsi and Alovera are well known for their positive effects of maintaining and restoring health. Furthermore, it is generally not common knowledge that Mango Seeds- used in asthma, diarrhoea, dysentery, haemorrhage, and menorrhagia and bleeding piles some plants extract like Laung used as pain-killers, however, medicinal plants have not lost their significance as natural alternatives to synthetic drugs even in the modern world. Since the number of commercially available drugs derived from plant sources is increasing day by day, there is a distinct need for an easy accessible data collection that provides detailed scientific information on such plants and its medicinal uses. AroMed India new medicinal plants database, hopes to improve the current situation. This distributed web-based information system stores, organizes and will provides combined text and multimedia data on AroMed India as well as pharmacological properties of medicinal plants, in particular from the multimedia point of view. From the technical point-of view our system presents 3-tier architecture, a WAMP server with MYSQL database and Joomla 1.5 Application as a front end.

Keywords *Aromatics and Medicinal Plant Database, Herbal Plants Database, Plants Chemical Properties, RDBMS, Genome Information, Trader's, Digitization, Bibliography Information*

1. Introduction

India has one of the richest medicinal and aromatic plant wealth in the world. Medicinal and aromatic plants are remarkable contemporary relevance for ensuring health security to the teeming millions [1]. It is expected that there are around 25,000 helpful plant-based formulations, used in folk medicine and known to rural communities in India [2]. Given the rapid growth of the herbal industry and medicinal plant related to drug development research work required all the information of those plants [3]. Ready information regarding as Identifying characters, pharmacological action/use, chemical properties, pricing, regulatory status and patent information etc., are not readily available in case of most of the medicinal and aromatic plants [4]. Non-availability of planting material and lack of awareness among the farmers on identification, improves the package of cultivation practices and farm processing of medicinal plants, are also lacking. Kinds of incentive required to encourage farmers to cultivate medicinal plant and what requires for the industry to encourage buying of medicinal plants from the farmers [5]. These are the questions one has to answer to achieving sustainable utilization of medicinal and aromatic plant resources is an objective that requires the application of a variety of methods, one of which is the development of practical guidelines for the sustainable exploitation of selected species [6] For this, Scientist will have to develop an Information Technology (IT)-based modern tools such as decision support systems or Artificial Intelligence System (AIS), and a toll which can be stored, retrieve and manage data, and can be improve cultivation, production and research work.

Computational methods have been frequently used by the private/public sector now a day [7]. Computer is the best way to data storage, accessible and easy to use by everyone. The proposed study is to develop data base management system for aromatic and medicinal plant related herbal industries and research sector at commercially level, which can be used at national and international level [8]. This methodology will provide the efficient information such as identification characters, chemical markers, pharmacological Information, cultivation information etc., which would be helpful to increase cultivation technology and Industrial research work [9].

The proposed system will be based on client-server approach, in which MS Access (Primary Storage) and MySQL database (Secondary Storage on Server) will be taken as back-end for data storage and Front end application will be designed in Joomla 1.5(CMS) or MS Visual Basic 6.0 for Menus, data screening and searching. The proposed system will be menu-driven and simply designed for data entry by the user. Recent and renewed interest in medicinal plants coupled to developments in information technology has fuelled an explosion in the range and content of electronic information concerning medicinal plants as a re-emergent health aid [10]. Recently reviewed diverse sources of such information in traditional abstracting services as well as in a variety of online electronic databases [11]. As a result of such developments, access to indigenous peoples and cultures concerning medicinal plants are greatly facilitated [12]. Furthermore, the active participation of such natural custodians and practitioners of valuable knowledge is guaranteed in the generation of research focusing on screening programmes dealing with the isolation of bioactive principles and the development of new drugs.

1.1. Aromed India Database Application

In the scope mentioned above, we designed AroMed India as a distributed information system relying on object-relational database technology. Our system is not limited for special scientific users but also useful to doctors, Students as well as home users and farmers, who is interested in medical plants and its products. Therefore, the access to this database is free of charge, but registration is required to be able to gather ideas about the user's reason why to access to AroMed India. The content of the database is designed in such a way that scientific users and researchers can benefit, as well as the interested home users. The demands on these different applications have been realized by

introducing two user main roles, one for the scientific user and one for the home user. The scientific user may be interested in botanical characteristics, pharmacology and Genome information, whereas the home user has a more general context approach. By using different weights for the queries, users can personalize AroMed India to one's own requirements (Table 1).

Table 1:

Home User's	Doctors, Pharmacist Farmers	Researcher, Biologist, Scientist	Students, Teacher's
Botanical interest	Botanical Interest	Botanical Interest	Application in: Physiology, and Plant biotechnology
Medicinal Plants Uses Information	Ayurvedic, Unani and siddha information	Medicinal uses and for Genome information	Self-Study
As a Home Remedy	For Identification	Genome Analysis Tools	Botanical Interest
Herbal Products	Pricing system for trade purpose	Development of plant drugs	As a Identification Key
Botanical guide	Medicinal uses and side effects	Target research	Molecular biology
For knowledge	For Business, import and export information	As an identification Key	Plant Genome Information

2. Materials and Methods

It is necessary to analysis all tools and technologies required to develop the proposed system. When I have considerate requirements to develop this project, there are two prospects, which have to examine, are following:

2.1. Text & Literature Requirement

Charaka samhita', 'Susrutha samhita', 'Vastuguna Deepika', 'Yogaratnakara', 'Vastuguna prakasika', and from medicinal plants treatises etc. (scientific journals, pharmacopoeia books & scripture).

2.2. Computational Requirements

Operating System	Windows XP and above
IDE	Joomla 1.5, internet explorer 6 or above
Back-End	WAMP SERVER, MYSQL

2.3. Minimum Hardware Requirements

Processor	500 MHZ
RAM	32 MB SDDR
HDD	20 GB or More

3. Result and Discussion

"AroMed India Database" has been design to exhibit the significant of commercially exploited Medicinal and Aromatic Plants of India. In this project no attempt have been made in reinventing a wheel but sincere attempts have been made in demonstrating a computer based "AROMATIC AND MEDICINAL PLANTS DATABASE" using the facilities of Joomla 1.5 CMS, a PHP based application for its commercially utilization through internet.

The project begins with the separate on different topics of as menu bar items. Each heading possesses a number of models as its menu items. The project provides a user-friendly environment revealing the functioning for each type of users like researchers, scientist, students, trader's and farmer's also.

In this project sincere efforts have been made to develop a simple and easy Computer Based Database of Medicinal Plants. The users can fill up his or her own data giving up appropriate constraints be able to manipulate the functioning of various highlights of system. The user provided with remarkable facilities of adding, printing, saving, deleting, modifying, closing as well as editing the data that has been entered currently or in the past. In addition to these the system also accepts complains, views, suggestions from the user's and facilitate to sort out those problems through online, using internet.

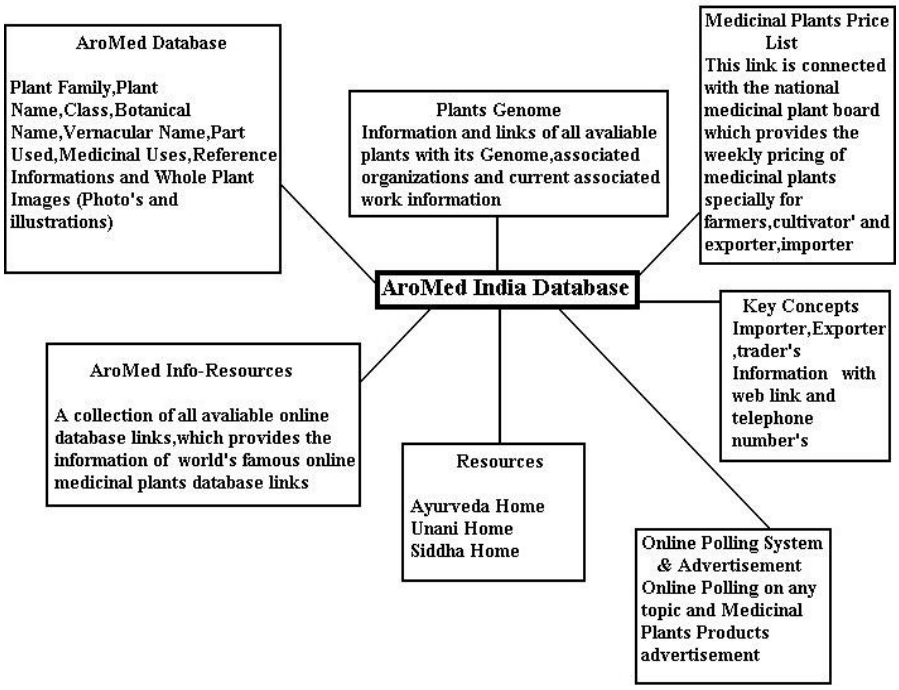

Figure 1: *Flow Diagram of AroMed India Database by Different User Group*

Figure 1 Gives an overview of the content organization with the main sections: of AroMed India Database AroMed database, AroMed Info Resources, Resources, Online Polling system, Key Concept, Online Pricing system. The AroMed Database section contains plants family wise information when you click on any selected family a list of plants associated with this family will appear and when you click on any plant name the bibliographic information will appear such as plant Image, Name, Family, Botanical Name, Vernacular Name in different languages and medicinal and therapeutic uses, Images and colored drawings help here to identify the text-described plant species. The AroMed Info Resources section provides the information of world's famous online database

information and its links if you click on any database it will connected with that online database directly. The Resources section is associated with Ayurveda ,Unani and Siddha web page which gives the details about what is Ayurveda?, What is Unani Medicine and Siddha Medicine?. The Pricing system gives the information of weekly medicinal plants price, this section is especially for trader who wants to buy and sale or import and exports their cultivated medicinal plants in the market. The Key Concept section provides the information of importer and exporter of India who is associated directly with medicinal and aromatic Plant industry, we provide trader's address, email id, contact number for trade inquiry and for business purpose and finally polling system section is an special part of this application with the help of this section we can collect view and answer's for a particular query from all of the user who is accessing AroMed database through Internet and can show the polling report at the same time.

3.1. Query Mechanism

AroMed India offers very simple search mechanism for browsing data and information. The Simple Search allows searching the whole database in a Google search engine like manner. In case of hits, the results are presented as resulted window web page.

This research work presents the experiences of creating the Information technology based database management system "AroMed India" (a Commercially exploited Medicinal and Aromatic Plants database of India) which is built to data collection and to provide interactive graphical user interface to the user for its information retrieval and related image, genome information ,medicinal uses and product information which is available in the market used by this plants and daily current price list of medicinal Plants and its importer and exporter information for its commercially exploitation.

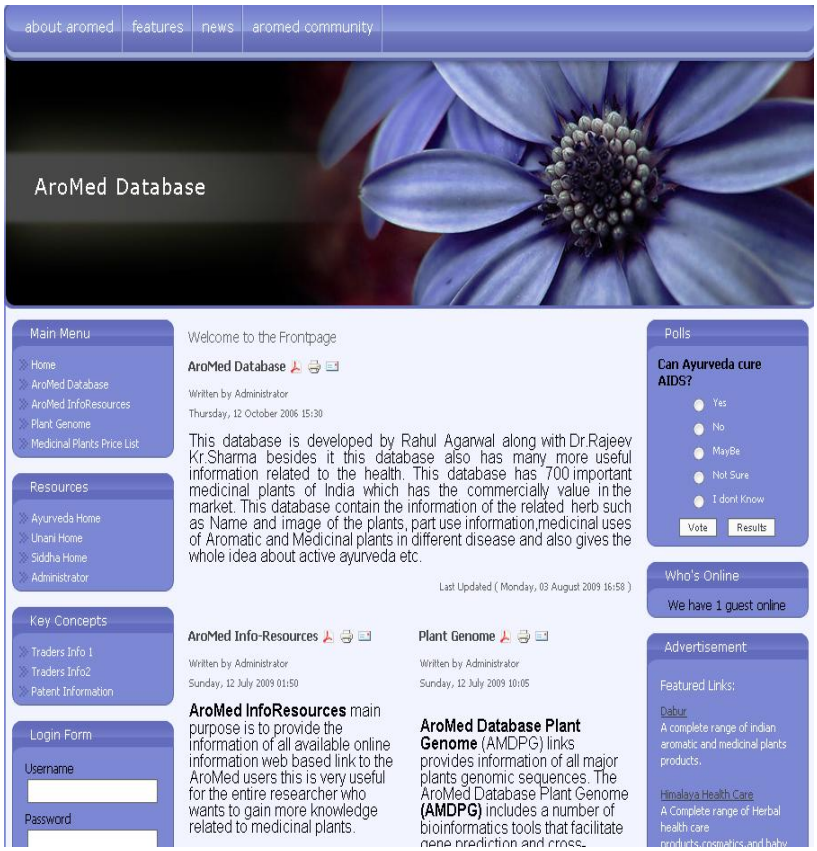

Figure 2: *AroMed India Database Main Web Page*

4. Conclusion and Future Prospects

Today's world running on technology and Internet. Every organization, company, research institute even most of the people are connected through internet and exchange many information using internet. So AroMed Database will also be run on internet through Joomla utility tools around the world with different languages and any user from different parts of the world can easily access its resources and can use its information on daily basis anytime from anywhere. And even Users can pass and share own views and idea's through online chatting, mailing, blogging world widely.

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Review Article **Open Access**

Agronomic Practices for High Trade Value Indian Aromatic Plants

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Abstract India has long tradition of perfumery trade in the world. The art of perfumery flourished and enriched under the patronage of Moghul kings during their rule in India. The perfumers of Kannauj (U.P.) were famous for the product of aroma by distilling rose, kewra, agarwood, and sandalwood etc. This was possible due to abundant availability of raw material from plants resources either wild or cultivated. Of the 18000 species of plants distributed in India, 1300 are aromatic. Out of this 65 aromatic plant species have large and consistent demand. Cheaper synthetic fragrance suppressed the natural aromas due to price advantage, but blenders have now realized that the complex chemical components of each essential oil resourced from plants can no way be matched. In the present communication agronomic practices for 20 high trade value Indian aromatic plants are reviewed.
Keywords *Agronomic practices, Aromatic plants, Essential oil*

Introduction

Essential oils represent the ‘essences’ or odoriferous constituents of plants. There are also called volatile oils being volatile in steam and at high temperature evaporate. Besides medicinal/therapeutic applications (carminative, diuretics, local stimulants, mild antiseptics, local irritants, antiseptics, or parasiticides), they find commercial applications as spices, flavoring of foods, confections, beverages, cosmetics, tobacco etc.

Plant texa of angiospermic families are major source of essential oils. About 2000 species distributed over about 87 plant families reported to consist essential oil. Pinaceae, Lauraceae, Rutaceae, Myrtaceae, Apiaceae, Lamiaccae and Astraceae are chief families for source plant material. Certain essential oil (e.g. in the liver of fish, musk etc.) are also resourced from animals. Depending upon the localization of essential oil in plant parts, essential oils are extracted from different morphological entities viz. flowers, fruits, seeds, stem, leaves, whole plant etc. Essential oil occur in specialized secretory structures such as glandular hairs, modified parenchyma or oils cells, oil tubes or vittae, as well as in internal lysigenous or schizogenous passages or glands. Chemically these are variable in nature depending upon the presence of hydrocarbon, alcohol, ester, aldehyde, ketone, phenols, esters, oxides and peroxides, and terpenoid in constitution.

The growing demand of essential oil is putting a pressure on the existing resources owning to limitation on the existing sources and threatened natural habitats; cultivation of aromatic plant is inevitable. The listed plant species in Table 1, economically viable and essential oil resourced from them are commercially exploited for different applications. This compilation1-7 in agronomic practices of certain Indian aromatic plants is intended to provide basic essential information for farmers and extension works in Table 2.

Table 1: *Important Aromatic Plants Species and Their Utility Pattern*

S. No.	Botanical Name	Trade Name	Commercial Application
1.	*Cymbopogon flexuous & C. pendulus*	Lemongrass	The oils used in perfumery and cosmetic industry and also in manufacture of Vitamin A.
2.	*Cymbopogon winterianus*	Citronella	Oil obtained from steam distillation of leaves in rich in citronellal and geraniol and is used in perfumery, cosmetics and mosquito repellent formulations.
3.	*Cymbopogon nardus & C. confertiflours*	Cymbopgon	The essential oil is used in perfumery and flavouring industries.
4.	*Cymbopogon nardus C. confertiflorus & C. jwarancusa.*	Jamrosa	Freshly harvested foliage and flowering shoots yield 0.4 % essential oil on steam distillation, which is used in perfumery/flavour/soap industry. The oil is good substitute of palmarosa oil.
5.	*Cymbopogon martini*	Palmarosa	It is used in perfumery and cosmetic industries, flavouring of tobacco and in soaps.
6.	*Mentha arvensis*	Menthol mint	Oil is source of natural menthol used in flavour and pharmaceutical industries.
7.	*Mentha piperita*	Peppermint	The leaves on distillation yield essential oil (0.4-0.5%). The oil is used in perfumery, food flavouring and pharmaceutical preparations.
8.	*Ocimum canum*	Basil oil	The herb yields 0.5-0.7% oil, useful in perfumery and flavouring industry.
9.	*Ocimum gratissimum*	Clocimum	It yields about 0.5% oil with 80-85% eugenol therein, useful in flavouring, pharmaceutical industry and synthesis of vanillin.
10.	*Pelargonium graveolens*	Geranium	The eaves and branches are steam distilled to get “oil of geranium” (Aporrox. yield 0.8%-0. 1%), used in high-grade perfumery product and soaps.
11.	*Vetiveria zizanioides*	Vetiver	The roots are steam distilled to get vetiver oil, which used in high-grade perfumers.
12.	*Salvia scalaria*	Clary sage	The flowers yield 0.15-0.25% essential oil rich in linalool and linalyl acetata. The oil is used in high-grade perfumes, cosmetics, flavouring liquors and as modifier in spice compounds.

S. No.	Botanical Name	Trade Name	Commercial Application
13.	*Pogostemon patchouli*	Patchouli	Oil is used in perfumery and cosmetic industry as a fixative as it Provides tenacity to other perfumes.
14.	*Humulus lupulus*	Hops	The female inflorescence of the plant, called hop cones, is chiefly used in the manufacture of beer.
15.	*Lavendula angustifolia*	Lavender	The flowering tops of lavender plant yield 1.2% -1.4% valuable essential oil, mainly composed of linalool and linaly acetate. The oil is used in high-grade perfumery. Lower quality oil goes for preparation of lavender water, toilet water and soaps.
16.	*Apium graveolens*	Celery	The seeds contain 2%-2.5% aromatic oil, dry seeds are used as spic in fresh and processed foods e.g. soups,sauces,pickles,meat,vegetable juices and in form of oleoresins. The seeds are also used in pharmaceutical applications and manufacture of commercial drug 'Ajmoda' used in Unani and Ayurvedic system of medicine. Leaves and stalk are used as salad vegetables.
17.	*Cinnamomum verum*	Cinnamon	Quill (tender inner bark) is used as a spice and medicine. In addition, the bark oil (55% -56% cinnamic aldehyde) and leaf oil 75% -95% eugenol) are obtained which are used as food as food flavour and in manufacture of toothpaste, perfumery and cosmetics.
18.	*Eucalyptus* sp.	Eucalyptus	The leaves are steam for ex traction of essential oil (1.0%-1.2% yield) which is widely used in soap, perfumery, pharmaceutical, cosmetic industry and in the manufacture of citranellal, citranellol and hydroxyl citranellal. The wood is used as mine props, railway sleepers, in Paper/pulp industry and as fuel.
19.	*Artemisia annua*	Wormwood	The plant is a source of artimisinin used for the manufacture of arteether-drug for treatment of malaria; also yield essential oil (0.3%).
20.	*Rosa damascena*	Damask rose	Rose products are used in high value cosmetic, perfumery, food, pharmaceutical Industry, pan masala and tobacco.

Table 2: *Important Aromatic Plants Species and Their Agronomic Profiles.*

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
1.	*Cymbopogon flexuous*	Tropical and sub-tropical plains.	Well-drained sandy loam, literate soil pH-5.5-9.0 and humid climate with sufficient sunshine.	Through vegetative slips during Feb / March, economic life: 4-5 yr.	Harvesting period May-Dec., 4-5 harvests/yr, 6-8 irrigation; fertilizer; N 150,P6O, K60, FYM 10t/ha.	In first 100-130 kg oil, second year onwards 175-200 kg oil/ha.
2.	*Cymbopogon winterianus*	NE region, southern region,Indo-gangetic plains	Well drained sandy loam or loamy soil, pH 5.8 - 8.0; sub-tropical to tropical climate, well-distributed rainfall 200-250 cm.	Vegetatively through slips during July / August and Feb / March ; about 55,000 slips/ha	Irrigation: 4-6 during rain free period, fertilizer: N-150, P60, K60 kg/ha/yr. Leaf blade is harvested 15 cm above the ground. First harvest comes 90 days after planting, subs equently at 3-4 months interval; economic life 4 yr	First yr: 150.2nd-3rd yr: 200,4th yr: 150 kg oil/ha.
3.	*Cymbopogon nardus*, & *C. confertiflours*	Northern and Central India	Sandy loam to loamy soil, ph range 6.5-8.5,tropical environmentcond itin, warm and humid climate with sufficient sun shine hours; 800 to 1500 mm annual rainfall.	Vegetatively through slips, 62,500 slips/ha. Planting period Feb/March, July/August.	1st cutting 130 to 150 days after planting, thereafter at 55 to 65 days interval; life span 4-5 yrs; irrigation: 8-10 per yr, fertilizers; FYM 10 t, N200 kg, P80 KG, k60 kg/ha.	1st year: 200-220 kg, 2nd year onwards: 250-280 kg oil/ha.
4.	*Cymbopogon nardus, C. confertiflorus* & *C. jwarancusa.*	UP and other region	Sandy loam, loam, wastes lands-tropical and sub-tropical. It can withstand water stress conditions though returns in irrigated conditions are higher.	Planting in Feb-March; 50,000 slip/ha.	Harvesting period: May to December at 90-95 days interval, 10 irrigation, fertilizer: N180, P60, K60 kg/ha.	First year: 160 KG and in second year onwards: 220 kg oil/ha.

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
5.	*Cymbopogon martini*	All regions except temperate hilly areas.	Well-drained sandy loams to loamy soils are ideal; the plant survives in sodic soil at 5-9.5, sub-tropical and tropical climate. It can also be cultivated in degraded soil, eroded lands, as well as marginal soils with reasonable returns. Propagated during rainy season through seed: 10-12 kg/ha.	Propagated during rainy season through seeds: 10-12 kg/ha.	Fertilizers: N 100, P 50 kg/ha/year. In poor red soil of Deccan plateau, N up to 250 kg/ha gives good result.4-6 irrigation (during rain free period). The crop is harvested 3-4 months after planting; 2-3 harvests obtained in the first year and 3-4 in subsequently year. Economic life 4-6 year.	First year: 60-80 kg, second year onwards: 100-150 kg/ha.
6.	*Mentha arvensis*	Punjab, Haryana, UP, Uttarakhand, MP, Bihar, Rajasthan.	Well-drained deep loamy soil; pH 6.5-8.0,tropical and subtropical climate, rainfall 950-1050 mm.	Vegetative propagation through suckers; 5q suckers for direct sowing and 1q/ha suckers are required for nursery and transplantation of seedlings.	It is a 6-7 months crop. First harvest 100-120 days after planting, second harvest after another 50-60 kg/ha.	150 to 200 kg oil/ha.
7.	*Mentha piperita*	Terai region and hill of UP, Himachal Pradesh, Sikkim.	Well-drained, rich deep loam soils; semi-temperate climate, average rainfall 95-105 cm, average temp. 15-30 ^{0} C.	Through runners or other vegetative parts during mid Dec.-mid Jan.	First harvest 110-130 days after planting, second after another 8-12 weeks; life span 1 year; irrigation: 10-12, fertilizer; N 125 kg, P60 kg, K60 kg, FMY10 t/ha. The crop is generally allowed to wilt for 24-28 hr before distillation. This reduces moisture content; allow proper packing of herb and effects savings in steam during distillation.	Apporox. 100 kg oil/ha.

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
8.	*Ocimum canum*	Punjab, UP, MP, Kerala, Tami Nadu.	Well-drained loam and sandy loam soil, tropical & subtropical climate; also grows on moderate acidic, saline and alkaline soil at Ph 6-8.	Through seed, 500 g/ha, planting in Feb-March and June-July.	Harvesting period: June, August to October; irrigation: 9; fertilizer N: P: K: 90:60:60, FYM 5 t/ha. It is a month crop.	180-210 kg oil/ha (during 7 months).
9.	*Ocimum gratissimu*	Punjab, UP, MP, Kerala, Bihar, Tamil Nadu.	Well-drained rich loam to poor laterite, saline and alkaline to moderately acidic soil, air to high rainfall and humid condition, tropical and subtropical climate at altitude up to 900m.	Through seed & tender cutting, planning material: 500 g/ha, transplanting during Feb-March and June-July.	First harvest 90-95 days after transplanting, thereafter at 65-75 days interval.2-3 harvests in the first year and 4 each in subsequent year during May, July, Sep, & Dec.Life span: 5year; irrigation: 8;fertilizer: FYM 10t,N: P: K: 90:60:60 kg/ha.	1st year: 175-180, 2nd year onwards: 200-250 kg oil/ha.
10.	*Pelargonium graveolens*	North and South India, both in plains and hilly region.	Well-drained fertile soil, pH: 5.5-7.5, and Mediterranean type of climate with warm winter and mild summer, average temp. 30-35^{o} C with low humidity, moderate rainfall up to 1000-1500 mm.	Through stem cutting, about 40000 plants/ha during November-February.	Harvest after about 5 months, subsequently at 3 months interval; grown as annual in North India Plains, up to 3 year in other irrigation as per need, fertilizer: 150-200 kg N, 60 kg and P60 kg /ha .2-3 weeding and regular hoeing are required.	30-35 kg oil/ha/year.

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
11.	*Vetiveria zizanioides*	North and south Indian plains	Light loam or medium loam loose soil; tropical and subtropical climate. It can survive in highly alkaline soils up to pH 10 and also grow in riverbeds and water logged conditions.	Vegetatively through slips; planting during February and July-August; 40,000 slips/ha.	Roots are harvested 18-10 months after planting. Soils of medium fertility do not require fertilizer. For red laterite soils in South India, fertilizer N20, P40 kg/ha is required as basal dose at the time of planting. Vertiver is cultivated as a rain fed crop; 1-2 irrigation: required if planted during dry period.	12-15 kg oil from month's crop.
12.	*Salvia scalaria*	Kashmir valley, HP and Uttarakhand.	Poor, dry and slightly acidic soils, temperature climate, bright light and 8-33^0 C temp during harvesting in June/July	Through seeds in Nov. or March / April using 3-4 kg seeds/ha. Propagation through seedling gives better result. Seedling raised in March /April are transplanted in Nov. and raised in Aug. / Sept. is transplanted in next season.	Plant does not require irrigation. 100 kg N/ha is required in 3 split doses. 25 kg N, 30 kg, P and 30 kg K are applied as basal dose. Flowering tops are harvested twice/ye i.e. in July and Sept.; remunerative life: 4 yr.	20 kg oil/ha.

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
13.	*Pogostemon patchouli*	NE India, Southern States where temperature is moderate.	Well-drained medium loam fertile soils are favourable,while tropical & subtropical regions are also Suitable.	Well-drained Medium loam Fertile soils are Favorable during Feb-April, Planting preferred in coconut	Spacing: 60cmχ60cmχ1m fertilizer: N 100, P 50, K50, K50 kg / ha, irrigation: 2-3 during Dec.-Feb. Perennial crop life span: 3-4 months interval. The leaves are dried in shade for 3-6 days. The leaves yield 1.8-3.0% oil on distillation. The plant in highly susceptible to root knot disease, which is controlled by adopting crop rotation and use of nematicides.	50 kg oil/ha.
14.	*Humulus lupulus*	Kashmir valley, Lahaul-Spiti in Himachal Pradesh and Champawat in Uttarakhand hills.	Sandy loam to clay loam pH 6-8.	Vegetatively through cutting, planted in Nov-Dec or March. Row to row and plant to plant spacing of 2m χ 2m each resulting in 2500 plants/ha with 5-6 m as the height of hop trellis.	Cow/mule dug or poultry manure is the traditional organic manure. Nitrogen is most important plant nutrient in addition to P & K along with micronutrients. After vines are trained, the field is irrigated repeatedly after picking; the cones are dried in a kiln, pressed and baled. The price of hops is determined by the concentration of alpha acids.	1st year: 2,500, 2nd yr: 5,000, 3rd yr: 6,250 kg/ha.

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
15.	*Lavendula angustifolia*	Kashmir Valley, Uttarakhand.	Well aerated dry and calcareous soil rich in nutrients, pH 7.0-8.4; cold winter and cool summer; rainfall 550-700 mm in the form of rain or snow. It can also be grow on gentile and steep slopes, poor eroded soils.	Through seeds during Nov. /Dec. also propagated through rooted cutting. Seeds germinate in April at 14-15^0 Scatting of 10 cm length are taken from 1yr old plants and firmly planted. Beds are covered with black polythene on protect from cold weather.	A basal dose of 20 kg N and 40 kg N are needed. The field requires regular weeding and hoeing. Old lavender plantations are regenerated during winters. Harvesting of flowers is done in dry sunny days during Aug/Sept.Oil is extracted by steam distillation.	15 kg oil/ha.
16.	*Apium graveolens*	Punjab, Haryana, UP and hilly areas having cold and dry climate.	Sandy loam soils, cool climate, winter season.	Direct planting or seeding 1.5-2 kg seeds / ha. Nov. to May (6 months crop)	Harvesting period: 2nd and 3rd week of May, 3-4 irrigation, fertilizer: N 100, P 40, K10 kg/ha. Maturity in 180-190 days. The seed is harvested as soon as colour change takes place. It is sun dried and cleaned before packing.	2t Seeds/ha.
17.	*Cinnamomum verum*	Coastal areas of Kerala, Orissa, Tamil Nadu, Karnataka, Goa, Maharashtra.	Medium to loam soils with sufficient organic matter; tropical humid coastal climate with well distributed rainfall.	Through seeds during May/June. One year old seedling transplanted at a distance of 2mχ2m.	Irrigation requited during rain free period till 1 yr old; fertilizer: 100g NPK per plant in 1st yr, 200 g in 2nd yr and 500 g in subsequent yr. harvest: 2 yr after coppicing during July/Aug and Dec./Jan., remunerative returns after 10 yr. quill by products yield 0.8% bark oil while leaves yield 0.6-0.8 % oil on distillation.	Bark 250 kg/ha/yr, leaf oil 100 kg/ha/yr.

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18.	*Eucalyptus*	Most part of India up to 1500 mean sea level altitude.	Well drained deep sandy to sandy loam soil preferred; also survives in marginal acidic and gravellier soils; tropical & subtropical climate; rainfall 200-250 cm, humidity about 85%, temp 16-30^{o} C.	Through seeds during March/April, seedling transplanted after 40-45 days.	Fertilizer: N80, K20 kg/ha/yr, irrigation during dry months; clipping of twigs after 6 months from planting, thereafter harvest every 3-4 months. Remunerative life: 10 yr.	First yr: 80 kg oil/ha; subsequent yr: 170-200 kg oil/ha. Dry wood: 8-10 t/ha.
19.	*Artemisia annua*	Himalayan region, subtropical northern plains.	Sandy loam-to-loam soils with proper drainage; light textured soil ideal; temperate climate with cold winter and moderate summer. It can also be cultivated during winter in northern plains.	Through seeds: in temperate region during March/April, in northern plains during Oct.; 35 days old seedling are transplanted.	Fertilizer: N150, P50, K50 kg/ha.Irrigation: during summers in tropical region, regular in subtrop.region; water logging to be avoided. Crop is harvested during Oct.-Nov, in tropical and May-June in subtopic. Region .The herbage is immediately steam distilled for essential oil. If artimisinin is the desired product, herbage is shade dried leaves and flowers separated from stalks for processing.	Artemisinin 5kg/oil 55 kg.

S. No	Botanical Name	Region	Climatic Environment	Propagation	Agripractices	Yield
20.	*Rosa damascena*	Jwala and Noorjehan are suitable for sub-tropical northern plains, mid hills and mild temperate region up to 1200 m altitude.	Wide range of soil, sandy loam to clay loam. It does well in deep rich loamy soil.	Through stem cutting, water-shots and seeds. The rooted cuttings are transplanted during monsoon while winter is ideal for establishment in field.	Irrigation is necessary during dry periods. Fertilizer: N: 120-150, P: 60-90, K: 40-50 kg/ha/yr.Harvest: in northern plans during March/April and in the hills during May. Economic life: 15-20 yr.	Fresh flowers: 3.5-5.0 t/ha; Oil recovery: 0.0250.030 %; Oil yield: 0.75-1.5 kg/ha; Rose concrete: 0.35-0.45%; Rose absolute: 0.15-0.20%; Rose water: AAA 1800-2000L/ha and AA grade 3,500-4,000 L/ha.

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