BIOINFORMATION
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Database

Volume 8(17)

CIBMAN: Database exploring *Citrus* biodiversity of Manipur

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Received August 17, 2012; Accepted August 20, 2012; Published September 11, 2012

Abstract:
The rich wealth of *Citrus* genetic resources makes India to enjoy a remarkable position in the "*Citrus* belt of the world". We have developed CIBMAN, a unique database on *Citrus* biodiversity of Manipur which comprises 33 accessions collected through extensive survey for more than three years. CIBMAN provides integrated access to *Citrus* species through sophisticated web interface which has following capabilities a) morphological details, b) socio-economic details, c) taxonomic details and d) geographical distribution. Morphological variability among *Citrus* accessions is due to variance in their genome which contributes to diverse agronomical traits and diverse bioactive compounds of high value. This diverse gene pool can be potential source for genetic improvement of existing cultivars and rootstocks. Systematic collection, characterization and conservation of the underutilized or lesser exploited varieties is required for incorporating in breeding program and conserve the germplasm from ever going on genetic erosion. This database will be useful for scientific validations and updating of traditional wisdom in bioprospecting aspects especially industrialization of *Citrus* found in the state. Further, the features will be suited for detailed investigation on potential medicinal and edible *Citrus* that make CIBMAN a powerful tool for sustainable management.

Availability: http://ibsd.gov.in/cibman

Background:
Citrus a delicious tropical fruit crop widely grows in the state of Manipur of North-East (NE) Region of India. The aromatic, nutritive and medicinal value of *Citrus* is integrally associated with day to day lifestyle of peoples of Manipur through ages. *Citrus* thrives in Manipur as cultivated, semi-wild and wild forms, with maximum diversity being maintained in home gardens. The commonly available *Citrus* fruits in the state are sweet oranges, pumelos, rough lemon, mandarins, wild orange, citron and other *Citrus* species. Various *Citrus* forms having acid taste is also widely available with ambiguities in their names. Frequently, people use to call the acid ones as champra or solom, if it is larger one. The rich germplasm of acidic *Citrus* group is under threat of loss as it is frequently removed from farmer's field or home gardens in exchange for crops of higher economic value. *Citrus* is valued for the fruit, which is either eaten alone as fresh fruit, processed into juice, pickles or added to dishes and beverages. The rich polyphenolic compounds of *Citrus* such as flavanoids can act as "functional ingredients" in promotion of health and prevention of degenerative diseases **[1-5].** *Citrus* is also potential source for compounds having high value in perfumery, cosmetic and chemotherapeutic purpose of controlling phytopathogens **[6].** The NE Region of India of which Manipur is a part is considered as "natural home for ***Citrus***" **[7].** Out of the 27 species reported in India, 23 are confined to North-East Region of India **[8]**. Easy hybridization amongst different *Citrus* species and genera and unique favourable agro-climatic and soil-physiographic setup of

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the region paves the way for origin of numerous forms growing in wild and semi-wild forms **[9].** *Citrus* plants in the NE region are still growing in deep forests undisturbed by biotic factors **[10, 11].** An important crop, *Citrus macroptera* or wild orange due to its habitat preference for wet and shady areas is growing inside deep forest of Chandel District of Manipur. Despite rich diversity in this biodiversity hotspot of the world, erosion of genetic resources of *Citrus* in the natural habitat is taking place due to various biotic and abiotic factors has resulted in loss of gene pool from nature and as well as from different centre of collections **[12].** Even though there is availability of large *Citrus* germplasm and suitable conditions that favours the growth, *Citrus* industry of the state is in poor shape as its productivity is extremely low. Region specific topographical problem, traditional way of cultivation, lack of scientific way of nutrients, diseases and insect-pest management and unavailability of quality planting material contributes to low productivity of *Citrus* in the state **[13].**

Database to retrieve the comprehensive information on *Citrus* will be extremely useful to modern research, and could enable new insights and discoveries concerning evolutionary relationship of gene and gene products anticipated in ethno-pharmacology. Some attempt has been made by Hazarika **[12]** for systematic documentation of the *Citrus* found in North-East India, but online interface will be certainly useful for the users to interpret more effectively and retrieve the desired information. Thus we have designed a sophisticated web based database to provide comprehensive information on *Citrus* found in Manipur.

CIBMAN is first resource that provides comprehensive retrieval of information related to *Citrus* bioresources found in Manipur and is a unique publically available web-interface that elaborates ***CI****trus* **B**iodiversity of **MAN**ipur (http://ibsd.gov.in/cibman) which provides access to the taxonomy, morphology (leaves, fruit and seed), geographical distribution, economic status, uses, references and links. **CIBMAN**s' sophisticated web based graphical user interface allows efficient retrieval of data. The reliable data content and links add an advantage in exploring data and that will make **CIBMAN** a comprehensive and sophisticated tool.

Methodology:

Surveys for availability of *Citrus* in nine districts of Manipur were conducted during 2008 to 2012. Data were obtained by performing personal visit to the market place, homeyards and forest. During the course of study, *Citrus* species were also collected from different habitats and preserved following the standard methods and identified up to genus level by following the standard taxonomic keys. The **genetic** resources were maintained at IBSD. The identification and authentication of the *Citrus* species were done at Botanical Survey of India (Shillong, Meghalaya); National Research Centre for *Citrus* (Nagpur, Maharashtra) and National Bureau of Plant Genetic Resources (Barapani, Meghalaya). *Citrus* crops are associated with socio-cultural activities of people of Manipur. Important *Citrus* species present in Manipur are *Citrus grandis, Citrus jambhiri, Citrus medica, Citrus macroptera, Citrus pseudolimon, Citrus reticulata, Citrus sinensis* and other *Citrus* species which are not properly known **[12, 14]**. There is high possibility of finding diverse form of *Citrus* since Manipur is located bordering to Myanmar, a region having high *Citrus* diversity. Proper exploitation of pumelos and acid group of *Citrus* for economic returns is required since farmers might opt for other crops thereby reducing the *Citrus* diversity. Kachai lemon a high yielding land race, cultivated in all the household of Kachai village of Ukhrul District, Manipur as community garden is a unique way for conserving the crop **[15].** Conservation by community gardening should be extended to *Citrus* species of lesser economic value and rarely available or endangered ones **[16].**

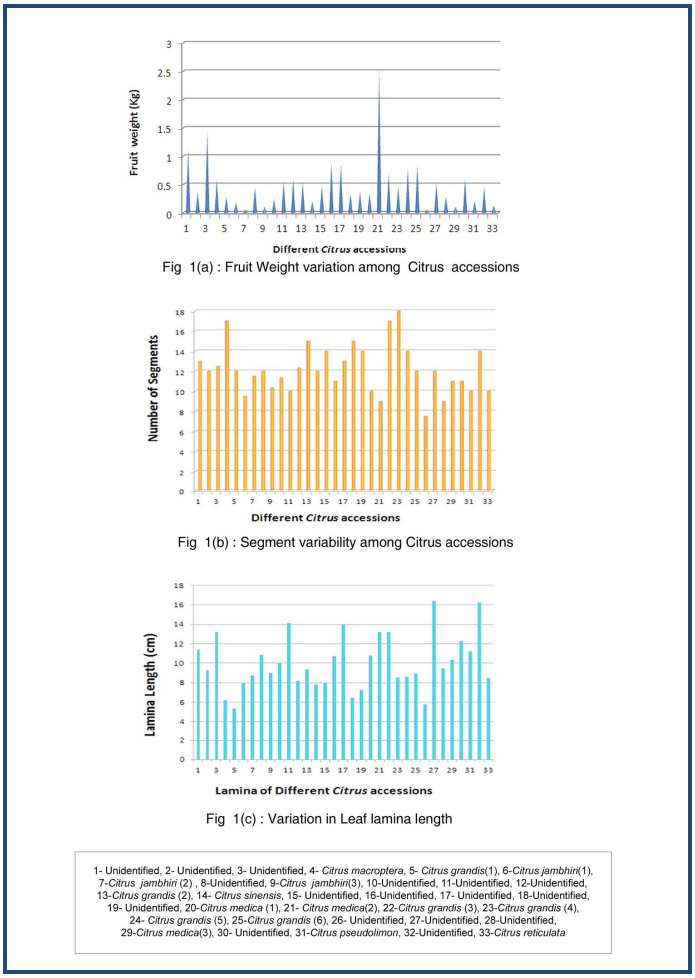

Figure 1: (A) Fruit weight variation among *Citrus* accessions**; (B)** Segment variability among *Citrus* accessions; **(C)** Variation in leaf lamina length.

In this study, the morphological characters like shape, size can give information about the diverse gene pool of *Citrus* available in the state (**Figure 1 (a, b & c).** The information provided also helps in differentiation between various *Citrus* accessions based on the phenotypes which are highly heritable and equally expressed in all environments. So far, there is limited information available on diverse germplasm of *Citrus* reported from the state.

Database Content

CIBMAN database contains an extensive compilation on *Citrus* biodiversity, which can be visualized and analyzed by graphical web-interface. The database comprises of 33 *Citrus* accessions of which most of them are used for edible and medicinal purposes. Extensive study on evolutionary relationship is under progress to understand the taxonomic

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conundrum retain since long. Further, biochemical and molecular data on gene and gene products will be updated after experimental validation of the data.

Figure 2: Architecture and outline of CIBMAN database

Design and Implementation:

The CIBMAN database was developed by extracting seamlessly integrating data from extensive questionnaire compiled in MS Excel. This data compiled in MySQL 5.1.41 (www.mysql.com) relational database along with cross references to taxonomy, morphology, gallery, references and links. **Figure 2** elaborates the architecture and outline of CIBMAN database. The MySQL database was normalized and indexed to ensure efficient and accurate data retrieval through the query option available in CIBMAN web interface. At present search by common name, scientific name and advance options are available to the users. The regular updates to the CIBMAN database to ensure that its' contents are up to date with periodic update in experimental and biochemical study.

The CIBMAN web interface developed in Apache 2.0 Handler CGI 1.1 (www.apache.org) runs on the Windows 2003 web server and utilizes the MySQL (XAMPP-win32-1.7.7) module to query and retrieve data from back end MySQL database (www.mysql.com). The graphical display of snaps was implemented with JAVA Script 1.6 programming (http://www.javascriptsource.com). The Google map API developer implemented for graphical representation of the distribution with respect to the GPS coordinates highlighting the specific site of sample collection.

Future Development:

The gene pool of *Citrus* requires systematic approach for its collection, conservation and characterization and its availability in the future. Large gene pool of wild relatives of cultivated *Citrus* crops is also potential source of important traits to face the unforeseen changes in climate and any further soil degradation, the sudden appearance of insect and virulent forms of pathogens. There is necessity of screening for any *Citrus* species having tolerance to pathogens, higher content of economically useful compounds and valuable horticultural traits. By incorporating such cultivars having valuable traits in somatic hybridization programs, crop genetic improvement of existing *Citrus* rootstocks and cultivars of higher bioactive compound with desired quality could be achieved **[17].** The rich *Citrus* germplasm can also be exploited for finding new antibiotic compounds that might be useful as drug for treatment of multi drug resistant pathogens that become resistant to existing antibiotic compounds. Diverse forms of *Citrus* available but most of them underutilized can give us some insight on potential *Citrus* based industry in the state of Manipur. It is the high time to establish field gene bank at different sites as revival policy and thereafter proper care needs to be taken for maintenance and better conservation of the *Citrus* diversity. To reduce the risk of germplasm loss, in addition to field gene banks, in *vitro* gene banks and cryopreservation techniques have to be developed **[12,16].**

Acknowledgement:

Authors are thankful to Department of Biotechnology (DBT), Govt. of India, New Delhi for providing financial support and working facilities in the Medicinal Plants and Horticultural Resources Division and Bioresources Database and Bioinformatics Division, Institute of Bioresources and Sustainable Development, Imphal.

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Edited by P Kangueane
Citation: Sanabam *et al.* Bioinformation 8(17): 838-840 (2012)
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