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EXPLORATION, COLLECTION AND CHARACTERIZATION OF CITRUS GERMPLASM - A REVIEW

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ABSTRACT

A large number of citrus species/progenitors of commercial citrus fruits are believed to have originated in India. Availability and wide gene pool in the form of genetic diversity is a prerequisite for crop improvement. Genetic diversity is the extent of genetic variability's among the individual in a single species and between the species. The diversity within a species needs to be preserved for improvement programme. Assessment of genetic vulnerability of any citrus species requires knowledge of the extent and distribution of genetic diversity. However, the number of citrus accessions worldwide are listed to be 6000 and in India 1495 inclusive of wild species, rootstock old cultivars, advanced cultivars, and breeding lines. In India collection and conservation of citrus species/types started long back. However, in mid of nineteen century it received major emphasis. In yesteryears, collection and conservation were primarily made for the quality fruits. While, the current research efforts are addressed to collection of gene pool with distinct desirable traits, which can be utilized for improvement of cultivars. Total 7 citrus species in India are reported as endangered citrus species. They needs special attention for future use.

Citrus in India is grown in 0.48 million ha area with a total production of 4.27 million tones countrywide basis, the most important commercial citrus cultivars in India are the mandarin (*Citrus reticulata* Blanco), followed by sweet orange (*Citrus sinensis* Osbeck) and acid lime (*Citrus aurantifolia* Swingle) sharing 41, 23, and 23 per cent, respectively, out of total production of all citrus fruits. Commercially, Kinnow mandarin is grown in Punjab, Haryana, Himachal Pradesh, western part of Rajasthan and Uttar Pradesh; Khasi mandarin in north-eastern region comprising states like Assam, Mizoram, Meghalaya, Manipur, Nagaland, Arunachal Pradesh; Darjeeling mandarin in Darjeeling; Coorg mandarin in Coorg area; Acid lime in Kheda district of Gujarat, Akola in Maharashtra, Periyakulam in Tamil Nadu; Nagpur mandarin in Vidarbha region (4 principal districts *viz.*, Aurangabad, Jalna, Parbhani and Nanded) of Maharashra and adjoining areas of Madhya Pradesh; Mosambi in Marathwada region of Maharashtra; Sathgudi in Andhra Pradesh (Triupati, Anantpur).

Citrus genetic diversity in India

In India, number of commercial citrus cultivars have shown their worth (Table 1). Citrus spp. L. (Rutaceae) and its relatives are claimed to have originated in south or south east Asia, the main centre being in eastern India. A vast reservoir of citrus diversity exists in this region in both wild and cultivated forms, but the erosion of these genetic resources is a cause of concern (Chadha, 1995; Singh *et al.*, 2001). North-eastern region, paradise of genetic diversity is also recognised globally as an area of mega-biodiversity. It is considered natural home of many citrus species. At the same time, ironically this region is also highlighted by past citrus explorers as hot spot area with regard to threat to existing valuable biodiversity. It warrants an urgent step for collection and conservation of the diverse flora to put check on otherwise continuing genetic erosion. Tanaka (1958) believed that sweet oranges originated in India in addition to many other citrus types. It is reported that some of the indigenous and wild mandarin types are found in south India, where as Hill

Table 1. Commercial citrus fruits in India

Species	Cultivars	Distribution
Mandarin (*Citrusreticulata* Blanco)	Nagpur mandarin, Khasi mandarin, Coorg mandarin, Kamala orange, Darjeeling mandarin and Kinnow	Maharashtra, Karnataka, Tamil Nadu, Madhya Pradesh, Punjab, Rajasthan, North-Eastern Region, West Bengal
Sweet orange (*C. sinensis* Osbeck)	Mosambi, Sathgudi, Blood Red Malta, Hamlin Sweet, Pineapple, Valencia and Jaffa	Deccan Plateau, Punjab, Andhra Pradesh, Tamil Nadu
Acid lime (*C. aurantifolia* Swingle)	Kagzi lime, Vikram, Pramalini, Jai Devi, Sai Sharbati, Baramasi Kagzi, Seedless lime (*C. latifolia* Tan.)	Andhra Pradesh, Maharashtra, Gujrat, Bihar, Karnataka, Tamil Nadu
Lemon (*C. limon* Burm.f.)	Baramasia, Assam lemon, Pant lemon, Gandhraj, Seville, Nepalli Oblong, Nepalli lemon, Italian, Eureka	All over the country in Homostead gardens
Grapefruit (*C. paradisi* Macf.)	Duncan, Marsh seedless, Ruby Red, Saharanpur special	Limited in cultivation
Pummelo	Chakotra, Gagar, Local selections (Red fleshed and white fleshed)	In home gardens
Gajanimma (*C. pennivesiculata* Tan.)	Gajanimma, Baduvapuli	Home gardens in South India
Belladikithuli (*C. maderaspatana* Tan.)	Valadipudi, Kichli, Belladikithuli	Commercial orchards in Guntur, Andhra Pradesh

lemon (Galgal) *C. pseudolimon* and Attani (*C. rugulosa*) are prevalant in the foot Hills of Himalayas in the north-west part of the country (Verma *et al.*, 1999; Singh and Singh, 2001). According to Singh (1981), NEH region and parts of north western India is considered as the best locations for collecting primitive germplasm of citrus (Singh, 1981). A large number of citrus species/progenitors of commercial citrus fruits are believed to have originated in India (Bhattacharya and Dutta, 1951, 1956; Dutta, 1958; Singh, 1967; Singh and Singh 1967, 1968 and Singh, 1977). Many of these species are wild (Bhattacharya and Dutta, 1956). As early as 1950 Vavilov reported the occurrence of sweet orange (*C. sinensis* Osbeck), mandarin (*C. reticulata* Blanco), citron (*C. medica* L.), sour lime (*C. aurantifolia* Blanco), Jenerutenga (*C. nobilis* lour), Rangpur lime (*C. limonia* Osbeck) and lemon (*C. limon* Burrm.) both in cultivated and wild form in the NEH region of India. The wide distribution of "Soh Nairiang" a wild sweet orange, wild Indian mandarin (*C. indica* Tanaka) (Bhattacharya and Dutta 1951,1956), *C. assamensis, C. ichangensis* Swingle, *C. latipes* Tanaka and *C. microptera* Mont in various parts of NE region of India growing upto an elevation of 2000 M elevation (Ghosh, 1977). This observation gave strong indication that the region may have been the natural home for these species. Later, more recently, Hore *et al.* (1997) and Singh *et al.* (2001) collected several types of citrus from Mizoram and Tripura Hills and Khasi, Jaintia and Garo Hills of Meghalaya in NEH region. Singh and Singh, 2003 reported 7 endangered citrus species (*C. indica* Tanaka, *C. assamensis, C. ichangensis* Swingle, *C. latipes* Tanaka, *C. macroptera* Mont, *C. rugulosa,* and *C. megaloxycarpa*) from India.

Exploration and Collection

In India collection and conservation of citrus species/types started long back, however,

Table 2. Total pool of Citrus germplasms maintained at genebanks of different citrus belts in India

	Site/Location	Number of accessions
1.	N.R.C. for Citrus, Nagpur, Maharashtra	537
2.	ICAR, RC, Umiam, Meghalaya	92
3.	RFRS (PAU) Abohar, Punjab	132
4.	Dr. PDKV, Akola, Maharashtra	18
5.	HRS (RAU) Birouli, Bihar	14
6.	RRS (IIHR) Chethali, Karnataka	126
7.	IIHR, Hessarghatta, Bangalore, Karnataka	76
8.	RFRS (Dr. PDKV), Katol, Maharashtra	33
9.	PAU, Ludhiana	76
10.	HRS (Maharashtra Agric. Univ.), Parbhani, Maharashtra	68
11.	HRS (TNAU), Periyakulam, Tamil Nadu	20
12.	Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra	66
13.	HRRS (YSPUHF), Dhaulkuan, Sirmour, H.P.	11
14.	FRS (Mahatma Phule Krishi Vidyapeeth), Srirampur, Rahuri, M.S.	34
15.	Citrus Research Station, AAU, Tinsukhia, Assam	36
16.	S.V. College of Agriculture, Tirupati, A.P.	115
17.	HRS (TNAU), Yerud, Tamil Nadu	41

Source: AICRP on citrus reports of different years and NRCC reports.

in the middle of nineteenth century it received major emphasis. In early part, collection and conservation were primarily made for the quality fruits, while current research efforts are for collection of gene pool with distinct desirable traits, which can be utilized for improvement of cultivars. *Ex situ* conservation is observed to be the best for citrus, since *in vitro* and cryo conservation in India is still regarded as sophisticated and not in practice for large scale conservation of citrus species. *Ex situ* conservation of citrus genetic resources began in the 1950s in India, but the number of accessions maintained in different field gene bank has declined mainly due to poor maintenance and insect, pest and disease. If you see the passport data of all citrus collection it clearly indicate that most of the earlier accessions of citrus were collected from one or other Institute within the country or imported from out side. Only few accessions were actually collected from field. Even in NRCC before 1999 all material of citrus were collected from different Institute mostly from IIHR, Bangalore, AICRP Tirupati and Abohar and Imported from USA, Australia, Japan, Nepal and France.

Looking the gaps in citrus collection and being a nodal agency for citrus research in country NRCC lead total 12 exploration mission under NATP(PB) and IPGRI/ADB project. A total of 384 *Citrus* collections were collected from different locations representing as many as 14 states *viz.*, Rajasthan, Punjab, H.P., Uttar Pradesh, Uttaranchal, Gujarat, Madhya Pradesh, Andhra Pradesh, Maharashtra, West Bengal, Meghalaya, Assam, Arunachal Pradesh and Sikkim (Singh and Singh, 2003). These collected materials were added to the pool of National gene bank. The germplasm of rootstocks and other wild and semi-wild nature were collected as seeds and currently being maintained in the field repository. On the other hand, the germplasm of scion cultivars were collected as budwood and being maintained under screenhouse to check for introduction of new viruses and virus like diseases. After indexing, these materials were planted in the field gene bank for onward evaluation. Distribution pattern of number of accessions collected from different states indicate highest accessions from Meghalaya followed by Uttaranchal confirming earlier reports of many workers (Bhattacharya and Dutta, 1951, 1956); Dutta (1958); Singh

(1967); Singh and Singh 1967, 1968), Singh (1977), Singh and Chadha (1993), Singh and Singh (2001), Singh (1999) and Singh *et al.* (2001) that maximum diversity exists in these region only.

Exploration and collection of citrus genetic diversity in North West India

Most of the citrus genetic diversity in northwest part of India comprising states like Rajasthan, H.P., U.P, Punjab and Uttaranchal are by and large maintained in home garden by farmers, except commercial cultivars namely Kinnow mandarin and sweet orange. Citrus germplasm collected in these areas are maintained at Fruit Research Station, PAU, Abohar Punjab, Horticultural Research Station, YSPUHF Dhaulakuan, H.P., GBPUAT, Pantnagar, Uttaranchal and NBPGR regional Station, Bhowali, Nainital, Uttaranchal In these areas, Kinnow mandarin is grown commercially, whereas sweet oranges and lemons are confined to a limited extent. In the foothills of western Himalaya hill, lemon locally known as galgal (*C. pseudolimon*) and Attanni, Chawanni (*C. regulosa*)) are commonly seen. Beside this, Malta type sweet orange (*C. sinensis*) was observed to grow at comparatively higher altitude in Uttaranchal. Locations like Poanta valley (Sirmour), Pathankot area of H.P., Doon valley, Nainital, Chamoli and Garhwal area of Uttaranchal have shown best genetic diversity of citrus. Poanta valley of H.P. and Doon valley of Uttaranchal are well known for genetic diversity of Purmelo and Galgal. Different types of Purmelo are maintained in home garden by the farmers. Pathankot area of H.P. is famous for local mandarin (Butwal) cultivation. Besides mandarin many rough lemon, strains were found to grow in the region. Seeds of rough lemon are even sold to Maharashtra for raising the large scale nursery. NBPGR regional station, Bhowali, Nainital surveyed Uttaranchal area and collected 54 accessions of citrus. Lower intensity of other citrus species was observed except *Citrus medica* group and galgal. Presence of Jambhiri, Jambhira, Khatta, Chukh, Madkakaree of Almora showed possible variability of citrus belonging to different groups according to Verma *et al.* (1999). Wild relatives of citrus spp known as *Glycomis pentophylla* was reported from Bajpur Khatima and from Majhere area of Nainital district of Uttaranchal (Verma *et al.*, 1999). In Sub-Himalayas tracts/ foothills of North-west India primitive types of citrus species commoly known as galgal and Attanni were reported to grow and rich diversity of citrus species were observed in the region. Latter in the year 1999-2001 NRCC lead three exploration mission in collaboration to NBPGR regional station, Bhowali, Nainital. In first exploration tour Sriganganagar (Rajasthan), Abohar (Punjab) Majra, Jagadpur, Dhaulakuan, Paonta valley (Sirmour, H.P.), Lakhanwala, Dhakarani, Kalsi ,Johri, Kalsi Bazar, Selakui, Bhaikhals, Jhajra and Hedwala (Dehradun, U.P.) Biharigarh, K. baag, Alsi baag, Rampur (Saharanpur, U. P.) were surveyed. Total 57 accessions were collected from these areas. The second exploration was concentrated in locations like Kabirpur, Ramnagar, Chiraigaon (Varanasi), Saidpur (Gazipur), Lalganj, Ranikisarai (Azamgarh), Jaunpur, Sihipur, Saidanpur (Jaunpur), Vindhyachal (Mirzapur), Chopan (Sonbhadra),Malihabad (Lucknow), and Jahanabad (Fatehpur, U.P.), Dogaon , Kainchi , Latibunga, Soneda, Bagar, Bajoon,Ghatgarh, Okhalkanda ,Jhargaon, Karail, Hadiya, Bhimtal (Nainital, Uttaranchal) and 43 accessions were collected.

During the exploration, two new types of citrus (probable hybrids) from Nanital area of Uttaranchal were brought to notice for the first time. It seems to be a cross between Malta and Pummelo. One potentially endangered citrus species (Athanni) was sighted growing at an height of 1465 m altitude Khutani to Dhari

road in Hadiya village, Bhimtal. Before 1992, this area was full of wild species, but today a majority of them have wiped out primarily because of deterioration in their natural habitat over a period of time, and becked up low preferences by the farmers owing to their low economic utility. However a wide range of variability was observed with respect to bearing habit, fruit shape and thorniness.

Third exploration was undertaken in Kumaon and Garhwal regions of Uttaranchal. A total of 28 accessions were collected from the places like Chamoli, Bageshwar, Almora, Champawat and Pithoragarh area of Uttaranchal states. These accessions of citrus germplasm belong to different citrus groups (mandarin, rough lemon, sweet orange and acid lime) and species comprising *C. pseudolimon* (2), *C. jambhiri* (9), *C. limonia* (2), *C. karna* (2), *C. reticulata* (3), *C. rugulosa* (2), *C. limettioides* (1), *C. sinensis* (1), *C. medica* (2), *C. grandis* (1), *C. limon* (1), *C. aurantifolia* (1) Mandarins are grown on commercial scale as rainfed crop in Pithoragarh. However, due to less rainfall during last few years in this area, farmers were forced to forgo this crop. This resulted in most of the mandarin plants started showing decline symptoms due to general neglect.

Mandal area near Gopeshwer in Chamoli is one of the potential areas for Malta cultivation. This material can be used for large scale nursery raising. Number of fruits per plant ranged from 800 to 2000 fruits. Fruit quality of Malta is quite good besides being very juicy. Malta and Galgal are grown on a large scale in this area. However, these two valuable cultivars are not finding proper preference by the farmers due to poor marketing facilities.

The exploration showed that the Nanital, Chamoli, Bageshwar, Almora, Champawat and Pithoragarh areas of Uttaranchal has tremendous diversity of rare and cultivated citrus species, but need to be conserved. Some policies are also needed to maintain them in form of field gene bank by providing the same natural habitat so that the diversity is not lost over the period of time, either because of change in climate or farmer's low preferences. Such materials would continue to be a valuable breeding materials for citrus researchers.

Exploration and collection of citrus genetic diversity in West India

In west India not much citrus diversity is reported except in acid lime. Exploration tour were conducted by NRCC from 14.8.2002 to 21.8.2002 to collect citrus germplasms from the citrus growing areas of Gujarat under NATP (PB) project. Total of 13 collections were made from the location like Kheda, Anand, Mehsana, Gandhinagar and Ahmadabad area of Gujarat. During the exploration, areas such as Kudsan, Raisan, Mansa, Lodhra in Mansa and Gandhinagar taluka of district Ghandhinagar; Nadiad, Vaso, Dabhan, Narsanda, Uttarsanda in Nadiad taluka of Kheda district; Vijapur, Pillvai, Chilur in Mehsana district and Napa, Umreth, Chilodiya, Bhogali, Bhaksol. Bhalej, Anand, in Borsad and Anand Taluka of district Anand and Hirapur, Taskohi and Ahmadabad in Ahmadabad district were visited. The habitat covered plain farmers field from latitudinal variation 115 - 135 m asl. Various material were collected in form of fruits. Samples were also collected from various sites differing in various physiographic land features. It was observed that acid lime cultivation has reduced in many places primarily due to inadequate rainfall. Kheda district of Gujarat once had highest area of acid lime cultivation but now farmers have lost their interest in acid lime cultivation since no crop regulation practice is adopted and resultant orchards give fruits in rainy season only when fruits are lowest in demand.

A large variability was observed within

and between the acid lime orchards during the exploration. Variability was observed in fruit size, tree vigour, and tolerance to canker under field conditions. Canker caused by *Xanthomonas citri* was observed not a constraint in the production of acid lime in these areas. However, *Phytopthora* infection was observed in few plants in field. Therefore, every efforts should be taken to control these diseases otherwise it will lead to infection to other plants.

The exploration indicated that Gujarat area has diversity of acid lime only. Therefore, exploration in this area need to be restricted for funding citrus diversity in acid lime only since not much citrus diversity was observed in other citrus species. Sustainence in acid lime production was very much evident from the production of good quality of acid lime fruits in this area without much problem of insect pests and diseases.

Exploration and collection of citrus genetic diversity in NEH Region

Northeast region of India is considered to be the natural home of many citrus species (Ghosh, 1977; Gupta and Yadav, 1999) and it is reservoir of various citrus species including mandarin orange (Tanaka, 1958; Bhattacharya and Dutta, 1956). Favourable climatic conditions aiding in easy hybridization amongst different species and genera has brought in about numerous forms growing wild and semi-wild in NEH Region (Hore *et al.*, 1997; Singh *et al.*, 2001). This region had remained isolated for a long time. Even today, the accessibility is rather poor in many parts of the region. In this region systemetic exploration and collection programme started during 1950 (s) and total 56 accessions were collected and planted at Burnihat (Assam) by Bhattacharya and Dutta (1956) from NEH Region. However all accessions were lost with time on due to poor maintenance at site. Letter on in the year 1980-1995, ICAR Research complex for NEH region, Barapani Meghalaya collected 92 citrus accessions mostly from NEH region and planted in field gene bank. But condition of this filed gene bank is also detericting. Basides this few accessions are maintained at AICRP, Tinsukia, Assam (Assam Ag., University). Citrus is almost universally present in various forms in the entire North eastern region of India. It has also been found to grow wild in Garo hills and Siang district of Arunachal Pradesh. Diverse forms of pummelo have been frequently observed growing in lower hills of Assam, Meghalaya and Tripura (Borthakur, 1993).

According to Karl (1981), there are 17 citrus species with 52 varieties/types of citrus occurring in NEH region and most of them are diploid having chromosome number 2N=18. Many edible species like *C. indica, C. ichangensis, C macroptera, C. latipes* are found to growing wild and semi-wild in the region. Beside this, different strains of citron (*C. medica*), sour pummelo (*C. megeloxycarpa*), rough lemon (*C. jambhiri*) and sour orange (*C. aurantium*) are also reported to grow in semi-wild condition in NEH region (Verma and Ghosh, 1979). Presence of 3 wild types of sweet orange (*C. sinensis*), namely Soh bitara, Soh nairiang and Tasi in Meghalaya and Arunachal Pradesh, respectively, provided a strong evidence that most of the citrus species originated in this region and later disseminated to otherparts of the world, claimed to be the epicentre of citrus production.

However, only two types of citrus are commercially grown in the region. Among the mandarin orange group, the Khasi mandarin covers the largest area in the region due to its quality of commercial values. Another variety Assam lemon, a seedless lemon, developed from a chance seedling is grown in sub-mountane tracts. Hore *et al.* (1997) surveyed Mizoram and Tripura and found maximum occurrence of diversity in the species *C. reticulata, C. limonia* and *C. grandis* in western parts of Aizawl district, Mizoram and Jampui

hills area as well as north Tripura bordering Mizoram. A rare species, *Citrus megaloxycarpa* was found to be confined n the Jampui hills of Tripura.

It was observed that abundance of the various species is location specific like *C. latipes* was found growing around Shillong only, *C. macroptera* was concentrated in Shella, Dawki area of Meghalaya and in Manipur and Mizoram. Tasi, a semi-wild sweet orange type, was concentrated in Basar area of Arunachal Pradesh. Singh *et al.* (2001) reported that Charapunji and Shella areas of east Khasi hills and Jowai area of Jaintia hill were potential areas for Khasi mandarin. They also reported about the number of endangered citrus species like *C. latipes, C. assamensis and C. megaloxycarpa* from the Muktapur area of Jaintia hills of Meghalaya. The other rare species of citrus, namely, *Citrus macroptera* and *C. latipes* were reported to be growing in semi-wild form at an altitude below 500 m and above 900m, respectively. Area bordering Bangladesh have greater variability in pummelo, Satkara and citron, while Kamrup district of Assam has different types of Assam lemon and pummelo.

During the year 2000 to 2002 NRCC lead 4 exploration tours to collect citrus genetic diversity from the region. First tour was carried out in Dawki (Jiantia Hills), Upper Shillong (East Khasi Hills), Barapani and Umroi (Ribhoi) areas of Meghalaya to collect citrus genetic diversity. A wide range of variability was observed in plant and fruit characters. During the exploration, it was observed that *C. latipes* was concentrated in and around Shillong only and maximum concentration was observed near upper shillong at the height of 1700 m. However, Satkara and Pummelo confined growing to lower altitude of less than 500 m. These areas are very rich in different citrus species, particularly endangered citrus species. Total of 16 accessions mostly endangered and threatened citrus types were collected, these materials are maintained in the screen house. After indexing, virus free materials were transplanted in field for their further characterisation.

Second exploration tour was undertaken in collaboration with NBPGR, Regional Station, Umiam Meghalaya. A large number of promient citrus growing areas were explored which consisted of Laityrra, Shella, Umray, Mawlanga,Walang, Laikor, Mowlai, Shillong (East Khasi Hills), Pomusa, Marsham, Pynther, Amlarem, Poengshakap,Umlari, Muktapur, Dawki, Sohlong, Sohlong (Jaintia Hills), Umbang, Umtrew, Umsening, Byrwa, Umroi Umrai, Nongrah, Khaprama, Umeit (Ribhoi, Meghalaya), Phulkibari, Rajapara (Kamrup, Assam), Ganolaphal, Chandigre, Darichickgre, Dalu, Barongapara (West Garo Hills), Gasuapara, Baghmara, Siju, Rongpeng, Karkula dingre, Karkula dingre, Maheshkhola, (South Garo Hills) Ghumaghat, Ranikor (West Khasi Hills) to collect the citrus germplasm.

A total of 79 collections were gathered from these area. During exploration natural diversity of citrus species was observed in Garo Hills of Meghalaya. These are considered to have originated by natural cross pollination. Many probable hybrids have been reported from time to time from this region (Dutta and Bhattacharya, 1956). During exploration 3 new types of citrus (probable hybrids) from near by Nokrek Biosphere reserve area of Meghalaya were identified and collected. It was extremely encouraging to see a wide range of variability. A good variation was found in terms of features, fruit weight, number of segments per fruit, fruit length, fruit width, number of seeds per fruit and rind thickness. Fruit colour was observed from yellow to reddish-deep yellow and greenish yellow. Other characters like bearing habit, fruit shape and thorniness habit showed large variability, which could be of immense utility, while shortlisting

them to activate germplasm repository.

Cherapunjee and Shella area of East Khasi Hills and Jowai area of Jaintia Hills are the potential areas for Khasi mandarin and other elite citrus species. However due to excessive rain coupled with indescriminate deforestion, these areas have turned into a wet wastland except Dawki and Muktapur areas of Jaintia Hills. Different types of citrus were collected from Muktapur area. From these areas 3 endangered citrus species *viz.*, *C. latipes, C. assamensis and C. megaloxycarpa* were collected. It was observed that *C. latipes* grew only at higher at an altitude above 900 m above msl. It is concentrated only in and around Shillong area. In contrast to this, Satkara (*C. macroptera)* was observed to growing only at lower altitude of less than 500 m in semi-wild form. It has good market value even better than Khasi mandarin. Areas bordering Bangladesh have greater variability for pummelo, Satkara and citron.

Garo Hills of Meghalaya is also one of the promising potential areas for locating citrus genetic diversity in its best form. Citrus gene sanctuary which is located in this area, is well protected and plants of different citrus species looked better in terms of growth with less infection of pests and diseases. Nokrek Biosphere Reserve has maximum genetic diversity of citrus. Three types of Memon Narang (*C. indica*) fruits from biosphere reserve area were collected. Variability was observed with respect to fruit size, colour and shape. Three new types, as probable hybrids were collected from this area. Siju and Baghmara area of West Garo Hills are another important areas from the point of view of locating genetic diversity. These areas are rich for pummelo, Khasi mandarin, Sharbati lime, Citron and Chinara. Areas bordering Bangladesh is famous for pummelo and Sharbati lime.

Third explorations was conducted in West Garo hills of Meghalaya, Kamrup, Golpara, Nagaon, Sunitpur, Lakhimpur, Dibrugarh, Jorhat, Golaghat area of Assam and West Siang District of Arunachal Pradesh to collect citrus germplasm from the North Eastern Hill region of India under IPGRI project funded by ADB. A total of 40 accessions of citrus germplasm belonging to different citrus groups viz., mandarin, rough lemon, sweet orange, citron, pummelo and acid lime and species *viz.*, *C. pseudolimon* (1), *C. jambhiri* (5), *C. karna* (2), *C. reticulata* (2), *C. macroptera* (1), *C. sinensis* (5), *C. medica* (5), *C. grandis* (10), *C. limon* (2), *C. aurantifolia* (1), *C. indica* (1), *Citrange* (1), *Poncirus trifoliata* (1) and New types (3) were collected representing diverse parts of NEH region. The Indian wild orange (*C. indica*) locally known as Memon Narang are well protected in citrus gene sanctuary. One of the exclusive merits of this species is that it is heavily liked by the local tribal people. Every family wants to keep at least one plant in their home garden. The fruits of this species are sold in market @ Rs. 5-10 per fruit against the commercial Khasi mandarin @ 1-2 Rs. per fruit. However, regeneration in Citrus Gene Sanctuary was observed to be very slow. The reason may be slow seed germination and their survival in natural condition. Citrus plants in reserve forest were observed to be very vigorous and healthy with no insect pest infestation. Beside citrus, mango and banana germplasm were also recorded in reserve forest. The mission has shown that the Tura area of Meghalaya and Basar area of Arunachal Pradesh has tremendous diversity of rare, wild and cultivated citrus species.

Basar area of Arunachal Pradesh is another area rich in citrus gene. Two indigenous sweet orange materials namely Tasi and Soh Nairiang were collected from this area. Besides these, one citron known as Tayum, largely cultivated by farmers was also collected. Tayum and Tasi fruits are loved by Adi tribes in these areas. The other citrus species in this

area have no commercial value and, therefore, are in danger of being lost or degenerated mainly due to farmers preference.

Fourth exploration was carried out in two main citrus growing belts of the country, where famous Sikkim mandarin and Darjeeling mandarin are grown on commercial scale. In Sikkim West, South and East Sikkim districts were covered. Whereas in case of West Bengal, Kalimpong, Mirik, Darjeeling and Bijanbbari areas of Darjeeling district were explored. A total of 12 accessions of citrus belonging to different citrus species were collected and brought for further regeneration at Nagpur.

In Darjeeling, best quality of mandarin is produced in area like Kalimpong and Mirik. These areas were observed as best area for production of high quality mandarin fruits. Besides mandarin, the other species like rough lemon, Rangpur lime and citron were observed growing in middle hills of Darjeeling, but without much useful variability.

Exploration and collection of citrus genetic diversity in Central India

Citrus genetic diversity in central India is maintained mostly by farmers as on farm conservation. In this region, all the three commercial citrus crops are grown in different pockets. Beside this rough lemon, Rangpur lime, sweet lime and lemon are also grown and because of demand, are maintained by farmers on their farms. In Central India four main field gene banks are established, one at NRCC, Nagpur, MPKV, Rahuri, Ahmednagar, Dr. PDKV, Akola and Katol. In all gene banks most of the accessions collected are from different Institutes. Details of gerpmplasm maintained in these gene bank were reported by Singh *et al.* (1999) and Pujari *et al.* (1999). Famous Nagpur mandarin is grown in Vidarbha region of Maharashtra and adjoining area of M.P. This commercial cultivar was first introduced in central India in the 18th century by Bhosale rulers. Most well known belt of Nagpur mandarin cultivation is the foot hill region of Satpura in central India from Jalgaon (MS) to Pandhurna (M.P.) However due to water shortage number of orchards have started drying up in Pandhurna area (MP) and Warud, Narkhed area (M.S.) in the year 2002. This has given great potential threat to existing genetic diversity in the region in this famous citrus belt of the country. Nagpur mandarin commercially grown in Warud, Morshi, (Amravati), Katol, Narkhed, Ramtek, Umred (Nagpur), Arvi, Karnaja (Wardha), Dhigras, Darwha (Yevatmal) Achalpur- Chandur Bazar (Amravati). Nagpur mandarin in this region is propagated by budding therefore less variability was observed in Nagpur mandarin.

Mosambi (*Citrus sinensis*) genetic diversity is also maintained by farmers in their orchards. Large variability was observed in farmers field. Acid lime is mainly grown in Akola, Nanded, Srigonda and Parbhani areas of Maharashtra. Variability in acid lime fruit size was observed since it is propagated by seed only. National Research Centre for Citrus Nagpur in its massive hunt for identifying superior clones, selected many clones of acid lime and Mosambi from existing gene pool.

In these areas NRCC leads three exploration tours first exploration commenced from 6th August and a total of 33 collections were made from Balapur area of Akola, Paithan, Gangapur and Khultabad area of Aurangabad, Badnapur and Ambad area of Jalna, Rahuri and Shrigonda area of Ahmednagar and Shirur area of Pune.

In second exploration a total of 19 collections were made representing the area like Amravati, Washim, Hingoli, Nanded, Beed, Jalna, Aurangabad and Ahmednagar area of Maharashtra. The sites were characterized by land features from plain land of farmers field to home garden.

Third exploration was undertaken in

Bundelkhand region from 4.8.2001 to 9.8.2001 in collaboration with NBPGR, New Delhi for collection of citrus germplasms. Areas explored consisted of : Kolva, Baruasagar, Babbina, Khajuraha, Mauranipur, Narayanbaag (Jhansi, U.P.) Niwadi, Nayakheda (Tikamgarh, M.P.) and Chhatarpur, (Chhatarpur, M.P.) Total 25 collections were gathered from these areas.

Exploration and collection of citrus genetic diversity in South India

Southern region includes, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. In south India, acid lime (*C. aurantifolia*.) is commercially grown in areas like Nellore, Gudur area of AP and Periyakulam area of Tamil Nadu. Whereas famous Coorg mandarin is grown in Coorg area of Karnataka. Sweet orange (*Citrus sinensis*.) cultivar, Sathgudi is famous in Anantpur area of A.P. All above genetic resources are maintained by farmers farm conservation. Other commercially important indigenous types include Gajanimma (*C. pennivesculata*, Tan), Kichli (*C. maderaspatana* Tan) and some wild mandarin types *viz*., Kodaithuli, Billikichili, Nakoor lemon, Mole Puli (sour orange type) are found in the area. In these areas most of the materials are maintained in three main field gene bank AICRP, Tirupati and IIHR, Bangalore and its regional station Chethalli. IIHR, Chethalli had maximum exotic collection. In the year 2002 NRCC made 19 collections from Tirupati, Chittoor and Nellore areas of Andhra Pradesh and reported the variability in collected material.

Characterisation of collected germplasm

Although most of the citrus species are indigenous to India, the information on characterization of citrus germplasm is lacking in country. Most of the information is available on phenotypic descriptions only. More detailed evaluation and characterization data are needed to assess the genetic diversity present. A wide range of variability was reported in citrus with respect to tree structure/canopy development shape of canopy, features of fruits, diseases and insect resistance as well as their utility as rootstock. Apart from variation in fruit size, colour also varied from yellow green to red orange in lime and shape from oblate to pyriform. At maturity, fruits of some cultivars were observed to have high acidity while others had almost none. Tree size also varied considerably and hence offers breeders the possibility of breeding them for various desirable characters. During the year 1999 to 2003 NRCC collected 384 accessions of citrus from different parts of country and most of them were characterized based on physico-chemical characters of fruits (Singh and Singh, 2003).

The evaluation of citrus germplasm comprising of 15 species and 8 hybrids maintained at ICAR Research Complex, Barapani farm indicated a wide range of variability in growth and physico-chemical characters within the different species and types (Sheo Govind and Singh, 2002). Considering the physico-chemical evaluation of fruits certain land races like Soh bitara and Kinnow mandarin in sweet orange and mandarin group, lemon mayer and galgal and citragequate can be exploited for commercial scale under mid hills altitude. The fruits of other species which have no commercial value as edible fruit can be utilized for the extraction of oil, citric acid as well as for manufacturing of pectin and in citrus improvement program as a parent.

Thirty three citrus types/cultivars belonging to 14 species and 3 probable hybrids indegenous to NEH region were studied at ICAR Research Complex for NEH Region with respect to their physico-chemical composition (Singh and Sheo Govind, 1999). Gandhraj had the maximum fruit weight (1316 g) and size, while lowest fruit weight (16.2 g) and size (2.26 x 3.4 cm) was recorded in Indian wild oranges. Minimum number of seeds were found to be

present in Assam lemon followed by sweet lime sour mutant. Segment ranged from 8.5 to 17 per fruit. The maximum acidity was recorded in Satkara (8.07%) followed by Soh myndung while minimum was in sweet lime (0.23%). Maximum juice per fruit was extracted in white fleshed pummelo. Peel thickness varied from 1.1 mm in Indian wild oranges to 26.8 mm in Gandhraj. A wide range of variation in respect of physico-chemical characters of fruits was observed within different types of fruits of same species and among different species.

Singh and Sheo Govind (2000) reported the shoot growth of 32 different citrus types at citrus germplasm block of ICAR Research complex for NEH region, Barapani. A wide range of variation in the shoot growth with regards to length and diameter of shoot, number and size of leaf as well as total dry matter of shoot was observed. Shoot growth in respect of length (19.43 cm) , diameter, leaf length (12.04 cm) and total dry matter content (2.97 g) of shoot was recorded significantly higher in white fleshed Pummelo (*Citrus grandis*) than all other species/ types of citrus including hybrids. Leaves/shoot (8.6) and distance between two internode was recorded maximum in sweet lime and Tangillo Dancy respectively which was significantly higher to all others. The breadth of leaf was recorded maximum in Karun Jamir as compared to other species/types of citrus.

Singh and Sheo Govind (2002) evaluated nine citrus hybrids which were introduced in the year 1983 to assess their performance under mid hills situations of Umiam, Meghalaya. The maximun height of plant was recorded in Citremon (495cm) while stem girth (55cm) and mean spread of plant was found to be highest in Kinnow and Citrangequate respectively. The vegetative growth in respect of plant height, stem girth as well as mean spread of plant was found to be minimum in Tangello plant. Fruit weight ranged 43.173 g to 165.62g (Citremon and Kara mandarin). Maximum (12.066) number of segment/fruit was recorded in Kinnow. Lowest peel thickness (2.42 mm) was observed in Troyer Citrange. TSS ranged 6.566 to 10.2. The results revealed that among rootstock Citrangequate and Citremon could be performed better as compared to other hybrids so far growth and yield is concerned at mid hills altitude situations of Meghalaya. In scion cultivars Kinnow show superiority over Kara mandarin in term of vegetative growth and yield. Pujari *et al.* (1999) evaluated citrus germplasm at Rahuri (M.S). Out of 140 accessions flowering were recorded only in 90 accessions. They reported rough lemon strains to be more vigorous. Mandarin group, trifoliate orange and its hybrids showed delayed flowering. Singh *et al.* (1999) also reported variability in vegetative growth and flowering in 30 accessions of citrus rootstocks, trifoliate orange and trifoliate hybrids collected from exotic and indigenous sources at Nagpur. Performance of different citrus rootstocks (Awtar Singh *et al.*, 2001) in citrus germplasm repository at NRCC, Nagpur showed large variability particularly in physico- chemical characteristicts of fruits. Verma *et al.* (1999) observed wide range of variability in citrus germplasm collected from Uttaranchal.

CONCLUSION

The present situation of citrus genetic diversity is alarming as enormous destruction in the natural habitat is taking place to fulfill various requirements of man kind. Special drives need to be launched in the remote areas to maintain a parallel repository of germplasm *ex situ,* so that if a diversity is extincted the species will be available for future utilization. At the same time every attempt should be made to conserve the purity of the existing germplasm *in situ.* Since some wild and rare citrus species (like *Citrus indica*) accessions failed to conserve in filed gene bank (author

personal observation). This will protect one alternative to other, if any one out of the two approaches failed. Such effort must be more confined to collection sites where seemingly the primitive types may still be found in an uncontaminated and pristine form. The inaccessible remote area and the forest possess great potential for primitive and wild citrus types, need our immediate attention. The unique area where a few citrus types tolerate adverse conditions (biotic and abiotic), may be thoroughly surveyed and collected as a rare collection for future utilization.

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