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Collection and characterization of *Citrus indica* Tanaka and *C. macroptera* Montr.: wild endangered species of northeastern India

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Abstract

Citrus indica and *C. macroptera* are the wild endangered species of *Citrus* occurring in northeastern India. Surveys were undertaken in this region for ascertaining distribution, studying variability and for collection of germplasm of these two species. *C. indica*, an endemic species of this region, was collected from the Citrus Gene Sanctuary located in buffer zone of Nokrek Biosphere Reserve in the Garo hills of Meghalaya. In addition, a putative natural hybrid of *C. indica* and *C. limon* was collected for the first time from the south Garo hills. *C. macroptera* had much wider distribution and was collected from Mizoram and Meghalaya states. In Jantia hills of Meghalaya, natural populations of this species are in a highly threatened state. The two species were unevenly distributed all over the explored territory. Morphological characterization of leaves, fruits and seeds indicated the presence of sizable variability within collected accessions of these two *Citrus* species. Indigenous technical knowledge gathered on the use and socio-economic importance indicated commercial potential for these two species in northeastern India. However, lack of cultivation of these species and clearing of forest cover at an alarming rate has led to an urgent need to adopt complementary conservation strategies to safeguard these species and to ensure their availability for future utilization. A major emphasis on developing methods for their propagation, multiplication and regeneration in *in situ* and *ex situ* conditions is required.

Introduction

South-east Asia, Australia, the intervening islands between Asia and Australia and central Africa are recognized as important centers of origin of *Citrus* and related genera (Tanaka 1958; Swingle and Reece 1967). In India, northeastern Himalayan region and foothills of the central and western Himalayan tracts are rich sources of *Citrus* genetic diversity. Natural undisturbed populations of *Citrus* genepool observed in northeastern India during collection periods from 1938 to 1948 led to the assumption that this area is center of origin of several *Citrus* species. As many as 17 *Citrus* species, their 52 cultivars and 7 probable natural hybrids are reported to have originated in the northeastern region of India (Bhattacharya and Dutta 1956). A recent study on genetic resources

of *Citrus* in northeastern India indicated the presence of 23 species, 1 subspecies and 68 varieties, thus according this area a special status as a treasure house of *Citrus* germplasm (Sharma et al. 2004).

Collection of *Citrus* genetic diversity from northeastern part of India was taken up intensively by Tanaka (1928, 1937) and Bhattacharya and Dutta (1956) during 3rd to 5th decade of last century. Several new *Citrus* species were identified, collected and documented from northeastern part of India during this period. Since then, no significant information has been added about the occurrence of any additional species of *Citrus* from this region. However, recent surveys and explorations enumerated the loss of some species from various pockets where these were earlier reported to occur (Singh and Singh 2003). Some *Citrus* species are still growing in a wild or semi-wild form in the forestlands. Natural populations of these species are shrinking drastically due to large-scale deforestation to meet the land requirement for cultivation in the Himalayan region (Ahuja 1996).

Citrus indica Tanaka and *C. macroptera* Montr. are two important wild species of northeastern India confined to selected pockets of the region. *C. indica* is supposed to be the most primitive species and perhaps the progenitor of cultivated *Citrus* (Singh 1981). *Citrus indica* was first recognized by Tanaka (1928). Later Tanaka (1937) stated 'It is really a wild citrus found in Nowgong district, Khasi hills and Manipur in Assam'. As no vernacular name of this wild species was available at that time, the name 'Indian wild orange', as it was then called was retained (Bhattacharya and Dutta 1956). This species is endemic to northeastern Himalayas and reported to be occurring wild in Naga hills of Nagaland, Kaziranga Reserve Forest in Assam and Garo hills of Meghalaya (Singh 1981). However, recent surveys conducted in these areas indicated the presence of this species only in the Tura ranges of Garo hills of Meghalaya (Upadhyay and Sundriyal 1998; Singh and Singh 2003). This protected area which falls in the buffer zone of Nokrek Biosphere Reserve has been designated as Citrus Gene Sanctuary (Singh 1981). Due to endemic and localized occurrence of this species in a particular microclimate, efforts for its collection and conservation are required.

Citrus macroptera commonly called as 'Melanesian papeda' was first reported by Father Montrouzier in the Island of Art situated a few miles northwest of the north end of New Caledonia (Swingle 1946). This species is distributed in Thailand, Indochina, Philippines, New Guinea, New Caledonia, Polynesia, etc. and thus it is considered to be native to these regions of southeast Asia (Bhattacharya and Dutta 1956). In northeastern India this species is more widely distributed compared to other endangered species such as *C. indica* and *C. latipes*. *C. macroptera* is reported to grow in the Khasi and Garo hills of Meghalaya, North Cachar, Karimganj and Karbi – Anglong districts of Assam, Mizoram, Tripura and Manipur (Bhatttacharya and Dutta 1956; Sharma et al. 2004).

Vast genetic diversity of wild and semi-wild *Citrus* germplasm of northeastern India has minimally been used for improvement programmes due to lack of their characterization. Major limitation in undertaking characterization of most of the endangered species is insufficient germplasm availability during collections. Sharma et al. (2004) also indicated wide gaps in the knowledge of useful characters of various *Citrus* species and varieties. Systematic efforts for collection, characterization and conservation of genetic variability of *C. indica*, are still lacking, while, preliminary physio-chemical characterization of *C. macroptera* collected from Mizoram and Tripura states has been reported by Hore et al. (1997). The present study is the first attempt to describe detailed distribution, collection, characterization, associated indigenous traditional knowledge, domestication and conservation status of the natural wealth of *C. indica* and *C. macroptera* in northeastern India.

Materials and methods

Two specific exploration missions were undertaken for collection of germplasm of these targeted species during November 2000 and October 2002. During the first trip parts of Assam, Meghalaya and Mizoram were explored while the second exploration concentrated on the buffer zone of Nokrek Biosphere Reserve in Garo hills of Meghalaya, covering east, west and south Garo hills.

General field observations, status of natural populations and indigenous knowledge on

importance and use of the species were recorded during these explorations. Collections were made following selective sampling strategy where sample collected from single plant was given an indigenous collection number (IC number) and treated as individual accession. Fresh fruits and leaves of each accession were collected in the cloth bags. Herbarium specimens and photographs of fruits and leaves were taken, wherever necessary. Unopened flower buds of *C. indica* were collected to extract pollen grains. Morphological characterization of leaves, fruits and seeds was done using descriptors developed for *Citrus* by International Plant Genetic Resources Institute (IPGRI), Rome, Italy. Characterization data of 13 leaf characters and 17 qualitative and 15 quantitative fruit and seed characters was recorded for the collected germplasm. Quantitative data was statistically analysed and mean, range, standard error and coefficient of variation was calculated.

Information on indigenous technical knowledge regarding importance and use of these species was collected by conducting personal interviews and interactions with local people including farmers, forest guards, officials of local government bodies and NGOs. Women and senior citizens were interviewed specifically to collect information on medicinal, religious and culinary uses of these species.

Results and discussion

Collection and characterization

Citrus indica was found distributed in the buffer zone of Nokrek Biosphere Reserve in east, west and south Garo hills of Tura, Willaims Nagar and Bakhmara districts of Meghalaya state (Figure 1). Distribution of *C. indica* was not uniform all over the explored territory; in some instances very small populations with isolated individual trees were observed, while in other cases the populations were large, with a high number of individuals occupying a range of few kilometers. A total of 14 accessions of *Citrus indica* were collected representing variability present in the area (Figure 2a).

Plant type in *C. indica* varied from a small thorny bush to a climber. Most of the plants observed were very robust and thorny without any symptoms of disease and pest (Figure 2b). Plants growing in the Citrus Gene Sanctuary area were observed to be more profuse and prolific bearers than those found outside the sanctuary. Fruits observed were at different maturity stages from dark green immature to greenish yellow, partially mature and deep orange, fully mature (Figure 2a and b). Simultaneous flowering and fruiting in *C. indica* during the collection period facilitated the collection of pollen of this rare and endangered species alongwith the seeds. Collected pollen has been cryopreserved in the cryogenebank of NBPGR for future use.

Flowering period of *C. indica* is from early September to January and fruiting starts from mid October and continues upto February. The quantitative and qualitative data for leaf, fruit and seed characters are presented in Tables 1 and 2, respectively. Mature fruits of *C. indica* were small with length and breadth ranging from 23×29 mm to 38×52 mm. Outer orange coloured fruit surface was smooth and shiny with conspicuous oil glands. Adherence of albedo to pulp was medium with yellowish fruit pulp. Mature fruit had spongy mesocarp and thin rind (Figure 2d). Number of segments varied from 9 to 12 while seeds per fruit ranged from 7 to 12. Spheroid, monoembryonic seeds were creamish in colour, with green cotyledons and brown chalazal spot colour. The variability was found to be maximum for fruit weight (cv = 29.90) and minimum for leaf lamina ratio (cv = 4.0). One of the accessions (IC-417293) was identified as promising based on higher fruit weight, size, density of oil glands and juice content.

During the mission, in the Rongschu area of Siju in Bakhmara district of South Garo hills, a probable natural hybrid showing several qualitative characters resembling *C. indica* and *C. limon* Burm. f. was found growing alongwith *C. indica* trees (Figure 2c). With the exception of leaf size, fruit weight, juice content, seed shape and number of seeds per fruit, all other leaf, fruit and seed characters of this hybrid were similar to that of *C. indica* (Table 1). Hybrid had lesser number of branches and thorns than *C. indica*. In terms of its qualitative and quantitative fruit characters significant dissimilarities were observed on comparison with *C. indica*. Hybrid fruits were about four times heavier while fruit size, juice content and number of seeds per fruit were more than double in the hybrid (Tables 1 and 2). However, it indi-

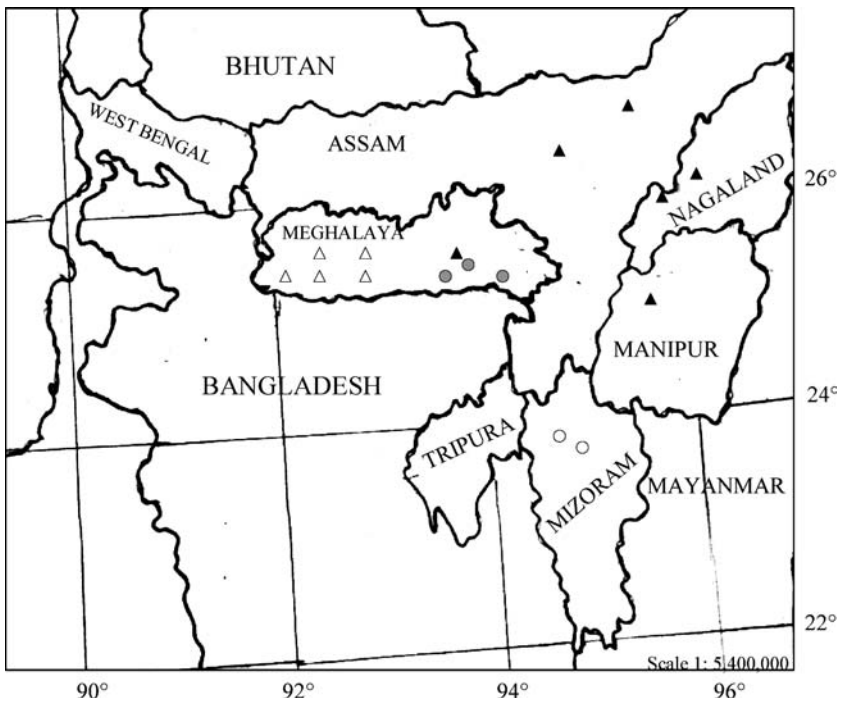

Figure 1. Map indicating collection sites of *Citrus indica* (triangles) and *C. macroptera* (circles) in northeastern India. Open triangles show the present collection sites while closed triangles indicate the sites from where *C. indica* was reported to occur earlier. Shaded circles indicate the sites where *C. macroptera* is presently under threat of extinction.

cated similarity to *C. indica* in terms of fruit shape, seed weight, seed size and shape, moisture content and viability. These similarities indicated that the hybrid might be derived as a result of crossing between *C. indica* and *C. limon* as qualitative and quantitative characters of leaf and fruits dissimilar to *C. indica* were similar to that of *C. limon* (viz. fruit colour, albedo adherence, leaf size, acidity in juice) indicating them to be the putative parents. However, along with morphological characters, molecular markers using techniques such as RAPDs, SSRs, AFLPs, etc. might help in elucidating the putative parents of the probable hybrid.

Large populations of *C. macropetra* were found concentrated in the Sairang area of west Aizawl in Mizoram while Shella valley area showed a sparse distribution where trees were scattered in the forest. Few trees were noticed growing in the homestead gardens of local inhabitants. Dwaki and Jaintia hills of Meghalaya bordering Bangladesh had a lesser occurrence of *C. macroptera* as earlier reported by Singh et al. (2001). However, as evident by the height, growth habitat, tree population and climate, Sairang area of Mizoram apparently was much more suitable for growth of this species. Mature fruits of three accessions of *C. macroptera* were collected from semi-wild conditions as a representative of the total prevalent variability.

C. macroptera was a tall (30–50 ft. in height), evergreen, profusely branched tree with dense foliage and with very sharp and long thorns (5–9 cm). Dark green leaves were longipetiolate with petiole longer than or of the same length as the leaf lamina (Figure 2e). Wide and winged obdeltate petiole was a typical distinguishing characteristic of this *Citrus* species. Fruit was spheroid with rounded apex and bumpy surface. At maturity fruit skin turned yellow with conspicuous oil glands with 52.6 glands per cm^2 (Table 2). Fruit was quite large with fruit length and breadth ranging from 54 to 94 mm and 58 to 105 mm, respectively and fruit weight ranging from 187.5 to 392.0 g. Fruit rind (mesocarp) was thick ranging from 8 to 11 mm and number of segments varied from 13 to 15 (Figure 2f). Fruits were juicy with an average juice content of 94.2 mL per fruit, with high percentage acidity (6.12%). The polyembryonic seeds (2–4 embryos

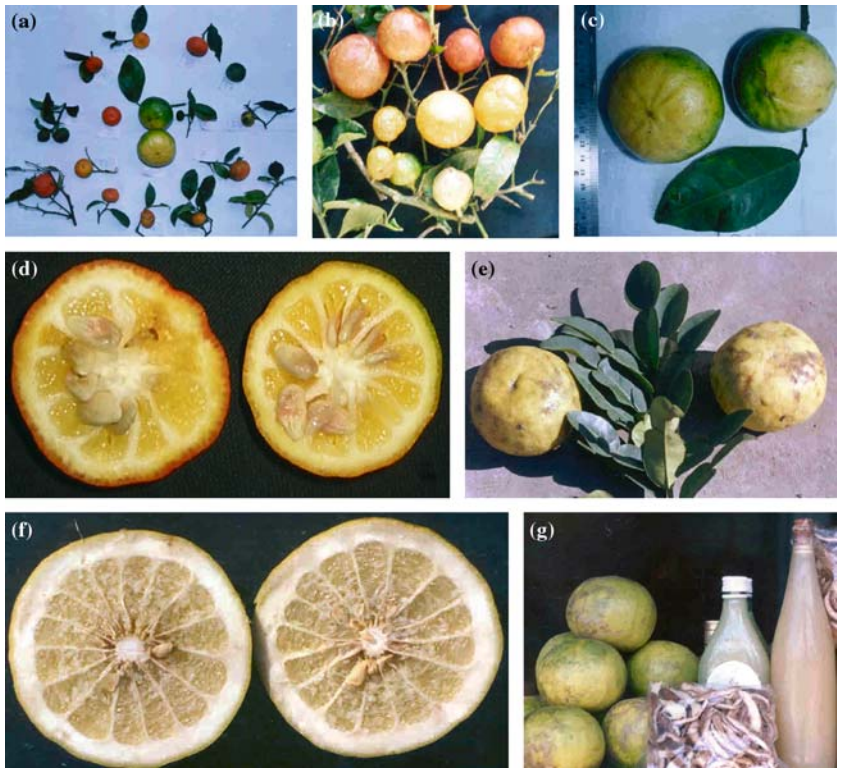

Figure 2. (a) Fruits and leaf variability in *C. indica* collected from Garo hills of Meghalaya; (b) fruits of *C. indica* at various maturity stages attached to hard and thorny branches; (c) fruits and leaf of probable natural hybrid of *C. indica* × *Citrus* sp. collected from Garo hills; (d) internal fruit structure of *C. indica* showing several bold seeds; (e) leaves and fruits of *C. macroptera* collected from Mizoram; (f) internal fruit structure of *C. macroptera*; (g) fresh fruits, bottled juice and dried fruit peels of *C. macroptera* being sold at a village shop in Mizoram.

per seed) varied from 7 to 14 per fruit. Seeds were bold with 10 seed weight ranging from 1.58 to 2.71 g and average length and breadth of 14.4 and 6.4 mm, respectively.

Fresh seeds of *C. indica*, *C. macroptera* and the hybrid showed high moisture content between 26 and 28.73% and high seed viability ranging from 90 to 100%. Studies are underway to determine the seed storage behaviour of these species.

Domestication and indigenous technical knowledge

Domestication of any wild species primarily depends upon the realization of its useful traits and consequently human intervention (Zeven and de Wet 1982). Domestication of plants also depends upon their utility, however, woody plants and fruit trees figure almost last in sequence of crops, which have been domesticated (Burkill 1951–52). Several species of *Citrus* still found in wild and semi-wild conditions in northeastern India (Singh and Singh 2003; Sharma et al. 2004) need proper attention for their identification, collection and domestication. Tanaka (1937) and Bhattacharya and Dutta (1956) reported no cultivation and use of *C. indica*, fruits being small and practically inedible hence holding no promise for commercialization. In the present exploration and collection trip undertaken in the villages near Nokrek Biosphere Reserve buffer zone area indicated the preliminary domestication and acclimatization process of *C. indica* near the Citrus Gene Sanctuary area. Remote villages of Daribogari and Seng Chang have microclimatic conditions similar to that of the Sanctuary, which is probably facilitating domestication of few seedling plants of this species in homestead gardens of farmers living in this area. However, plants of *C. indica* growing outside the buffer zone area showed no flowering and fruiting

Table 1. Leaf, fruit and seed characters of *C. indica*, *C. macroptera* and the probable hybrid.

Characters	*C. indica*				*C. indica* × *Citrus* sp. (probable hybrid)				*C. macroptera*			
	Mean	Range	SE	CV	Mean	Range	SE	CV	Mean	Range	SE	CV
Leaf												
Leaf lamina length (mm)	85.8	65–110	7.32	19.08	97.4	85–112	4.7	10.80	89.7	72–115	7.23	22.50
Leaf lamina width (mm)	40.2	27–53	4.12	23.20	47.2	36–60	3.89	18.43	51.2	39–73	5.10	16.72
Ratio leaf lamina length/width	2.12	2.0–2.22	0.04	4.00	2.08	1.87–2.36	0.08	8.40	1.75	1.6–2.06	0.14	18.45
Fruit												
Fruit weight (gm)	23.92	17.8–34.1	3.19	29.90	99.68	82.2–112.4	5.66	12.71	294.49	187.54–392.0	36.99	28.08
Fruit length (mm)	29.2	23–38	3.04	23.27	60.4	55–65	1.86	6.88	75.6	54–94	7.11	21.05
Fruit breadth (mm)	38.8	29–52	4.28	24.64	69.4	60–75	1.96	6.33	83.20	58–105	6.98	20.32
Density of oil glands on fruit surface (per cm^2)	46	34–54	3.63	17.66	53.6	47–62	3.26	13.62	52.6	42–60	3.09	13.14
Fruit rind (mesocarp) thickness (mm)	2.6	2–3	0.24	21.06	3.2	3–4	0.2	13.75	9.2	8–11	0.58	14.17
Number of segments per fruit	10.6	9–12	0.50	10.75	11.6	11–13	0.4	7.71	14.2	13–15	0.37	5.89
Juice content per fruit (ml)	20.6	15–25	1.80	19.59	56.6	50–65	2.71	10.71	94.2	52–128	12.62	29.98
Acidity (%)	2.33	1.97–2.43	0.15	14.12	3.05	2.5–3.6	0.19	13.88	6.12	5.25–6.8	0.28	10.24
Seed												
Number of seeds per fruit	9	7–12	0.89	22.22	25	16–38	3.59	32.12	9	7–14	1.41	35.14
Number of embryo per seed	1	–	0	0	1	–	0	0	3	2–4	0.31	23.57
10 Seed weight (gm)	1.68	1.45–2.16	0.12	16.88	1.82	1.57–2.34	0.14	17.30	2.19	1.58–2.71	0.20	20.46
Seed length (mm)	10.8	9–13	0.73	15.21	10.4	9–12	0.51	10.96	14.41	12–17	0.81	12.61
Seed breadth (mm)	7.2	6–8	0.37	11.62	7.2	6–8	0.37	11.62	6.4	4–8	0.51	17.81
Seed moisture (%)	28.19	23.07–36.66	2.30	18.26	26.35	23.07–30.24	1.29	11.01	28.73	27.52–29.51	0.37	5.82
Seed viability (%)	96	90–100	2.44	5.70	97	90–100	2.0	4.61	94	90–100	2.44	5.82

Table 2. Qualitative characters of fruit, seed and leaf of *C. indica* and *C. macroptera*.

Characters	*C. indica*	*C. indica* × *Citrus* sp. (probable hybrid)	*C. macroptera*
Fruit			
Fruit shape	Obloid	Obloid	Spheroid
Fruit base	Truncate	Truncate	Concave
Fruit apex	Truncate	Truncate	Rounded
Fruit skin (epicarp) colour	Light to dark orange	Yellow	Yellow
Fruit surface texture	Smooth	Smooth	Bumpy
Adherence of albedo (mesocarp) to pulp	Medium	Strong	Strong
Nature of oil glands	Conspicuous	Conspicuous	Conspicuous
Density of oil glands	Intermediate	Intermediate	Intermediate
Oil glands size	Small	Small	Small
Albedo colour	White	White	White
Fruit pulp (flesh) colour	Yellow	Yellow	Light yellow
Seed			
Seed shape	Spheroid	Semi-deltoid	Semi-deltoid
Seed surface	Smooth	Smooth	Wrinkled
Seed colour	Cream	Cream	Yellow
Cotyledon colour	Medium green	Medium green	White and green
Chalazal spot colour	Brown	Brown	Cream
Seed embryony	Monoembryonic	Monoembryonic	Polyembryonic
Leaf			
Vegetative life cycle	Evergreen	Evergreen	Evergreen
Leaf division	Simple	Simple	Simple
Intensity of green colour of leaf blades	Medium	Medium	Dark
Leaf lamina attachment	Sessile (petiole absent)	Sessile (petiole absent)	Longipetiolate (petiole longer than or same length as leaf lamina)
Leaf lamina shape	Ovate	Ovate	Orbicular
Leaf lamina margin	Dentate	Dentate	Entire
Leaf apex	Acuminate	Acuminate	Emarginate
Absence/presence of petiole wings	Absent	Absent	Present
Petiole wings width	–	–	Medium
Petiole wings shape	–	–	Obdeltate

inspite of healthy vegetative growth. This indicates the lack of adaptation of this species to the man made habitats which according to Zeven and de Wet (1982) is a complex phenomenon. In *C. indica* adaptations leading to domestication and to enable the species to enter into reproductive phase, are still to be achieved. Speed of domestication depends on the duration of a generation and intensity of selection pressures (Zeven and de Wet 1982). Due to increasing demand of *C. indica* fruits for medicinal and social uses, its cultivation is getting extended in this area. In addition, this hardy species found free from any pest and disease infestation in natural habitat, may prove to be a promising rootstock for commercial *Citrus* species once adapted to the varied climates. Commercialization of this species would also lead to its protection and utilization as a medicinal plant for which it is being valued by local people in Garo hills.

Singh (1981) reported that *C. indica* was locally known in Garo language as 'Memang Narang', which means ghost (Memang) orange (Narang) and suggested this as a vernacular name for Indian wild orange. This is now a well-known fruit in the Garo hills of Meghalaya and is commonly being used by local people especially belonging to Garo tribe as a medicine for chronic diseases. Whole raw fresh fruits and dried fruit powder are taken as a medicine for deadly communicable diseases like small pox. In addition, fruit is also consumed to cure jaundice and other related stomach ailments of human beings and domestic animals. Besides this the fruit is highly valued in religious rituals of Garo people especially during their last rites. Interestingly, it is customary that a fresh fruit of

this species is placed on the dead body before conducting the last rites to keep away the ghost from the other family members, thus deriving its name. Fruits occasionally brought to the market by the tribals fetch a higher price in comparison to other edible citrus types. This might be the reason why some farmers have taken up their cultivation on small scale.

C. macroptera is locally known as Sat-kara (Sat meaning seven or its multiples and kara meaning segments) as most of the fruits possess 14 segments. This species is found wild as well as in semi-domesticated form in the forest. The plants were rarely observed in the backyards of local people living in the villages bordering the forest area. This species inspite of producing very hard and large fruits, which are too acidic in taste for eating raw as a table fruit, has shown some promise. Fresh fruits, bottled juice and dried fruit peel were being used by local people for edible purposes and sold on small village shops in Sairang near west Aizawl in Mizoram (Figure 2g). Juice mixed with sugar is used as a squash and relished by local people as it gives soothing effect to the stomach. Fresh fruits in small quantity and dried fruit peels are used in non-vegetarian preparations to give a typical acidic flavour to the curries. Interestingly, a small state-owned fruit processing plant was found functional where these preparations were being made from fruits of *C. macroptera* for selling to local inhabitants. It was also interesting to note that some farmers in the area were willing to cultivate plants for their own consumption. However, in other parts of northeastern India no commercialization of this species was reported.

Conservation

Erosion of genetic resources of *Citrus* due to various biotic and abiotic factors has resulted in loss of gene pools from nature and as well as from different centers of collections (Chadha 1995). *Citrus* genetic diversity is being lost at alarming rate in its natural habitat of northeastern India due to large-scale deforestation, practice of Jhum or shifting cultivation and cutting of trees for urbanization and other developmental activities in this socio-economically neglected area till now. Equally serious problem contributing to loss of genetic diversity of *Citrus* species is the preference of farmers for the edible and economically viable species. All these factors are causing serious threat to the genetic resources of many *Citrus* species in this area of diversity, which has been classified as a hot spot with regard to threat to citrus biodiversity (Singh and Singh 2003). As per the International Union for Conservation of Nature and Natural Resources (IUCN) norms seven Indian *Citrus* species are endangered as indicated by threat perception analysis (Singh and Singh 2003). These species include *C. indica*, *C. macroptera*, *C. latipes*, *C. assamensis*, *C. ichangensis*, *C. megaloxycarpa* and *C. rugulosa*. However, two species viz. *C. indica* and *C. macroptera* need special and immediate attention for conservation due to their endemism and high degree of threat perception. *C. macroptera* is in a highly endangered state in some pockets especially in Shella valley area and Jantia hills of Meghalaya (Figure 1) bordering Bangladesh where forest cover is disappearing at an alarming rate to allow large scale developmental activities. Figure 1 indicates the sites from where *C. indica* and *C. macroptera* were collected during present and earlier explorations. Also shown are the sites from where *C. indica* has ceased to exist according to earlier reports and *C. macroptera* is under the threat of extinction.

To protect these invaluable genetic resources of citrus, initiative was taken way back in 1981 by establishing the Citrus Gene Sanctuary in the Garo hills of Meghalaya covering an area of about 10,266 hectares (Singh 1981). This unique Citrus Gene Sanctuary probably first of its kind in the World is endowed with highly specified microclimate with a combination of tropical and mild temperate seasons and experiencing very high humidity and rainfall. Gene sanctuary is a part of buffer zone of Nokrek Biosphere Reserve and spreads over the east, west and south Garo hills of Meghalaya. *Citrus* species growing inside the gene sanctuary are still safe, however, the slow regeneration of these species and increasing human intervention around the gene sanctuary area are the causes of serious concern. Conservation of *Citrus* genetic resources needs urgent attention so as to protect the existing genetic diversity and to promote cultivation of rare and endangered species that are of great relevance in socio-economical structure of tribal populations of this area. Sharma et al. (2004) suggested the creation of more nature reserves, gene sanctuaries and gene parks, and

inclusion of *Citrus* species in the social forestry system to safeguard the genetic resources and to allow their further evolution under the natural stresses. Till these measures are implemented the *ex situ* conservation of rare and endangered species should urgently be undertaken to ensure safe storage of germplasm by developing suitable long-term storage methods. Initiatives have been taken for studying seed storage behaviour of these species and for devising long-term storage strategies using cryopreservation for both the species at the author's laboratory (NBPGR, India).

In view of importance of these species in citriculture and potential as a future commercial horticulture crops, concentrated efforts are required to collect, characterize and conserve the wild and semi-wild species of *Citrus* of northeastern India. These efforts should go along with the major emphasis on developing methods for propagation, multiplication and regeneration of these species under *in situ* and *ex situ* conditions to facilitate their effective utilization in *Citrus* improvement programmes.

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References

Ahuja A. 1996. Loss of biodiversity and need for conservation. In: Gujral G.S. and Sharma V. (eds), Changing Perspectives of Biodiversity Status in the Himalaya. The British Council, New Delhi, India.

Bhattacharya S.C. and Dutta S. 1956. Classifications of *Citrus* fruits of Assam. ICAR Sci. Monograph 20: 1–110.

Burkill I.H. 1951–52. Habits of man and the origins of the cultivated plants of the Old World. Proc. Linnean Soc. Lond. 164: 12–42.

Chadha K.L. 1995. Status report of tropical fruit species in south Asia. In: Arora R.K and Ramanatha Rao V. (eds), Proceedings of Expert Consultation on Tropical Fruit Specially Asia, MARDI Serdang, Kuala Lumpur. IPGRI Office for South Asia, New Delhi, pp. 45–60.

Hore D.K., Govind S. and Singh I.P. 1997. Collecting of Citrus germplasm from the Mizoram and Tripura hills of India. Plant Genet. Resour. Newslett. (IPGRI/FAO) 110: 57–59.

Sharma B.D., Hore D.K. and Gupta S.G. 2004. Genetic resources of *Citrus* of north-eastern India and their potential use. Genet. Resour. Crop Evol. 51: 411–418.

Singh B. 1981. Establishment of First Gene Sanctuary for Citrus in *Garo* Hills. Concept Publishing Company, New Delhi.

Singh I.P., Singh S., Singh K. and Srivastava R. 2001. Exploration and collection of citrus germplasm from NEH region (Meghalaya) of India. Indian J. Plant Genet. Resour. 14: 70–73.

Singh I.P. and Singh S. 2003. Exploration, collection and mapping of citrus genetic diversity in India. Technical Bulletin No.7, National Research Centre for Citrus, Nagpur.

Swingle W.T. 1946. Botany of Citrus. In: Citrus Industry Vol. 1. University of California Press, USA, pp. 129–474.

Swingle W.T. and Reece P.C. 1967. The botany of Citrus and its wild relatives. In: Reuther W., Batchelor L.D. and Webber H.J. (eds), The Citrus Industry. University of California Press, Berkeley, pp. 190–340.

Tanaka T. 1928. On certain new Species of citrus. Studia Citrologica 2: 155–164.

Tanaka T. 1937. Citrus fruits of India. Allahabad Farmer 2: 53–57.

Tanaka T. 1958. The origin and dispersal of citrus fruits having their centre of origin in India. Indian J. Horticult. 15: 101–115.

Upadhyay R.C. and Sundriyal R.C. 1998. Crop gene pools in the Northeast Indian Himalayas and threats. In: Pratap T. and Sthapit B. (eds), Managing Agrobiodiversity-Farmers Changing Perspective and Institutional Responses in the Hindu Kush-Himalayan Region. ICIMOD & IPGRI, Kathmandu, Nepal, pp. 167–173.

Zeven A.C. and de Wet J.M.J. 1982. Dictionary of Cultivated Plants and Their Regions of Diversity. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.