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RESEARCH ARTICLE

Socio-economic importance, domestication trends and *in situ* conservation of wild *Citrus* species of Northeast India

S. K. Malik · Susheel Kumar · I. P. Singh · O. P. Dhariwal · Rekha Chaudhury

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Abstract Northeast India is rich in *Citrus* genetic diversity representing several wild and cultivated species. Besides commercially cultivated species, several wild, semi-wild and domesticated species namely *Citrus indica*, *C. macroptera*, *C. ichangensis*, *C. latipes*, *C. megaloxycarpa* and *C. assamensis* are found to grow in Northeast India. These species have great potential in improvement of Indian *Citrus* industry being source of genes for combating biotic and abiotic stresses. The natural diversity of these genetic resources is shrinking at alarming rate due to large scale deforestation, shifting cultivation practiced in these areas and climate change scenario in this important hotspot of biodiversity. Socio-economic importance of each species have been studied during surveys and interesting domestication trends were observed based on their cultural and economic significance, which led to "*in situ* on farm" conservation of *C. indica*, *C. macroptera* and threat to *C. megaloxycarpa* and *C. ichangensis*. Consequently, genetic resources of most of these species are facing severe threat of extinction necessitating policy interventions and adoption of dynamic conservation and management strategies. Species specific conservation strategy especially through "*in situ* on farm" conservation has been proposed with suitable sites in Northeastern Indian states based on population structure and suitability of habitat. Farmers' support through "The Biological Diversity Act, 2002" and "The Protection of Plant Varieties and Farmers' Rights Act, 2001" of India would bring the required impact on management of these complex genetic resources of Citrus. In the present study, we have documented the current status, socio-economic potential, domestication trends and associated traditional knowledge of these wild and semi-wild *Citrus* species occurring in Northeast India and enumerated suitable conservation strategies and desired policy interventions for their dynamic conservation and sustainable utilization.

Keywords *Citrus* · Domestication · Ethnobotany · Genetic diversity · *In situ* conservation

S. K. Malik (✉) · S. Kumar · R. Chaudhury
Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi 110012, India
e-mail: skm1909@gmail.com; skm@nbpgr.ernet.in

I. P. Singh
National Research Centre for Citrus, Nagpur 440 010, Maharashtra, India

O. P. Dhariwal
Division of Exploration and Collection, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012, India

Introduction

Citrus L. (Rutaceae) is one of the most important commercial fruit crops of the world, which includes some of the major fruit species, such as *Citrus medica* L. (citron), *C. maxima* (Burm.) Merr. (pummelo),

C. reticulata Blanco (mandarin), *C.* × *aurantium* L. (sour orange), *C.* × *sinensis* (L.) Osbeck (sweet orange), *C.* × *paradisi* Macf. (grapefruit), *C.* × *limon* (L.) Burm. f. (lemon), and *C.* × *aurantiifolia* (Christm.) Swingle (lime) (Swingle and Reece 1967; Mabberley 2004). Citrus is believed to have its primary centre of origin in South and Southeast Asia, particularly in the region extending from Northeast India, Southeastward through the Malayan Archipelago to China and Japan, and Southward to Australia (Tanaka 1954; Webber 1967; Scora 1975, 1988). *Citrus* fruits are widely cultivated throughout the tropical and sub-tropical regions of the world (Webber 1967). In India, it is the third most important fruit crop grown after mango and banana with an estimated production of 8.60 million metric tones in a total area of 0.923 million ha (Anonymous 2009). Cultural, socio-economic, dietary, nutraceutical and medicinal importance of *Citrus* fruits are well documented (Dugo and Di Giacomo 2002; Mabberley 2004; Malik et al. 2006).

Domestication of wild plant species has been a historical phenomenon continuing since the origin and evolution of agriculture. Wild species are domesticated to fulfill various human needs which change with time. Wild class of organisms merges into the domesticated class by continuous series of degrees of intimacy with man (Higgs and Jarman 1969). Domestication of plants depends upon its utility at a point of time and woody plants and fruit trees have been listed in the last only before industrial plants in the sequence of crops which have been domesticated (Burkill 1951–1952). Domestication of a species due to its market and social importance also ensures *in situ* on farm conservation at farmland of small and marginal farmers. Domestication played an important role in conserving the local plant diversity on farmlands in West Usambara Mountains in Tanzania for several species (Msuya et al. 2008). Casas et al. (2007) studied the *in situ* management of plant populations and their domestication *in situ* in Mesoamerica and also analyzed the patterns of morphological, physiological and genetic effects of artificial selection in plant populations under *in situ* management in this region. Artificial selection operating on *in situ* managed populations of the species analyzed is causing initial domestication (Casas et al. 2007). *In situ* domestication of plants could be relevant to understand early processes of domestication and current conditions of *in situ* conservation of plant genetic resources.

The taxonomy and phylogeny of *Citrus* are complicated and controversial, mainly due to the sexual compatibility between *Citrus* and its related genera, apomixis (adventive nucellar polyembryony), high frequency of bud mutations, long history of cultivation, and wide dispersion (Frost and Soost 1968). Consequently there has been no consensus among the Citrologists as to the actual number of species that constitute the genus. Swingle's system of *Citrus* classification (Swingle and Reece 1967) recognized 16 species on the basis of biological species concept. Tanaka (1954, 1977), based on horticultural species concept, recognized up to 162 species. Advanced studies based on the biochemical and morphological characterization, suggested that there are only three basic species, i.e. citron (*C. medica* L.), mandarin (*C. reticulata* Blanco), and pummelo [*C. maxima* (Burm.) Merr.] within the subgenus *Citrus*, whereas the other edible *Citrus* e.g. lemon, lime, sour orange, sweet orange, grapefruit, etc. have been considered as apomictically perpetuated biotypes with probable hybrid origin (Scora 1975; Barrett and Rhsodes 1976). The concept of basic species in *Citrus* was well supported by Mabberley (1997, 2004), and gained further support from data derived from karyo-morphology and molecular markers like microsatellites, RAPD, ISSR, AFLP, RFLP, and coding and non coding regions of chloroplast DNA and ITS region of nuclear ribosomal DNA (Federici et al. 1998; Nicolosi et al. 2000; Araujo et al. 2003; Barkley et al. 2006; Liang et al. 2007; Bayer et al. 2009; Jena et al. 2009; Marak and Laskar 2010; Hynniewta et al. 2011; Kumar et al. 2012).

India has a distinct position in the 'Citrus belt' of the world due to remarkable diversity in citrus genetic resources, both in cultivation and wild. Apart from the most commonly cultivated species/hybrids, such as citrons, lemons, limes, mandarins, sour oranges, sweet oranges, pummelos, and grapefruits, some species, viz. *C. indica* Tanaka (Memang narang), *C. macroptera* Montr. (Melanesian papeda), *C. latipes* (Swingle) Tanaka (Khasi papeda), *C. ichangensis* Swingle (Ichang papeda), *C. megaloxycarpa* Lush. (Sour pummelo) and *C. assamensis* Dutta et Bhattacharya (Ada jamir) were recorded to occur in wild/semi-wild/semi-domesticated state in Northeast India (Brandis 1874; Hooker 1875; Bonavia 1880–1890; Lushington 1910; Bhattacharya and Dutta 1956; Nair and Nayar, 1997; Singh and Singh 2001; Sharma et al. 2004; Malik et al. 2006; Pandey et al. 2008; Hazarika 2012).

Citrus genetic diversity collection from Northeastern parts of India was taken up intensively by Tanaka (1928, 1937) and Bhattacharya and Dutta (1956) during 3rd to 5th decade of last century. Most of the new *Citrus* species were identified, collected and documented from Northeast India during this period. Since then, no significant information has been added to our knowledge about the occurrence of any additional species of *Citrus* from this region. However, recent surveys and explorations enumerated loss of some species from various pockets where these were earlier reported to occur (Singh and Singh 2003; Sharma et al. 2004). Some of the *Citrus* species still grow in a wild or semi-wild form in the forestlands. The genetic diversity and natural habitats of the wild and semi-wild genetic resources of *Citrus* in Northeast India is degenerating at an alarming rate due to large scale deforestation, shifting or jhuming cultivation and development projects being undertaken in the Himalayan region (Ahuja 1996; Talukdar et al. 2005; Kumar et al. 2010; Hazarika 2012). Farmers and local tribal populations rarely prefer to maintain and grow wild and semi-wild species of *Citrus* in their fields, home gardens and community lands due to less or negligible commercial values, which subjects most of these wild species to severe threat of extinction. Despite designating some area as *Citrus* Gene Sanctuary in Garo Hills, Meghalaya as back as in 1981 by NBPGR (Singh 1981), the dynamic conservation and management strategies have not been implemented in this important hotspot of biodiversity to protect species with substantial socio-economic, cultural and religious values for local people. Policies and provisions made through various Government acts to fulfill international and national commitments and to support farmers for *in situ* on farm conservation and sustainable utilization of *Citrus* genetic resources need urgent implementation. Therefore, there is an urgent need to collect, characterize and conserve this vast genetic diversity and document the ethno-botanical as well as indigenous technical knowledge about the *Citrus* species particularly from Northeastern India.

Hence, in the present study, we have documented the socio-economic importance, horticulture potential and domestication trends of these wild and semi-domesticated species of *Citrus* occurring in Northeast India. Present status of genetic resource management, associated traditional knowledge, *in situ* conservation strategies and policy issues for their long-term conservation and sustainable utilization have also been discussed.

Materials and methods

Several surveys and exploration trips were conducted in *Citrus* growing areas and in the protected forests of Northeast India in the states of Arunachal Pradesh, Assam, Meghalaya, Mizoram, Sikkim, Manipur, Nagaland and Tripura during 2002–2011 (Fig. 1). Systematic survey studies included recording of number of *Citrus* species, area under these, status and size of population, indigenous traditional knowledge on importance and uses, market demand, biological status, indicative domestication trends and cultivation practices. *In situ* conservation efforts being undertaken by tribal farmers and public or private agencies was assessed and evaluated in the surveyed area. Information was gathered from farmers, local people and forest guards during consecutive years. Collections of desired germplasm were made following selective sampling strategy in which plant material collected from single plant was treated as individual accession. Detailed passport data of each collected accession was recorded as per the format developed at NBPGR, New Delhi. Herbarium specimens of all the collected genotypes were prepared and documented in National Herbarium of Cultivated Plants at NBPGR, New Delhi.

Besides the natural forest sites, community lands and farmers' fields and orchards, visits were undertaken to various Citrus field genebanks existing in India during last 10 years. Germplasm status in relation to these wild species in the existing field genebanks was also assessed and various implications on conservation status were studied. Ethno-botanical information regarding importance and use of these wild, semi-wild and domesticated species of *Citrus*, viz. *C. indica*, *C. macroptera*, *C. ichangensis*, *C. latipes*, *C. megaloxycarpa* and *C. assamensis* were collected by interacting with local people. Specifically, senior-most persons of tribes in a village, local vendors and traditional healers were interviewed to collect information on religious, cultural, medicinal and dietary uses of these species. Local and weekly markets (Haats) of villages were surveyed to understand economic value of fresh fruits and their products. Livelihood support to small and marginal farmers

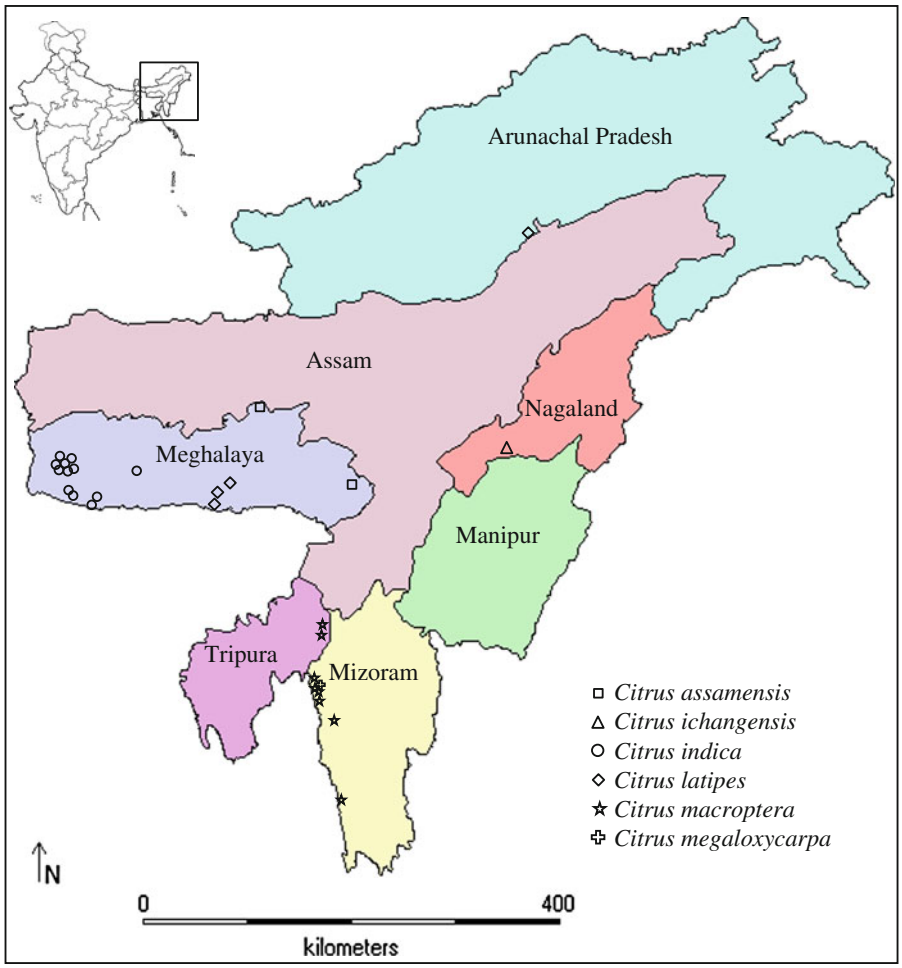

Fig. 1 Map indicating collection sites of wild and semi-wild species of *Citrus* in Northeast India (*Inset* India showing Northeast states in square)

through these species either by sale of fresh fruits and processed products was assessed. During surveys villages having small scale indigenous fruit processing units and market value chain of these fruits and their products were prioritized to study the commercial importance of these species and livelihood support to local people.

Results and discussion

Northeastern part of India, an important hotspot of biodiversity harboring cultivated, wild and semi-wild populations of *Citrus* species is also known as 'Treasure House of Citrus Genetic Wealth'. Bhattacharya and Dutta (1956) recorded 17 species, 52 cultivars and seven probable natural hybrids of *Citrus* in Northeast India. Later, in a systematic account on Indian *Citrus*, Nair and Nayar (1997) included 18 species, which comprised of eight species under sub-genus *Citrus*, viz. *C. aurantifolia*, *C. aurantium*, *C. indica*, *C. limon*, *C. maxima*, *C. medica*, *C. reticulata*, *C. sinensis*, three species under sub-genus *Papeda*, viz. *C. ichangensis*, *C. latipes*, *C. macroptera* and seven other indigenous species of probable hybrid origin and uncertain taxonomic affinities, viz. *C. jambhiri*, C. *karna*, *C. limetta*, C. *megaloxycarpa*, *C. madurensis*, *C. assamensis* and *C. nobilis*. Sharma et al. (2004) reported the occurrence of 23 species, one sub-species and 68 varieties of *Citrus* in Northeast India. In this study, they included 18 species as classified by Nair and Nayar (1997) and five new species of *Citrus* (*C. juko*,

C. kinesu, *C. sechen*, *C. serotina*, and *C. tanaka*) and one sub-species (*C. sechen* subsp. *sjanchen*) as earlier reported by Kokaya (1983). These five new species and one sub-species of Kokaya (1983) have been included in the recent reviews by Sharma et al. (2004) and Hazarika (2012) with no recent report of their collection or evidence of existence in India putting forth the ambiguous scenario of *Citrus* status in India. However, earlier Nair and Nayar (1997) did not report these five new species in their systematic studies on Indian *Citrus*. Existing literature and our surveys lead us to conclude that presently 18 species of *Citrus* comprising 65 varieties and 10 probable natural hybrids may be the best estimate of present status of *Citrus* resources found to grow in India majority occurring in the Northeastern states (Malik et al. 2012). Besides the ambiguous reporting on presence of *Citrus* species, biological status have also been wrongly presented in case of *C. medica* and *C. jambhiri* which have been reported to be wild and endemic requiring high conservation priority (Hazarika 2012). However, during present surveys, these two species have been found to be most widely cultivated and used as common rootstock in Uttarakhand, Himachal Pradesh and Punjab States and all over North-east India (Malik et al. 2012).

Several important species of *Citrus* namely *C. indica*, *C. macroptera*, *C. ichangensis*, *C. latipes*, *C. megaloxycarpa* and *Citrus assamensis* are found to occur in Northeast India in wild, semi-wild and domesticated state. Genetic resources of these species are important for studies on *Citrus* taxonomy and phylogeny. Some of these species are of greater significance in socio-economy and livelihood support to local people as well as for source of genes for agronomic traits like disease resistance, cold tolerance, etc. for use in genetic improvement programmes (Table 1). Surveys and explorations revealed the localized and sporadic presence of plants of these species in Northeastern states (Fig. 1). Distribution pattern, socio-economic importance, domestication trends and *in situ* conservation of six wild and semi-wild species of *Citrus* from Northeast India are discussed in detail.

Table 1 Important characteristics and *in situ* conservation areas proposed for wild and semi-wild species of *Citrus* in Northeast India

Species	Vernacular name	Important traits	Potential threats	Proposed area for *in situ*/on farm conservation
Citrus indica (Indian wild orange)	Memang narang/ Memang athur	Resistant to greening disease	Endemic, endangered, low regeneration, require microclimate, jhooming cultivation	Nokrek Biosphere Reserve, Garo Hills of Meghalaya
Citrus latipes (Khasi papeda)	Soh-kymphorshrich/ Soh-shyrkhoit	Resistant to greening disease and tolerant to cold	Endemic, endangered, low regeneration	Upper Shillong area of Meghalaya
Citrus macroptera (Melanesian papeda)	Satkara/Hatkara	Resistant to greening, psorosis and exocortis virus diseases	Endangered, habitat destruction	Shella area of Meghalaya, Sairang area of Mizoram and Jumpui Hills of Tripura
Citrus ichangensis (Ichang papeda)	Ketsa Chüpfü	Highly cold resistant	Endangered, deforestation, no cultivation	Khonoma village, Naga Hills, Nagaland
Citrus megaloxycarpa (Sour pummelo)	Amilbed/ Hukuma-tenga/ Holong-tenga/ Solong	Resistant to exocortis virus diseases	Endangered, no-socio-economic value	Jaintia Hills of Meghalaya and Mamit area of Mizoram
Citrus assamensis (Ada jamir)	Soh-sying/Ada jamir	Adopted to very high rain fall and humid atmospheric conditions	Endangered, endemic	Dawki area of Jaintia Hills, Meghalaya

Citrus indica Tanaka (Indian wild orange)

Citrus indica, an endangered and endemic wild species, is native to Northeast India. It was reported from the Garo Hills in Meghalaya and other parts of Northeast India in Assam, Mizoram, Manipur and Nagaland (Bhattacharya and Dutta 1956; Singh 1984, 2002; Shampru and Chauhan 2000). However, recent surveys conducted in these regions indicated that *C. indica* is perhaps now restricted only to the Tura ranges of Garo Hills in Meghalaya. This is a habitat specific species, which flourishes only in a particular microclimate of Citrus Gene Sanctuary located in the Garo Hills. A maximum number of individuals ranging from 15 to 25 were recorded in two populations namely at Daribokgre in East Garo Hills and Rongwak in South Garo Hills covering an area of 2 km^2. However, limited number of plants (6–8 plants per population) was located in Chandgre, Mandalgre, Sasatgre, Nokrek peak, Dura Kalakgre and Rengsangre in and around Nokrek Biosphere Reserve. Tribals of buffer zone of Nokrek Biosphere Reserve propagate plants in their home gardens and backyards through seeds due to its socio-economic and medicinal properties.

The species is characterized by small bushy tree or sometime of straggling nature (Fig. 2A). Fruits are small, spherical or oblate shape, surface deep orange red to scarlet, juicy with large plumpy seeds (Fig. 2B, C). *C. indica* is locally known as 'Memang-narang' among the Garo tribe; in Garo language 'memang' means ghost and 'narang' means fruit, i.e. the fruit of ghosts. The Garo people place fresh fruits of *C. indica* on the dead body before conducting the last rites to ward off the ghost from the other family members (Malik et al. 2006). Whole fresh fruit, its juice and dried powder are used for curing viral diseases, jaundice, intestinal disorders and deadly communicable diseases like small pox (Malik et al. 2006). Fresh fruits are sold in the local market @ Rs. 5–10 per fruit (Fig. 2D). *C. indica* is resistant to greening disease; hence it would prove to be a potential gene source for *Citrus* breeding programmes and needs to be exploited as rootstock.

Citrus macroptera Montr. (Melanesian Papeda)

Citrus macroptera is one of the endangered wild species, locally known as Satkara and Hatkara due to presence of fruit segments in multiple of seven and eight, respectively. It is considered to be native of Southeast Asia (Dutta 1958). In Northeast India, *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 (Bhattacharya and Dutta 1956; Sharma et al. 2004, Malik et al. 2006). The present survey reported that *C. macroptera* is commercially cultivated on small scale in some areas of Mizoram (Aizawl, Kolasib, Mamit and Lunglei), Tripura (Jumpui Hills) and in Meghalaya as demands of fresh fruits, juice and dried fruit peels for various purposes is increasing in the local market of Mizoram, encouraging the locals to grow this plant in home gardens and fields.

The plant is a medium to large tree, erect, profusely branched and densely foliaged with very long stout spines (Fig. 3A). Fruits are more or less oblate to globular in shape, yellowish in colour and smooth surface (Fig. 3B). Seeds of this species are large having cuneate or triangular shape. Fresh fruits, dried peels and extracted juice of *C. macroptera* are utilized by tribals in Mizoram for edible purpose and sale in the local markets. Juice mixed with sugar is used as a squash and relished as it gives soothing effect to the stomach. Fruits have medicinal value and used for treatment of stomach pain and alimentary disorders. A small scale fruit processing unit is used by farmer groups for the production of fruit juice of Satkara (Fig. 3C–E). For preparation of processed juice, fruits are carefully de-peeled using specially designed knife. Fruit peel is left for drying and freshly de-peeled fruits are crushed for juice extraction using indigenously designed and developed wooden juice extractor and then filtered in alumunium containers. This filtered juice is sufficiently boiled and sugar is added before cooling it overnight. Cooled juice is packed in bottles to be sold in the local markets. Processed juice of Satkara is sold in markets of Aizawl, Kolasib, Mamit in Mizoram and other Northeastern states and also exported to Bangladesh (Fig. 3F). Dried fruit peel is packed in small polybags and sold in the local market. It is used in non-vegetarian preparations for acidic flavor and softening of meats. Dried albedo of fruit is also utilized for feeding domesticated pigs by local people. Due to enormous commercial and medicinal uses of fruits, this species has important role in socio-economy and livelihood support of local people.

Fig. 2 *Citrus indica* **A** Domestication in homestead garden of village Sasatgiri in East Garo Hills; **B** plant bearing mature fruits; **C** matured fruits and its internal structure; **D** fresh fruits being sold in local market of Tura, Garo Hills

Besides this, *C. macroptera* may be a promising rootstock being a source of genes for resistance to greening, psorosis and exocortis virus diseases in Citrus improvement programme.

Citrus ichangensis Swingle (Ichang Papeda)

Citrus ichangensis is an endangered and wild species of sub-genus *Papeda* and locally known as "Ketsa Chüpfü" in "Angami" tribal language (one of the sixteen tribal languages existing in the tribal state of Nagaland, India) among the local tribal people. Earlier, Bhattacharya and Dutta (1956) reported this species as growing absolutely in the wild state in nearby Khonoma village in Barail range of the Naga Hills at high altitude. This species has been originally reported in 1913 by Swingle from Hupeh Province of China and distributed in West-Central and South-Western China in Hupeh, Szechwan, Kweichow and Yunnan provinces (Swingle and Reece 1967). Our recent survey in 2011 in State of Nagaland, India revealed that this species is still growing in the forest near Khonoma village in the Naga Hills at altitude of 1,861 m above msl in natural condition (Fig. 1). In India, natural distribution of this species is restricted to this area; however, Hazarika (2012) erroneously reported it to be an extinct species from Northeast India. This forest is dense, however, disturbed due to human activities such as rearing of Mithuns (*Bos frontalis*)- a domesticated free range bovine and due to collection of other forest produce by local tribal population. This wild species was found to be growing

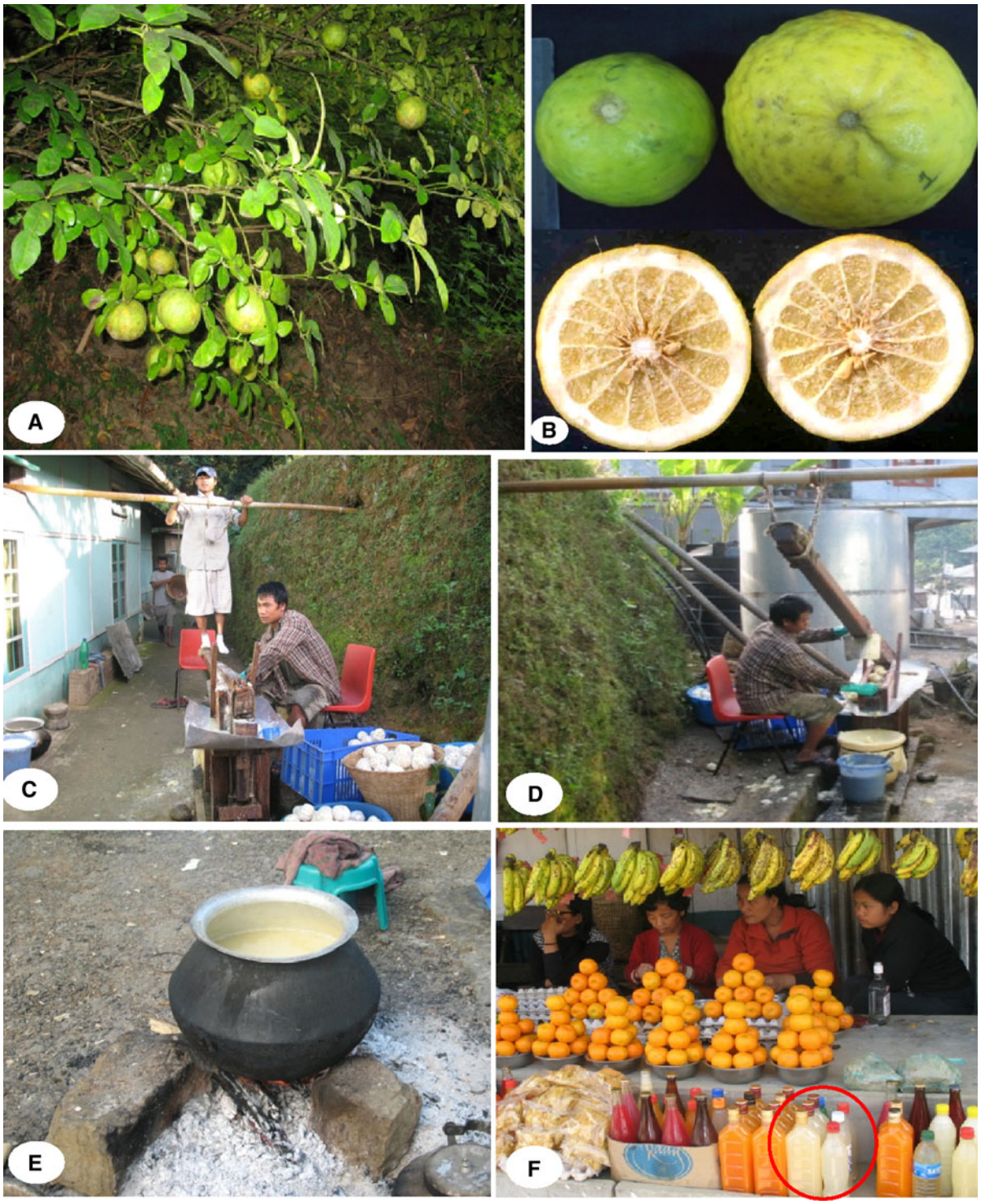

Fig. 3 *C. macroptera*: **A** Branches bearing mature fruits; **B** mature fruits and its internal structure; **C**, **D** fruit juice being extracted using indigenously developed wooden fruit processing unit; **E** boiling and subsequently cooling of juice; **F** fresh fruits, bottled juice and dried peels being sold in local market of Mizoram

along with other trees especially of *Docynia indica* (Indian crab apple). Natural regeneration of Ichang papeda was evident by the presence of different ages of trees including juvenile seedlings in the population. This may be due to the heavy fruit set of this species in natural habitat and large number of bold sized seeds in each fruit. Fruits being uneconomical are not harvested by local inhabitants and at maturity drop at the forest bed. Nutritionally rich seeds are consumed by monkeys, squirrels, birds and other small forest animals. Seeds which receive humid and suitable substrata germinate into seedling plants, albeit slowly in the forest.

Ichang papeda is a medium sized very hardy tree usually 10–18 ft. hight, having very large and sharp spines up to 7.0 cm long and broadly winged petioles as long as leaf lamina. Fruits are oblong in shape with gradual tapering at both the ends, size 6.0–8.0 cm in height, 4.0–6.0 cm in diameter. Fruits are full of extremely large and thick seeds, 1.2–2.2 cm long, 1.0–2.1 cm broad and 0.8–1.6 cm thick (Fig. 4A, B). Seeds have dented and reticulate impressions on the rounded end with white testa, brown colour chalazal spot, white cotyledons and are monoembryonic in nature.

The fruits are inedible having very less quantity of sour and acrid juice; therefore, it has no socio-economical value for local inhabitants. This is one of the hardiest *Citrus* species being highly cold resistant, which can contribute in development of cold resistant varieties. Some of the cold resistant hybrids using this species have been developed in China namely "Ichandarin" and "Yuzu" (*C. ichangensis* × *C. reticulata*) and "Shangyuan" (*C. ichangensis* × *C. grandis*) (Swingle and Reece 1967). Due to its limited and low population size intensive and immediate conservation efforts are required to save it from imminent loss from present habitat in the state of Nagaland through *ex situ* as well as *in situ* conservation strategies (Malik et al. 2012).

Citrus latipes (Swingle) Tanaka (Khasi Papeda)

Citrus latipes is an endangered wild species endemic to Northeast India. It is locally known as Soh-kymphorshrieh or Soh-shyrkhoit by Khasi tribe in Khasi Hills of Meghalaya. It grows primarily in the Khasi and Naga Hills (Dutta 1958). During the surveys undertaken by authors this species was observed in Shillong and Upper Shillong region with maximum of 60–80 individuals in each population. Few individuals were found growing in wild and semi-wild state in Shella valley of East Khasi Hills, Dawki area of Jaintia Hills and in Citrus gene sanctuary area of West Garo Hills in Meghalaya.

It is a medium to large tree, spreading habit and densely foliaged having long stout spines. Leaf lamina is ovate-lanceolate or broadly lanceolate with sub-acute apex. Petiole is broadly winged as equal or slightly longer than lamina, oblong-spathulate shape, obtuse apex and crenate margin. Fruit is almost globular or spherical shaped, moderately depressed at both ends with many large ovoid seeds (Fig. 4C). Fruits are traditionally used by locals for preparing pickles and treatment of viral diseases, intestinal disorders and jaundice. Due to these uses, occasional backyard and homestead garden plantation has been raised by local tribals in Khasi Hills of Meghalaya. *C. latipes* thrives well in Upper Shillong region of Meghalaya at altitude between 1,400 to 1,700 m above msl indicating tolerance to cold climate and is resistant to greening disease, potential characters of use in Citrus breeding programme.

Citrus megaloxycarpa Lush. (Sour Pummelo)

Citrus megaloxycarpa is indigenous to India and found to grow in Assam, Tripura and Jaintia Hills of Meghalaya (Hore et al. 1997; Singh et al. 2001; Sharma et al. 2004). It is commonly known as Amilbed in Western India. This is a historical Citrus fruit which finds reference in Mughal history of India as early as in the sixteenth century (Babar memoirs 1519). In Northeast India, several forms of sour pummelo viz. Bor-tenga, Hukum-tenga, Holong-tenga and Jamir-tenga are found to grow in semi-wild and domesticated state. The present surveys to regions of Assam, Mizoram and Tripura where from this species was reported earlier revealed that very few plants were found to be surviving.

Citrus megaloxycarpa is medium sized spreading tree, with moderately dense thorny foliage. Fruit is almost spherical to slightly turbinate, light yellow surface, smooth to sub-warty surface having many ovate to cuneate shaped seeds (Fig. 4D). Fruit juice of this species is reported to be very sour and morphologically it is very similar to pummelo, hence it is

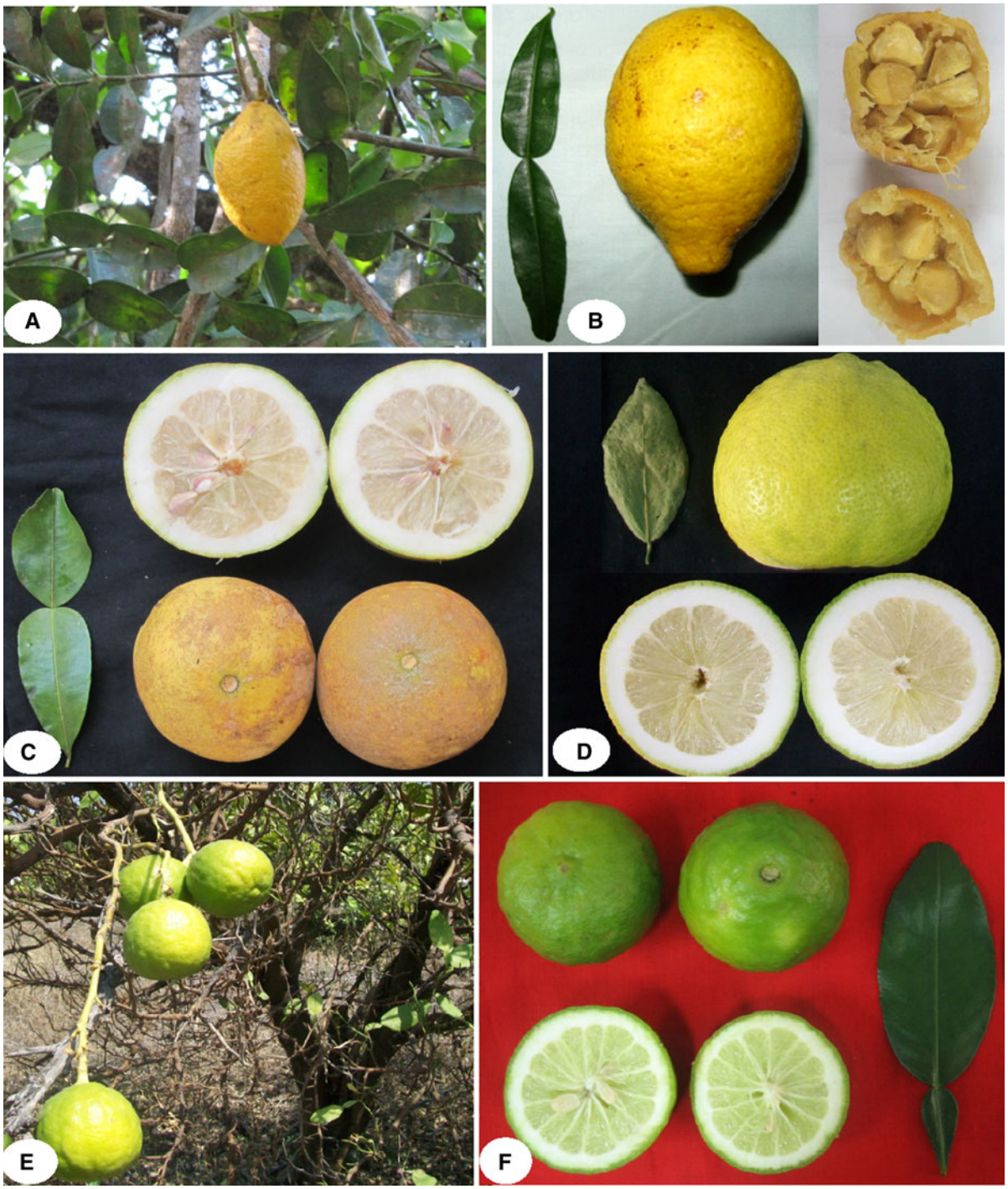

Fig. 4 Matured fruits and its internal structure of *C. ichangensis* (**A**, **B**); *C. latipes* (**C**); *C. megaloxycarpa* (**D**); *C. assamensis* (**E**, **F**)

known as "Sour Pummelo". Fruits have very little localized commercial and socio-economic importance for edible and nutritional purpose and hence this species is facing severe threat of extinction as farmers are reluctant to grow plants in their home gardens and fields.

Citrus assamensis Dutta et Bhattacharya
(Ada jamir)

Citrus assamensis is native to Northeast India and locally known as Ada-jamir (Ada- Ginger, Jamir- Citrus) in Assam and Soh-Sying (Soh- Fruit, Sying- Ginger) in Khasi Hills of Meghalaya. The species was first reported by Bhattacharya and Dutta (1956) and recorded to be growing in Barak valley, Sylhet and Cachar in Assam, Dawki area of Jaintia Hills and East Khasi Hills in Meghalaya (Dutta 1958; Singh et al. 2001; Sharma et al. 2004). Presently, few individuals of this species were found surviving as natural wild in the Dawki area of Jaintia Hills and East Khasi Hills of Meghalaya.

The plant is medium to large spreading tree, moderately branched and foliaged with long stout spines (Fig. 4E). Fruit is almost spherical to slightly turbinate, light lemon yellow colour with slight greenish tinge and smooth surface having many ovate shaped seeds (Fig. 4F). The fruits of this species are used by the local people for its aromatic flavor and very sour juice which are also processed to prepare squash and pickles. Fresh fruits give peculiar ginger like flavor which led to its local names. This species is adapted to very high rain fall and humid climatic conditions and hence could be used as rootstock in *Citrus* breeding programmes. The naturally growing populations of this species are under threat and found to be continuously shrinking at alarming rate due to less or negligible attention towards its commercial, horticultural and socio-economic importance.

Domestication trends

Domestication is a continuous ongoing evolutionary process, acting on incipient and semi-domesticated plants (Casas et al. 2007). Plant species when enter into cultural and economic main stream tend to the process of domestication facilitating dynamic "*in situ* on farm" conservation. *In situ* management of useful wild and semi-domesticated plants in the agricultural fields by the local people in Mesoamerica has been well documented by Casas et al. (2007). Due to continual alteration of both phenotypic and genotypic frequencies by selecting desirable phenotypes of useful plants, artificial selection is influencing evolution of plant populations, and because these processes are intentionally regulated by human actions, they should be considered as domestication processes. *In situ* management and artificial selection have been hypothesized as mechanisms that may facilitate domestication of plants (Casas et al. 1997; Zarate et al. 2005).

Species gain socio-economic importance and domestication process get trigger first by the tribal and marginal farmers primarily depending upon the demographic status of the species. Domestication of any wild species primarily depends upon its interaction with human beings and subject to the realization of its useful traits (Zeven and de Wet 1982). Several bottlenecks encountered during domestication of species and founder effect works especially for the species where number of domesticates in the process of domestication are less (Emshwiller 2006). In the domestication process of wild and endangered species discussed in the present paper these factors played very important role as number of individual in the founding population are very less increasing the duration of overcoming bottlenecks during domestication. In the present context enhanced period of domestication has been observed for *C. indica* which is endemic with limited population in comparison to *C. macroptera*. *C. indica* is still facing domestication bottlenecks especially adaptation and acclimatization of reproductive phase when brought outside the buffer zone of Nokerek Biosphere Reserve, with time these limiting factors would be probably overcomed. Acclimatization and adaptation according to Zeven and de Wet (1982) is a complex phenomenon and this is reflected in *C. indica* by the lack of adaptation of species to varied habitats. The adaptations for the species to enter into reproductive phase are still not achieved and hence remain a bottleneck for its wider domestication. Speed of domestication depends on the duration of a generation and intensity of selection pressures (Zeven and de Wet 1982). However, due to commercial, medicinal and socio-economic uses the demand of *C. indica* and *C. macroptera* fruits is increasing and their cultivation area is getting extended even in newer areas. Earlier the limited demand of fruits was being met by the limited fruits collected by the tribals from wild plants of Citrus Gene Sanctuary deep in the reserve forest. It is recorded that tribal and marginal farmers have willingly domesticated *C. indica* in their homestead gardens and backyards to earn some additional income due to the high market demand of this fruit. *C. indica* has specific microclimatic nitch as presently it is only observed in the villages of buffer zone of Nokrek Biosphere

Reserve covering East, West and South Garo Hills and still need efforts to complete the domestication process especially outside the Reserve, in contrast *C. macroptera* due to its higher adaptability and larger distribution have been found growing in the homestead gardens and farmers orchards at Kolasib, Aizawl, Mamit, and Lunglei districts of Mizoram.

Commercialization and social significance of these two *Citrus* species has driven the tribal farmers and local people to their cultivation with inherent on farm conservation. Similar pattern has been observed in the west Usambara Mountains in Tanzania for several species where domestication played an important role in conserving the local plant diversity on farmlands (Msuya et al. 2008). Complete neglect of *C. megaloxycarpa* and *C. ichangensis* by the local people and farmers is primarily due to their no market demand and economic use and hence for want of proper protection and domestication these species have assumed the status of highly threatened species. Comparative brief account of four species related to indigenous use, domestication and *in situ* "on farm" conservation at different periods is presented in Table 2. The model suggested in the present study (Fig. 5) based on detailed analysis of several factors elaborates the pattern of market value led domestication by stakeholders greatly helping the valuable plant species (*C. indica* and *C. macroptera*) and adversely affecting non-economic species (*C. megaloxycarpa* and *C. ichangensis*) leading to their unintentional and slow elimination from natural habitat and ecosystem. The present case study highlights the changing status of plant species in natural habitat with the time depending on positive or negative impact of human interaction with species which has a bearing on its future conservation efforts and survival in nature.

In situ conservation and policy issues

India is one of the 12 mega diversity rich countries of the World possessing three hotspots of biodiversity (Conservation International-Biodiversity hotspots: http://www.biodiversityhotspots.org). Northeast India falls under the "Indo-Burma Region" of hot spot of biodiversity, which is the richest and one of the most threatened places for plant life on the earth. Several *Citrus* species and their natural hybrids have been reported to originate and exist in this area; however, this vast indigenous *Citrus* diversity of India has not been used to its full potential for *Citrus* improvement programmes. In India, focus has remained on acclimatizing and popularizing readily available exotic cultivars and selecting promising genotypes from indigenous germplasm with commercially important characters. Several potential indigenous *Citrus* types could not get desired attention as regards to domestication, diversification and commercialization due to their apparent undesirable horticultural traits and remained at the mercy of local inhabitants who protected them in their backyards and homestead gardens to fulfill localized domestic, religious and cultural needs and occasionally for limited livelihood support. Consequently, the natural diversity of Indian wild, semi-wild and endangered species of *Citrus* is facing severe threat of genetic erosion due to large scale deforestation, urbanization, shifting or jhuming cultivation and developmental projects. Farmers and local people do not prefer to grow some of these species in the fields and home gardens due to limited commercial value. Target 7, 8 and 9 of objective II of "Updated Strategy for Plant Conservation 2011–2020" of convention on biological diversity (CBD), emphasizes conservation of plant diversity and focuses on achieving *ex situ* and *in situ* conservation of at least 75 % of known wild, endangered and socio-economically important species along with associated indigenous knowledge (Anonymous 2010). India is one of the pioneering countries in formulating and implementing the *sui-generis* system of management of biodiversity as expected from the parties of CBD in 1992. The Biological Diversity Act, 2002 of India emphasizes on the various provisions of biodiversity conservation, sustainable use, protection and rehabilitation of threatened species and traditional knowledge associated with these species (Anonymous 2004). However, the ground implementation of these provisions has hardly been observed by the stakeholders even after a decade of formulation of legislation and empowerment of the National Biodiversity Authority of India with these laws. Impact is limited probably due to the involvement of multiple public agencies and lack of defined comprehensive approach to achieve conservation and sustainable utilization of these complex genetic resources. Active involvement of stakeholders such as local tribal communities and farmer groups who are the actual growers, protectors and users of these resources need to be ascertained to have positive outcome especially in the case of wild and semi-wild

Table 2 Status of indigenous use, domestication and *in situ* "on farm" conservation of *C. indica*, *C. macroptera*, *C. megaloxycarpa* and *C. ichangensis*

Species and local name	Historical perspective (Tanaka 1937, 1954; Bhattacharya and Dutta 1956)	Species status in year 2000/2002 (Singh and Singh 2003; Sharma et al. 2004; Malik et al. 2006)	Species status in year 2010/2011 (Kumar et al. 2010, 2012; Hynniewta et al. 2011; Malik et al. 2012)
C. indica Tanaka (Indian Wild Orange) Local Garo name –Memang Narang	Wild endangered species occurring only in the forests of Khasi Hills, Garo Hills, Assam, and Nagaland with no local name and no edible use	Found only in the east and south Garo Hills used by Garo tribe as medicinal and cultural fruit Domestication initiated in the buffer zone of Nokrek Biosphere Reserve near Citrus Gene Sanctuary in Garo Hills	Fruits being used by Garo tribe as medicine and in religious rituals and being sold in local market fetching Rs. 8–10/- per fruit Domestication efforts increased and diversified to the West Garo Hills Market value driven domestication ensuring dynamic *in situ* "on farm" conservation of species.
C. macroptera Mont. (Melanesian Papeda) Local names—Satkara, Hatkara	Wild and semi-wild species in the Khasi and Garo Hills of Meghalaya, North Cachar, Karimganj and Karbi-Anglong districts of Assam, Mizoram, Tripura and Manipur Local use of fruit by Muslims for curries and fruit juice	Vanished from several parts of Assam and Khasi and Garo Hills due to cutting of forest area for shifting cultivation Processing and marketing of fruits in Sairang area of Mizoram for fruit juice, pickle and fruit peel Domestication of species was noticed in the backyards and homestead gardens in Mizoram	Domestication and cultivation in commercial orchards in several districts of Mizoram Several small scale indigenous processing units have been installed to process the fruits. Juices, pickle and packed fruit peel being sold in small village markets and being exported to South-Asian countries Species domesticated and being protected in dynamic "*in situ* on farm" conservation mode
C. megaloxycarpa Lush. (Sour Pummelo) Local names- Amilbed, Shisuphal, Bor-tenga	Historical fruit species indigenous to India referred in Baber Memoir (1519) Distributed widely in Assam Valley plains and existed commonly in villages as curiosity plant only as fruits are very sour and worthless Species has historical and taxonomic significance	Reported from Jantia Hills, Tripura and Mizoram as wild and endangered species No utility of fruits eventually no domestication efforts Species faces threat of extinction as continuously vanishing from wild and semi wild locations	Only 4 plants were located in a village Dampui near Mamit district of Mizoram No domestication efforts noticed even old plants are decaying as no care is taken Fruits do not have any cultural or economic significance, highly endangered status and extinction is paramount if no conservation and restoration efforts made
C. ichangensis Swingle (Ichang papeda), Local name in Angami—Ketsa Chüpfü	True wild species, found to grow absolutely in the wild state in nearby Khonoma village, Barail range of the Naga Hills at high altitude No socio-economic value	Found only in Khonoma village, Barail range of the Naga Hills Species faces threat of extinction as continuously decreasing from natural population due to no economic value	Present survey in Nagaland revealed that this species is still growing in the forest near Khonoma village in the Naga Hills at altitude of 1,861 m Natural population size decreases Fruits do not have any economic value, highly endangered status and extinction is paramount if no conservation and restoration efforts made

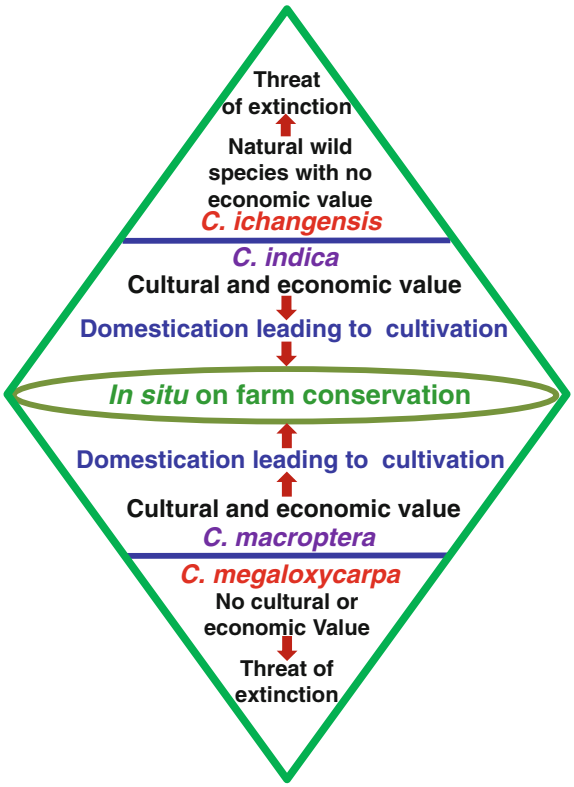

Fig. 5 Domestication viz-a-viz *in situ* on farm conservation model for four wild endangered species of *Citrus*

Citrus species. Small and marginal farmers nurturing plants of such species in the remote tribal villages of Northeastern India need support from such provisions to sustain these trees and conserve the rare germplasm on field. Legislative provisions such as access and benefit sharing are complicated and are unlikely to benefit these small tribal farmers in short and medium run. Till now the discussion and implementation has been limited at international and national level on "Access to Genetic Resources and Benefit Sharing out of their Utilization" which seems to be the most lucrative and sensational provision of these laws addressing interest of some elite scientific and business community but not reaching to the real farmers or tribal communities which are the mentors and custodians of these genetic resources.

Conservation of genetic resources of diverse genus like *Citrus* needs appropriate planning and complementarities of approaches both *in situ* and *ex situ*. In the present scenario most appropriate strategy for *Citrus* germplasm conservation is to adopt immediate *ex situ* conservation (i.e. field genebank and cryogenebank) complemented with *in situ* conservation (*In situ* on-farm conservation, gene sanctuary and National Parks) for wild and semi-wild species of *Citrus*. Presence of high degree of polyembryony in many *Citrus* species provides the opportunity of conserveing germplasm as true-to-type in the form of seeds (Malik and Chaudhury 2006). However, seeds of many *Citrus* species show non-orthodox seed storage behavior and therefore, cannot be stored using conventional −20 °C storage methods. In this case, cryopreservation has been extensively used for long-term conservation of such species using a range of explants like zygotic and somatic embryos, embryonic axes, shoot apex and cell suspension. Germplasm of wild and semi-wild species of *Citrus* have been successfully cryostored at cryogenebank of NBPGR, New Delhi (Malik et al. 2012).

Species specific conservation strategy is to be adopted in genus Citrus depending upon biological status, population size, diversity and endemism. Some of the species namely *C. indica*, *C. latipes* and *C. assamensis* are endemic to this area requiring growth in the specific microclimate. In contrast, *C. macroptera* and *C. indica* due to their increasing commercial, religious and cultural importance are now being prefered for cultivation by the farmers and tribal people in some parts of the Northeast India. *In situ* and *in situ* on-farm conservation strategies are proposed for species like *C. indica*, *C. macroptera*, *C. latipes*, *C. ichangensis*, *C. assamensis* and *C. megaloxycarpa* (Table 1). *Ex situ* conservation in the field genebanks have been tried for wild and semi-wild species of *Citrus* but it has not been of much success due to the requirement of specific microclimate for better survival and overcoming/breaking juvenility. Therefore, conservation of wild and semi-wild species of *Citrus* may be attempted using dynamic *in situ* approach for that suitable areas have been suggested for each species in different parts of Northeast India (Table 1; Fig. 6). Farmers and tribal communities of these areas may be encouraged to maintain and grow few plants of these wild species in their fields, backyards or in community lands for reasonable incentive as per the provisions already made in the "Protection of Plant Varieties and Farmer's Right Act, 2001" of India. Provisions for establishment of "National Gene Fund" to support the conservation and sustainable use of genetic resources including *in situ* and *ex situ* conservation especialy in the agro-biodiversity hotspots have been made in this act (Anonymous 2001). Areas should be marked in the

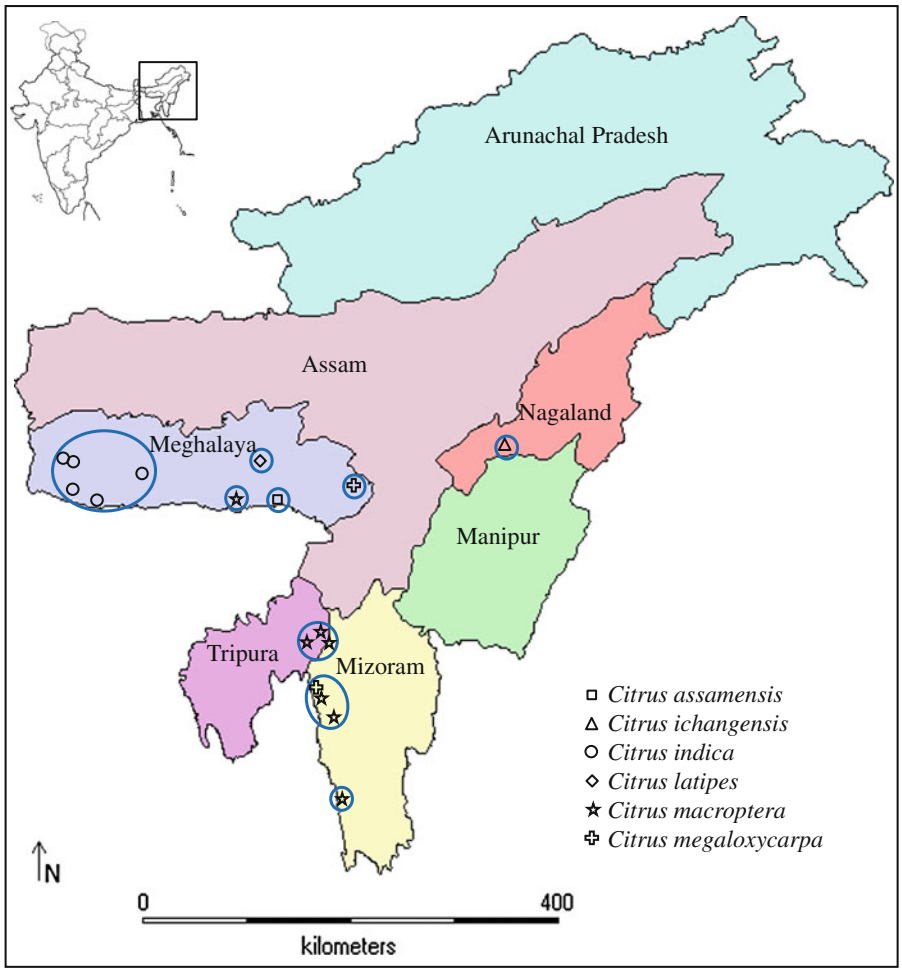

Fig. 6 Map indicating *in situ* sites for conservation of wild and semi-wild species of *Citrus* in Northeast India (*Inset* India showing Northeast states in square)

habitat for growing these *Citrus* species along with other forest species for sustainable and dynamic habitat conservation sites in the form of "Gene Mangement Zones" or "Gene Sanctuaries". 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 ha (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. This "Citrus Gene Sanctuary" deserves the status of Heritage Site under the provisions existing in "The Biological Diversity Act, 2002" of India (Anonymous 2004). There is an urgent need to develop "Citrus Parks" or "Citrus Biodiversity Gardens" in other states of Northeast India where germplasm of diverse *Citrus* species may be maintained and made economically viable by generating resources within 4–5 years of planting. It would help in exchange, conservation and sustainable use of *Citrus* germplasm for genetic improvement as well as knowledge sites for students, researchers and teachers and tourism site for national and international visitors.

Conclusion

Wild and semi-wild species of *Citrus* in Northeast India have diverse social, economic and scientific importance. Sincere *in situ* conservation efforts are

urgently required to safe guard existing genetic diversity for posterity and utilization in *Citrus* improvement programmes. Policy interventions, implementation of Farmers' Rights and generating awareness among local tribal and farmer communities would support the task of dynamic conservation and preservation of associated indigenous knowledge. Bioprospecting for confirmation of medicinal and nutritional traits in these *Citrus* genotypes and use of modern biotechnological tools would further enhance the possibilities of utilizing rare genes to mitigate the challenges of climate change and biological stresses encountered by *Citrus* industry in India.

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