RESEARCH ARTICLE

Assessment of genetic variability in the endangered Citrus macroptera Mont. ("hatkora") from Mizoram, north-east India

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Abstract Assessment of genetic variability in the available germplasm is the prerequisite for development of improved genotypes through planned breeding programmes. In view of this, 39 ''hatkora'' (Citrus macroptera Mont.) genotypes collected from different locations of Mizoram, India were evaluated for physicochemical characteristics during 2013–2015. The study reveals that there was significant variation among the collections in these particular traits. Individual fruit weight ranged from 277.78 to 617.69 g; fruit diameter 9.32–12.52 cm; fruit length 7.74–10.19 cm, fruit volume 238.33–583.33 cc; pulp weight 153.75–320.94 g; pulp:peel ratio 1.50–3.24 and seed number 9.33–23.6. Similarly, the chemical parameters also varied significantly among different germplasms. The juice content varied from 13.45 to 32.53 %, ascorbic acid 34.81–73.64 mg/100 mL, TSS 6.15–9.10 %, acidity 5.03–8.75 %, total sugars 5.16–7.97 % and sugar:acid ratio 0.75–1.52. From the analysis of genetic parameters, it could be concluded that characters like fruit weight,

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fruit length, fruit diameter, fruit volume, pulp weight, pulp–peel ratio, juice, Total soluble solids (TSS), acidity, ascorbic acid, total sugars, and sugar–acid ratio could be used as selection criteria for development of effective and productive plant types in ''hatkora''. Wide range of variation in physico-chemical parameters of ''hatkora'' fruits indicated the great scope of individual plant selection based on these characters for future genetic improvement programme.

Keywords Citrus macroptera · Genetic diversity · Mizoram - North-east India - Physico-chemical characteristics

Introduction

The genus Citrus L., the sole source of the Citrus fruits of commerce, belongs to the orange subfamily Aurantioideae of the family Rutaceae and is grown in tropical and subtropical areas of the world (Webber [1967\)](#page-9-0). The genus includes some of the most commercially important fruits viz. mandarin (Citrus reticulata Blanco), sweet orange [Citrus sinensis (L.) Osbeck], grapefruit (Citrus paradisi Macf.), lemon [Citrus limon (L.) Burm. f.] and lime [Citrus aurantiifolia (Christm.) Swingle]. India enjoys a remarkable position in the "Citrus belt of the world" due to her rich wealth of Citrus genetic resources, both wild and cultivated (Malik et al. [2013;](#page-9-0) Nair and Nayar [1997\)](#page-9-0).

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The north-eastern region of India is a rich treasure of various Citrus species. Natural and undisturbed populations of Citrus genepool observed during collection trips from time to time confirms the assumption that this area might be the centre of origin of several Citrus species. A vast reservoir of Citrus diversity exists in wild, semi-wild form and is found scattered here and there without commercial cultivation and much care (Hazarika [2012](#page-8-0)). Citrus plants growing in deep forests undisturbed by abiotic factors have also been reported from the region, thus bestowing this area with a special status of ''treasure house'' of Citrus germplasm (Sharma et al. [2004](#page-9-0)).

As per IUCN norms, seven Indian citrus species fall under the category of endangered species as indicated by threat perception analysis which include C. indica Tanaka, C. macroptera Mont., C. latipes Tanaka, C. assamensis Dutta et Bhattacharya, C. ichangensis Swingle, C. megaloxycarpa Lushington, and C. rugulosa Tanaka, (Singh and Singh [2003;](#page-9-0) Malik et al. [2006;](#page-9-0) Hynniewta et al. [2011](#page-9-0)). Two species, C. indica and C. macroptera need special and immediate attention for conservation due to their endemism and high degree of threat perception (Malik et al. [2006](#page-9-0); Hynniewta et al. [2011](#page-9-0)).

Citrus macroptera Mont., commonly known as "satkora" or "hatkora" is found confined in evergreen forests of N. E. India and moist deciduous forests of the north Himalayas and Assam (Cachar, Karbi Anglong, Nagaon and Sivsagar) with great diversity (Nair and Nayar ([1997\)](#page-9-0). A rich genetic diversity of "hatkora" exists in north-eastern region of India including bordering areas of Bangladesh, Meghalaya, Mizoram and Manipur (Singh and Singh [2006\)](#page-9-0). It is also reported of growing this species in semi wild form in Shella and Dawki area near Cherrapunjee in Meghalaya, Chandel district of Manipur and Mizoram. In Mizoram, ''hatkora'' plants are found naturally in marginal lands, forest areas and homestead gardens in semi wild and wild state. Although, this species have wider distribution in Mizoram but the commercial cultivation is confined mainly in Kolasib, Aizawl, Lunglei, Mamit, and Serchhip district, where the elevation and agro-climatic condition are lower and warmer which is suitable for its growth and yield performance.

As majority of ''hatkora'' are from seedling origin, therefore they showed a tremendous variation in their morphological and physico-chemical traits among its population. Due to lack of selection of any superior germplasm, the farmers have been planting trees of seedling origin of unknown yield potential and quality. But, these trees show wide variation in their fruiting, yield and quality. However, the information on extent of genetic variation among ''hatkora'' accessions for different morphological characters in north-east India is not available. Only little efforts have been made for genetic characterization of diversity in the naturally grown ''hatkora'' population in the wild and semi wild state. In order to enrich the information and acquaint the citrus breeder to interpret phenotypic values in terms of potential genetic gain, an attempt has been made to elucidate the genetic variability of ''hatkora'' accessions with respect to various physical and chemical characteristics of the fruits from natural population of Mizoram, north-east India.

Materials and methods

Mizoram, having an area of $21,081$ km²., lies between $21^{\circ}56'$ N and $24^{\circ}31'$ N latitude and $92^{\circ}16'E$ and 93°26'E longitude. The state has international borders of Bangladesh in the west and Myanmar in the east and south. On the northern side it borders with three states of India viz. Tripura in North West, Assam in north and Manipur in north east. Being sandwiched between Bangladesh and Myanmar, its location is geographically and politically significant (Fig. [1\)](#page-2-0).

The ''hatkora'' trees are found scattered throughout the states from homestead garden to forest areas. Considering its vast spread, the surveying of "hatkora" orchards and collection of fruits from different locations of Mizoram comprising of 39 different orchards was conducted during the fruiting season of 2014–2015 to identify the elite germplasms among natural population. The detailed about of germplasm and their sources are described in Table [1.](#page-3-0) The collected specimens were brought to the Laboratory, Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Mizoram, India for analysis of physico-chemical characters.

For measuring the physical parameters of the fruits, 20 randomly selected fruits were taken from each replication. The data on physical parameters like fruit weight, pulp weight, peel weight and seed weight were recorded as per standard procedures with the help of an

Fig. 1 Map of the study area

electronic balance. The fruit volume was measured by dipping the fruit in the water through water displacement method and expressed in cc. The specific gravity was measured by dividing the fruit weight by the fruit volume and was expressed in g/cc. Quality parameters like juice, TSS, acidity, ascorbic acid, reducing, nonreducing and total sugars were estimated following standard procedures. The juice content of the fruit was determined by extracting the juice with the help of mechanical juice extractor. The standard method (AOAC [1995](#page-8-0)) was followed to determine the titrable acidity, reducing, non-reducing and total sugars of fruit. Visual titration method (Freed [1966](#page-8-0)) was followed for the estimation of ascorbic acid content of the fruit pulp and the result was expressed in mg per 100 g. The data obtained from different observations during field experimentation and laboratory analysis were subjected to Fisher's method of analysis of variance (ANOVA) by following completely randomized design. Significance and non-significance of the variance due to different treatments were determined by calculating the respective 'F' value and comparing with the appropriate value of 'F' at 5 % probability level (Panse and Sukhatme [1985](#page-9-0)). By comparing different treatments among themselves critical difference were calculated at 5 % probability level.

Results and discussion

Fruit quality is a complex trait, which depends upon a number of other parameters and their interaction. The ANOVA of 39 ''hatkora'' collections identified in this investigation revealed significant differences in various physico-chemical parameters of the fruits (Tables [2](#page-4-0), [3](#page-6-0)). The rich variation could be due to highly heterozygous and diverse genetic background of parents.

The fruit weight of the germplasms ranged between 277.78 and 617.69 g. The highest fruit weight was recorded in MZU-HAMP-HS-21 (617.69 g), while the lowest was recorded in MZU-HAMP-HS-2 (277.78 g). The variation in fruit weight among different germplasms has also reported by Madhumathi and Sekhar ([2015\)](#page-9-0) in sweet orange. Hazarika et al. ([2013](#page-8-0)) also observed significant variation in fruit weight among 15 "hatkora" germplasms.

Among the different collections, the maximum fruit length was recorded in MZU-HAMP-HS-7 (10.19 cm). It was followed by MZU-HAMP-HS-21 (9.87 cm), and MZU-HAMP-HS-17 (9.56 cm). The lowest fruit length was recorded in MZU-HAMP-HS-39 (7.74 cm). This variation in fruit length might be due to different genetical constitution of the individual genotypes (Hazarika et al. [2013](#page-8-0)).

The accessions varied significantly with respect to fruit diameter. Among all the germplasms, the highest fruit diameter was recorded in MZU-HAMP-HS-21 (12.52 cm). The lowest fruit diameter was recorded in MZU-HAMP-HS-2 (9.32 cm). Our study is in close conformity with the findings of Hazarika et al. ([2013\)](#page-8-0) who also reported variation in fruit volume among the "hatkora" accessions. The variation in fruit diameter among different germplasms has also reported by Prakash et al. [\(2010](#page-9-0)) in jamun.

Table 1 Germplasms and their sources

Sl. no.	Germplasm	Latitude	Longitude	Elevation
1.	MZU-HAMP-HS-1	N23°49'06.9"	E092°44'39.4"	1114 m
2.	MZU-HAMP-HS-2	23°49'07.2"	E092°44'39.5"	1108 m
3.	MZU-HAMP-HS-3	N23°49'07.5"	E092°44'39.2"	1108 m
4.	MZU-HAMP-HS-4	N23°49'07.5"	E092°44'39.2"	1107 m
5.	MZU-HAMP-HS-5	N23°49'07.4"	E092°44'39.0"	1107 m
6.	MZU-HAMP-HS-6	N23°49'07.6"	E092°44'39.5"	1109 m
7.	MZU-HAMP-HS-7	N24°03'51.1"	E092°36'14.1"	66 m
8.	MZU-HAMP-HS-8	N24°03'50.5"	E092°36'14.0"	66 m
9.	MZU-HAMP-HS-9	N24°03'50.4"	E092°36'14.0"	65 m
10	MZU-HAMP-HS-10	N24°03'50.4"	E092°36'14.1"	64 m
11.	MZU-HAMP-HS-11	N24°03'49.8"	E092°36'11.7"	64 m
12.	MZU-HAMP-HS-12	N24°03'49.8"	E092°36'11.6"	63 m
13.	MZU-HAMP-HS-13	N24°03'49.8"	E092°36'11.6"	61 m
14.	MZU-HAMP-HS-14	N24°03'49.9"	E092°36'11.4"	62 m
15.	MZU-HAMP-HS-15	N24°03'49.8"	E092°36'11.6"	63 m
16.	MZU-HAMP-HS-16	N24°03'49.8"	E092°36'1.4"	60 m
17.	MZU-HAMP-HS-17	N24°03'49.5"	E092°36'11.3"	61 m
18.	MZU-HAMP-HS-18	$N24^{\circ}03'49.5''$	E092°36'10.7"	63 m
19.	MZU-HAMP-HS-19	N24°03'51.5"	E092°03'11.3"	57 m
20.	MZU-HAMP-HS-20	N24°03'51.2"	E092°36'11.3"	59 m
21.	MZU-HAMP-HS-21	N24°03'52.5"	E092°36'11.0"	51 m
22.	MZU-HAMP-HS-22	N24°03'52.4"	E092°36'11.2"	60 m
23.	MZU-HAMP-HS-23	N24°03'52.7"	E092°36'10.5"	63 m
24.	MZU-HAMP-HS-24	N24°03'52.7"	E092°36'09.9"	63 m
25.	MZU-HAMP-HS-25	N24°03'52.9"	E092°36'09.9"	62 m
26.	MZU-HAMP-HS-26	N24°03'52.8"	E092°36'09.9"	61 m
27.	MZU-HAMP-HS-27	N24°03'52.8"	E092°36'09.8"	60 m
28.	MZU-HAMP-HS-28	N24°03'52.7"	E092°36'09.7"	60 m
29.	MZU-HAMP-HS-29	N24°03'51.8"	E092°36'08.7"	59 m
30.	MZU-HAMP-HS-30	N24°03'51.9"	E092°36'08.8"	61 m
31.	MZU-HAMP-HS-31	N24°03'51.6"	E092°36'08.9"	58 m
32.	MZU-HAMP-HS-32	$N24^{\circ}03'49.9''$	E092°36'09.7"	57 m
33.	MZU-HAMP-HS-33	N24°03'48.8"	E092°36'08.5"	58 m
34.	MZU-HAMP-HS-34	N24°03'49.8"	E092°36'10.5"	60 m
35.	MZU-HAMP-HS-35	N24°03'48.7"	E092°36'09.6"	58 m
36.	MZU-HAMP-HS-36	N24°03'48.6	E092°36'09.8"	58 m
37.	MZU-HAMP-HS-37	N24°03'49.5"	E092°36'10.7"	60 m
38.	MZU-HAMP-HS-38	N24°03'49.9"	E092°36'10.8"	61 m
39.	MZU-HAMP-HS-39	N24°03'50.2"	E092°36'11.3"	62 m

The fruit volume of the germplasms ranged between 238.33 and 583.33 cc. Among all the germplasms, the significantly highest fruit volume was recorded in MZU-HAMP-HS-21 (583.33 cc), while, the lowest was recorded in MZU-HAMP-HS-2 (238.33 cc). The variation in fruit volume among different germplasms has also reported by Nayak et al. [\(2013](#page-9-0)) in mango.

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Table 2 continued

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The highest specific gravity was recorded in MZU-HAMP-HS-5 (1.41 g/cc), and the lowest was in MZU-HAMP-HS-34 and MZU-HAMP-HS-9 (0.92 g/cc). The variation in specific gravity of the fruits among different germplasms has also reported by Singh et al. [\(2010](#page-9-0)) in mango.

Among all the germplasms, the peel weight ranged between 249.05 and 311.33 g. The maximum peel weight was recorded in MZU-HAMP-HS-15 (311.33 g). The lowest peel weight was observed in MZU-HAMP-HS-7 (249.05 g). Similarly, the significantly highest pulp weight was recorded in MZU-HAMP-HS-21 (351.61 g), while, the lowest was recorded in MZU-HAMP-HS-2 (153.75 g). The variation in pulp weight among different germplasms has also reported by Prakash et al. [\(2010\)](#page-9-0) in jamun, Singh et al. ([2010\)](#page-9-0) in mango.

There was no significant difference among the germplasms with respect to pulp peel ratio (Table [2](#page-4-0)). Among the germplasms, MZU-HAMP-HS-6 (1.74) and MZU-HAMP-HS-26 (0.95), recorded the highest and lowest value of pulp peel ratio.

Peel thickness also showed considerable variation among the germplasms and maximum of 1.75 cm was observed in MZU-HAMP-HS-21. Among the germplasms, the lowest peel thickness was recorded in MZU-HAMP-HS-36 (0.76 cm). There is earlier report of considerable variation in peel thickness of C. macroptera (Singh and Singh [2006](#page-9-0)). The variation in peel thickness among different pomegranate germplasms has also reported by Madhumathi and Sekhar [\(2015](#page-9-0)) in sweet orange.

The highest pulp thickness was observed in MZU-HAMP-HS-21 (10.78 cm) while, the lowest was recorded in HAMP-HS-6 (8.05 cm). Our study is in close conformity with the findings of Hazarika et al. [\(2013](#page-8-0)) who also reported variation in pulp thickness among 15 ''hatkora'' accessions.

There was no significant variation among the germplasms with respect to number of segments. However, MZU-HAMP-HS-8, MZU-HAMP-HS-16, MZU-HAMP-HS-21, MZU-HAMP-HS-25 and MZU-HAMP-HS-34 recorded the highest (14.33) and MZU-HAMP-HS-33 (11.67) lowest number of segments respectively.

Similarly, the number of seeds per fruit was lowest in MZU-HAMP-HS-7 (11.67). Germplasm MZU-HAMP-HS-33 recorded the highest no. of seeds (39.17) . Hazarika et al. (2013) (2013) also reported variation

in seed number among 15 ''hatkora'' accessions from Mizoram.

The lowest seed weight was noticed in was observed in MZU-HAMP-HS-7 (4.93 g), whereas it was highest in MZU-HAMP-HS-33 (22.35 g). Our study is in close conformity with the findings of Hazarika et al. ([2013\)](#page-8-0) who also reported variation in seed weight among the ''hatkora'' accessions. The variation in seed weight among different germplasms has also reported by Prakash et al. ([2010\)](#page-9-0) in jamun and Singh et al. ([2015\)](#page-9-0) in guava.

For an ideal variety lower weight and small size of seed are the desirable characters. These observations revealed a positive correlation among pulp weight, seed weight and fruit weight. The genotypes produced higher pulp weight may be due to higher fruit weight and less seed weight. This clearly indicated that, during selection of any genotype based on fruit, the breeder should give emphasis on fruit pulp content rather than fruit weight alone. This finding is in conformity with Hazarika et al. ([2009\)](#page-8-0).

The data presented in Table [3](#page-6-0) showed significant variations in chemical characters of fruits. The juice content was found maximum in MZU-HAMP-HS-17 (32.53 %). Germplasm MZU-HAMP-HS-4 recorded the significantly lowest juice content (13.45 %). This finding is in agreement with Madhumathi and Sekhar (2015) (2015) in sweet orange, Singh et al. (2001) (2001) .

The TSS content of the fruits varied from 6.15 to 9.10 %. MZU-HAMP-HS-21 recorded the highest of 9.10 %. The lowest TSS was recorded in MZU-HAMP-HS-1 (6.15 %). The variation in TSS may be due to different genetical constitution of the individual genotypes. Fruits growing in arid region with limited water tended to more accumulation of dry matter and lower moisture may result in higher TSS in fruits (Meghwal and Azam [2004\)](#page-9-0). Singh and Singh ([2003](#page-9-0)) reported variation of TSS among different citrus accessions. The breeders during selection of superior genotypes should emphasize total soluble solids content of the fruit. The variation in TSS among different germplasms has also reported by Singh and Misra [\(2010](#page-9-0)) in bael and Srivastava et al. ([2014](#page-9-0)) in sweet cherry.

Ascorbic acid content is also one of the most important criteria in determining the superiority of ''hatkora'' germplasms. MZU-HAMP-HS-21 showed the highest ascorbic acid content (73.64 mg/100 g), while the lowest was recorded in MZU-HAMP-HS-32

(34.81 mg/100 g). It is a fact that, if TSS increases, the ascorbic acid also increases because the precursor of ascorbic acid is glucose-6-phosphate (Prakash et al. [2010\)](#page-9-0), which also confirmed from our study. The variation in ascorbic acid among different germplasms has also reported by Madhumathi and Sekhar [\(2015](#page-9-0)) in sweet orange and Srivastava et al. ([2014\)](#page-9-0) in sweet cherry.

Titrable acidity of the fruits ranged between 5.03 and 8.75 %. MZU-HAMP-HS-17 (5.03 %), recorded the lowest titrable acidity. The highest value with respect to acidity was recorded in MZU-HAMP-HS-26 (8.75 %). This is a fact in many fruits that, if total soluble solids are increasing definitely acidity will be decreased. This may be major factor for minimum acid content in MZU-HAMP-HS-17, MZU-HAMP-HS-21and MZU-HAMP-HS-7, whereas variation among genotypes for acidity percent might be due to total soluble solids content and genetic make of plant (Prakash et al. [2010](#page-9-0)) which has also proved in our study.

Similarly, sugar content also varied significantly among the collections. The highest value of total sugar was recorded in MZU-HAMP-HS-21 (7.97 %). The lowest total sugar was recorded in MZU-HAMP-HS-24 (5.16 %). The variation in sugar content among the "hatkora" germplasms may be due to different genetical constitution of the individual genotypes (Hazarika et al. 2013).

The Highest value of reducing sugar was recorded in MZU-HAMP-HS-29 (4.82 %) while, the lowest was recorded in MZU-HAMP-HS-10 (3.09 %). The variation in reducing sugar among different germplasms has also reported by Madhumathi and Sekhar [\(2015](#page-9-0)) in sweet orange.

There was no significant difference among the germplasms with respect to non-reducing sugars.

The highest sugar:acid ratio (1.52) was recorded in MZU-HAMP-HS-17. Among all the germplasms, MZU-HAMP-HS-24recorded the lowest value of sugar: acid ratio (0.75). Our study is in close conformity with the findings of Hazarika et al. (2013) who also reported variation in sugar:acid ratio among the "hatkora" accessions.

Among the 39 ''hatkora'' germplasms in the present study, the highest value of TSS:acid ratio was recorded in MZU-HAMP-HS-17 (1.75), and the lowest was recorded in MZU-HAMP-HS-6 and MZU-HAMP-HS-26 (0.91). The variation in TSS:acid ratio among different germplasms have been also reported by Madhumathi and Sekhar ([2015\)](#page-9-0) in sweet orange.

The results of the present investigation revealed that there was significant variation in physico-chemical characteristics among different ''hatkora'' germplasms. Preference of consumers always depends on physical parameters of fruits like fruit weight, fruit diameter, pulp content and pulp:peel ratio of any fruit. In ''hatkora'', more fruit weight, bigger size, more pulp content and pulp:peel ratio, greater is the acceptability by the consumer. In addition, consumers prefer the fruits with less seed. Likewise, among the biochemical constituents of the fruits, consumers always prefer the fruits with high juice content, ascorbic acid, low acidity and high sugar:acid ratio. Similarly, for development of a new variety, breeders also choose germplasms with these desirable qualities. From the summary of the present investigation, it has observed that, among all the germplasms of ''hatkora'' collected from different locations of Mizoram, HAMP-MZU-HS-21, HAMP-MZU-HS-17 and HAMP-MZU-HS-7 having all the desirable physical and chemical parameters from the consumers as well as breeders. Therefore, from the present investigation, it can be concluded that HAMP-MZU-HS-21, HAMP-MZU-HS-17 and HAMP-MZU-HS-7 can be considered as elite ''hatkora'' germplasm for use in various purposes from Mizoram, north-east India.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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