Genetic diversity and molecular evolution of Naga King Chili inferred from internal transcribed spacer sequence of nuclear ribosomal DNA

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A B S T R A C T
Sequences of the Internal Transcribed Spacer (ITS1-5.8S-ITS2) of nuclear ribosomal DNAs were explored to study the genetic diversity and molecular evolution of Naga King Chili. Our study indicated the occurrence of nucleotide polymorphism and haplotype diversity in the ITS regions. The present study demonstrated that the variability of ITS1 with respect to nucleotide diversity and sequence polymorphism exceeded that of ITS2. Sequence analysis of 5.8S gene revealed a much conserved region in all the accessions of Naga King Chili. However, strong phylogenetic information of this species is the distinct 13 bp deletion in the 5.8S gene which discriminated Naga King Chili from the rest of the Capsicum sp. Neutrality test results implied a neutral variation, and population seems to be evolving at drift–mutation equilibrium and free from directed selection pressure. Furthermore, mismatch analysis showed multimodal curve indicating a demographic equilibrium. Phylogenetic relationships revealed by Median Joining Network (MJN) analysis denoted a clear discrimination of Naga King Chili from its closest sister species (Capsicum chinense and Capsicum frutescens). The absence of star-like network of haplotypes suggested an ancient population expansion of this chili.
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1. Introduction

Chili is one of the most important crops of the world and has the distinction of being the first plant to be cultivated in the new world. It has been a part of the human diet since at least 7500 BC. Though tropical South America is believed to be the original home of chili (Greenleaf, 1986), chilies are now grown worldwide. The cultivated species of the genus are Capsicum annuum, Capsicum chinense, Capsicum frutescens, Capsicum baccatum, Capsicum pubescens. Chili fruits are rich sources of metabolites such as carotenoids (provitamin A), vitamins (C and E), flavonoids and capsaicinoids that are beneficial for human health (Maga, 1975; Ramchiary et al., 2014). Naga King Chili is considered as India’s hottest chili and was formerly acknowledged as the world’s hottest measuring 1,001,304 Scoville Heat Units (SHU) (Guinness Book of World Records, 2006; Kehie et al., 2012a). However, this chili was superseded by the Infinity chili in 2011, followed by the Naga Viper, the Trinidad Moruga Scorpion in 2012, and the Carolina Reaper in 2013 (Hottest Chili, 2015). Naga King Chili which is known in local Angami dialect as ‘Kedi Chüsi’ which literally means the ‘King of Chilies’, is also known by other local names such as Naga Mirchi, Bhut Jolokia, and Umorok (Kehie et al., 2013). Naga King Chili is native to the Northeastern states of India. Since time immemorial, people of Northeastern region, particularly the Naga people have close sodality with this chili. Nagas are keened to have utilized this chili as remedial agents for treating myriads of ailments. The Government of Nagaland has patented this chili and has registered as the proprietor with the Government of India under Geographical Indication Registry (Kehie et al., 2014). Although earlier studies have treated this chili as C. frutescens, the taxonomic relationship of ‘Naga King Chili’ based on RAPD markers have placed ‘Naga King Chili’ in a taxonomic position between C. chinense and C. frutescens with ‘Naga King Chili’ clustering more closely to the C. chinense group (Bosland and Baral, 2007). Internal Transcribed Spacers (ITS) sequence analysis reported by Purkayastha et al. (2012) showed distinct genetic differences between Bhut Jolokia and the species of C. frutescens and C. chinense. The occurrence of high cross pollination and adaptation to micro-climatic conditions has led to the formation of variants and landraces within the species (Kehie et al., 2012b). However, study on its genetic diversity and molecular evolution remains unexplored as far as our knowledge is concerned. Molecular approach using ITS of nuclear ribosomal DNA (nDNA) has been widely used for resolving phylogenetic relationships among closely related species of angiosperms. Eukaryotic nDNA has two internal transcribed spacers ITS1 and ITS2. The two spacers and the 5.8S subunit are collectively known as the ITS region and it has become an important nuclear locus for molecular systematic investigations of flowering plants. They have frequent insertions/deletions which could be phylogenetically informative (Baldwin et al., 1995). The popularity of the ITS region can
be attributed to the relatively high rate of nucleotide substitution in the transcribed spacers, permitting the systematic comparison of relatively recently diverged taxa (Ghada et al., 2013). The ITS region is influenced by concerted evolution which homogenizes the tandem copies within individuals, thus making ribosomal DNA amenable for phylogenetic inference (Hillis and Davis, 1988; Chiang and Schaal, 2000). However, variations within individuals have been reported primarily as a result of slow concerted evolution (Harris and Crandall, 2000; Coté and Peculis, 2001). In this study, we attempted to explore and investigate the genetic diversity and molecular evolutionary history of Naga King Chili using nrDNA.

2. Methods

2.1. Taxon sampling and genomic DNA isolation

Samples of Naga King Chili were collected from different geographical regions of Northeastern India viz., Nagaland, Manipur and Meghalaya (Table 1). Total genomic DNA was extracted from frozen fruits of Naga King Chili following the method described by Doyle and Doyle (1987) with some minor modifications. The isolated DNA concentration was checked by agarose (1%) gel electrophoresis. The ITS sequence alignments were performed using ClustalX version 2.1 (Thompson et al., 1997). Alignments were subsequently adjusted manually using BioEdit version 7.2.5 (Hall, 1999). The length and GC content of ribosomal DNA sequences of Naga King Chili. DNA amplification was performed in an Applied Biosystems GeneAmp® PCR System 2700, Rotkreuz, Switzerland. PCR reaction volume was 25 μL containing 5 μL of template DNA (50ng/μL), 2 μL 10× PCR buffer (Tris with 15 mM MgCl₂), 1.5 mM MgCl₂, 5 μL primer at 10 pmol, 5 μL dNTPs at 2 mM, 0.2 μL Taq DNA polymerase at 3 U/μL, and 1.3 μL ddH₂O. The thermocycling protocol consisted of a single denaturation step at 95 °C for 5 min, followed by 40 cycles of 1 min denaturation at 94 °C, 1 min annealing at 57 °C, 2 min extension at 72 °C, with a final incubation step at 72 °C for 10 min. To confirm successful amplification and size of the amplified fragment, 10 μL of the PCR product was run on 1% agarose gel in 1× TAE buffer, stained with ethidium bromide, and visualized under UV light. GeneRuler 100 bp Plus DNA Ladder, ready-to-use, 100–3000 bp (Fermentas, In., Glenn Burnie, MD, USA). The PCR amplified product approximately 750 bp corresponding to ITS1, 5.8S, ITS2 and 26S partial sequence were sequenced at the Macrogen Inc. (Seoul, Korea). Amplicons were sequenced directly in both sense and antisense directions. The amplification primers were used as the sequencing primers.

2.3. Sequence alignments and phylogenetic analyses

All sequence information has been deposited in the GenBank database (accession no. KP006656, KP006667, KP006658, KP006659, KP006660, KP006661). Sequences of the whole ITS region of Naga King Chili were used to determine ITS1, 5.8S and ITS2 boundaries by homologous blast with published ITS sequences in the GenBank database. The ITS sequence alignments were performed using ClustalX version 2.1 (Thompson et al., 1997). Alignments were subsequently adjusted manually using BioEdit version 7.2.5 (Hall, 1999). The length and GC content

Table 1

| Species/cultivar | GenBank accession no. | Geographic origin | Elevation (m) | References |
|------------------|-----------------------|-------------------|--------------|------------|
| Naga King Chili  | KP006659              | Phipema, Nagaland, India | 750          | This study |
| Naga King Chili  | KP006658              | Kohima, Nagaland, India | 1444         | This study |
| Naga King Chili  | KP006660              | Ruzaphema, Nagaland, India | 500          | This study |
| Naga King Chili  | KP006657              | Jalukie, Nagaland, India | 347          | This study |
| Naga King Chili  | KP006661              | Shillong, Meghalaya, India | 1525         | This study |
| Naga King Chili  | KP006655              | Imphal, Manipur, India | 786          | This study |

Sources of Capsicum ITS sequences and their geographical origin.

| Source/location | GenBank sequences |
|-----------------|-------------------|
|              | Capsicum spp.       |
| Dibrugarh, Assam, India | HQ705983          |
| Jorhat, Assam, India | HQ705984          |
| Sonitpur, Assam, India | HQ705985          |
| Karbianglong, Assam, India | HQ705986      |
| Ukhurul, Manipur, India | HQ705987          |
| Kohima, Nagaland, India | HQ705988          |
| Tezpur, Assam, India | HQ705989          |
| Sonitpur, Assam, India | HQ705990          |
| Mexico, North America | AY66841          |
| Badajoz, Spain | GU944973          |
| Utah, USA | AF244708          |
| Madison, USA | AY875740          |
| Madison, USA | DQ314158          |

| Species/cultivar | GenBank accession no. |
|------------------|-----------------------|
| Naga King Chili  | KP006656              |
| Naga King Chili  | KP006657              |
| Naga King Chili  | KP006660              |
| Naga King Chili  | KP006658              |
| Naga King Chili  | KP006661              |

| ITS | 5.8S | ITS2 | Entire region |
|-----|------|------|---------------|
| %GC | Length | %GC | Length | %GC | Length | %GC | Length |
| Naga King Chili | KP006659 | 65.5 | 240 | 53.52 | 142 | 68.48 | 237 | 63.87 | 620 |
| Naga King Chili | KP006658 | 62.5 | 240 | 53.52 | 142 | 68.64 | 236 | 62.78 | 618 |
| Naga King Chili | KP006660 | 65.14 | 241 | 53.52 | 142 | 68.64 | 236 | 63.81 | 619 |
| Naga King Chili | KP006657 | 65 | 240 | 53.52 | 142 | 68.64 | 236 | 63.59 | 618 |
| Naga King Chili | KP006661 | 64.16 | 240 | 53.52 | 142 | 69.06 | 236 | 63.19 | 618 |

Table 2

Length and G + C content of ribosomal DNA sequences of Naga King Chili.

| Species/cultivar | GenBank accession number | ITS1 | 5.8S | ITS2 | Entire region |
|------------------|--------------------------|------|------|------|---------------|
| %GC | Length | %GC | Length | %GC | Length | %GC | Length |
| Naga King Chili | KP006659              | 65.5 | 240 | 53.52 | 142 | 68.48 | 237 | 63.87 | 620 |
| Naga King Chili | KP006658              | 62.5 | 240 | 53.52 | 142 | 68.64 | 236 | 62.78 | 618 |
| Naga King Chili | KP006660              | 65.14 | 241 | 53.52 | 142 | 68.64 | 236 | 63.81 | 619 |
| Naga King Chili | KP006657              | 65 | 240 | 53.52 | 142 | 68.64 | 236 | 63.59 | 618 |
| Naga King Chili | KP006661              | 64.16 | 240 | 53.52 | 142 | 69.06 | 236 | 63.19 | 618 |
Selection neutrality was tested by both Tajima’s D (Tajima, 1989) and Fu and Li’s D* and F* methods (Fu and Li, 1993). Demographic parameters were assessed using the distribution of pairwise sequence differences (mismatch distribution) of Rogers and Harpending (1992) and site frequency spectra (distribution of the allelic frequency at a site) of Tajima (1989) using the program DnaSP software version 4.0 (Rozas et al., 2003). Median joining (MJ) network analysis was conducted using software NETWORK 4.6.1.0 (Bandelt et al., 1999; available at http://www.fluxus-engineering.com).

### 3. Results

#### 3.1. ITS sequence analysis

ITS sequence alignment of Naga King Chili and other Capsicum sp. sequences from the GenBank revealed sequence variability in Capsicum sp. It was observed that the variable of ITS1 exceeded that of ITS2. Sequence alignment results of Naga King Chili showed two sites deletion viz., 10 bp and 5 bp (indicated by arrow) in the ITS1 sequence region (Fig. 1); these deletions were also observed in sister species viz., C. chinense and C. frutescens, which were clustered more closely to Naga King Chili. Sequence analysis of 5.8S gene which is 142 bp length showed a much conserved region in all the accessions of Naga King Chili. However, sequence comparison of 5.8S with other Capsicum sp. revealed a distinct phylogenetic signal which discriminated Naga King Chili from the rest of the Capsicum sp. This phylogenetic information is attributable to its distinct 13 bp deletion (indicated by arrow) in the 5.8S gene.

#### 3.2. Sequence diversity, length variation and GC content of ITS sequences

The ITS sequence (ITS1-5.8S-ITS2) obtained from Naga King Chili showed variations in both length and composition (Table 2). The ITS1 spacer was 240 bp in all accessions except accession KP006660, which was 241 bp, with an average of 240.16 bp. The length of ITS2 spacers in all the accession was found to be 237 bp except the accession KP006659, which was 236 bp with an average of 236.16 bp. The GC content of ITS1 ranged from 52.5 to 55.5%, while in ITS2 the GC content ranged from 68.48 to 69.06%. The 5.8S rDNA sequence region had a conserved length of 142 bp, and its GC content varied from 52.81 to 53.52% and was significantly lower than ITS1 and ITS2 in length and GC content. The length of the entire ITS region ranged from 618 to 620 bp with GC content ranging between 62.78 to 63.87%.

### Table 3

Maximum composite likelihood estimate of the pattern of nucleotide substitution.

|      | A | T | C | G |
|------|---|---|---|---|
| (a)  |   |   |   |   |
| A    | – | 0.09 | 0.21 | 42.89 |
| T    | 0.13 | – | 17.96 | 0.18 |
| C    | 0.13 | 7.34 | – | 0.18 |
| G    | 30.61 | 0.09 | 0.21 | – |
| (b)  |   |   |   |   |
| A    | – | 0.04 | 0.05 | 51.54 |
| T    | 0.05 | – | 0.05 | 0.05 |
| C    | 0.05 | 0.04 | – | 0.05 |
| G    | 48 | 0.04 | 0.05 | – |
| (c)  |   |   |   |   |
| A    | – | 0.04 | 0.08 | 62.5 |
| T    | 0.03 | – | 7.89 | 0.06 |
| C    | 0.03 | 3.92 | – | 0.06 |
| G    | 25.28 | 0.04 | 0.08 | – |
| (d)  |   |   |   |   |
| A    | – | 1.74 | 3.36 | 35.86 |
| T    | 1.86 | – | 14.21 | 2.93 |
| C    | 1.86 | 7.35 | – | 2.93 |
| G    | 22.81 | 1.74 | 3.36 | – |

(a) ITS1 spacer, (b) 5.8S gene and (c) ITS2 spacer, (d) ITS entire region of ribosomal DNA. Each entry shows the probability of substitution (r) from one base (row) to another base (column). For simplicity, the sum of r values is made equal to 100. Rates of different trans- and transition substitutions are shown in bold and those of transversional substitutions are shown in italics.

### Table 4

Nucleotide diversity, sequence polymorphism and neutrality test based on ribosomal DNA of Naga King Chili.

|      | ITS1 | 5.8S | ITS2 | ITS Entire region |
|------|------|------|------|-------------------|
| N    | 6    | 6    | 6    | 6                 |
| S    | 20   | 1    | 5    | 26                |
| h    | 4    | 2    | 4    | 6                 |
| h_H | 0.687 | 0.333 | 0.867 | 1                 |
| h_W | 0.03650 (SD = 0.01855) | 0.000308 (SD = 0.000308) | 0.000928 (SD = 0.000928) | 0.01843 (SD = 0.00920) |
| n   | 0.02861 (SD = 0.01486) | 0.00235 (SD = 0.00152) | 0.00876 (SD = 0.00260) | 0.01499 (0.00572) |
| k   | 6.867 | 0   | 2.067 | 9.267 |
| P(JC) | 0.02995 | 0.00236 | 0.00883 | 0.01525 |
| P(i) | 0.333 | 0.333 | 0.333 | 0.667 |
| P(i) | 0.00138 | 0 | 0.00140 | 0.00107 |
| P(i) | 0.02861 | 0.00235 | 0.00876 | 0.01499 |
| Tajima’s D | –0.93302 (P < 0.10) | 0.93302 (P < 0.10) | 1.03194 (P < 0.10) | –1.13197 (P < 0.10) |
| Fu and Li’s D* | –1.36967 (P < 0.10) | –0.95015 (P < 0.10) | –0.21471 (P < 0.10) | –1.17008 (P < 0.10) |
| Fu and Li’s F* | –1.148775 (P < 0.10) | –0.96473 (P < 0.10) | –0.25113 (P < 0.10) | –1.27884 (P < 0.10) |
| Fu’s Fs | 1.798 | –0.003 | –0.439 | –1.075 |

Fig. 1. Aligned mtDNA ITS sequence (ITS1-5.8S-ITS2) of Capsicum sp. The polymorphic sites in all accessions of Naga King Chili in comparison with other species are shown with (*). Sites deletion of 10 bp, 5 bp and 13 bp are shown with arrow.
3.3. Nucleotide composition variation, mutational events and test for neutrality

The ITS sequence (ITS1-5.8S-ITS2) analysis showed that the nucleotide frequencies are 18.85% for adenine (A), 17.56% for thymine (T), 33.95% for cytosine (C), and 29.64% for guanine (G). The transition/transversion rate ratios are $k_1 = 12.233$ (purines) and $k_2 = 4.231$ (pyrimidines). The overall transition/transversion bias is $R = 3.746$. There was a total of 618 positions in the final dataset.

The analysis in Table 3 indicated that in Naga King Chili, transitions were more frequent than transversions at the intergenic spacer in ITS. The results showed that G/A and A/G transition were more frequent than C/T and T/C. The composition variation of nucleotide sequence alignment of ITS within a matrix of 621 characters showed the existence of 592 conserved sites, 26 variable sites containing 3 informative sites and 23 singleton sites. The sequence variation in the intergenic spacer ITS of the ribosomal DNA was detected with 6 haplotypes observed for 6 accessions of Naga King Chili. The haplotype diversity ($H_d$) was found to be 1 whereas nucleotide diversity ($\pi$) was 0.01499. Furthermore, the average difference between pairs of nucleotide ($k$) was calculated (9.267) which showed polymorphism (Table 4).

Neutrality test of Tajima (Tajima, 1989) and Fu and Li (Fu and Li, 1993) statistics were employed to assess whether the sequencing data shows evidence of deviation from neutrality. We obtained negative values which were not significant, which is an indication of neutral variation, and population seems to be evolving at drift–mutation equilibrium with no evidence of selection.

Further examination to assess deviation from neutrality, Fu statistics (Fu, 1997) was employed to detect neutrality. Sequence analysis showed (Table 4; Fig. 2) positive and negative values which were statistically not significant for this parameter. Fu’s $F_s = -0.003$ for 5.8S gene, Fu’s $F_s = -0.439$ for ITS2 and Fu’s $F_s = -1.075$ for ITS entire region. Fu and Li and Fu statistics indicated no evidence for changes in population size or any particular pattern of selection in the regions examined in Naga King Chili. Furthermore, our suggestions were confirmed by the values of raggedness index “r” higher than 0.05 showing values of 0.1067 for the intergenic spacer of ribosomal DNA (ITS) (Fig. 3).

3.4. Mismatch distribution

Analysis of mismatch distribution in the ITS sequences of rDNA confirmed a neutral variation. A frequency graph between alleles showing multimodal aspect of the curve of mismatch indicated demographic equilibrium and not a recent demographic expansion (Fig. 3).

3.5. Haplotype network analysis

The haplotype network was reconstructed based on the whole ITS sequence data of Naga King Chili combining with ITS sequences of Capsicum sp. downloaded from GenBank. Median joining networks were drawn for the haplotypes identified in 19 ITS sequences of Capsicum sp. The network illustrated the relationship between 18 haplotypes (Fig. 4). Evolutionary relationships among haplotypes were inferred using MJN approach, which showed a clear separation between lineages of Naga King Chili (H1, H2, H3, H4, H6, H7, H8, H9, H11, H12, H13) and the rest of the other haplotypes of Capsicum sp. (H5, H10, H14, H15, H16, H17, H18). Haplotype network showed that majority of the Naga King Chili accessions seem to emerge from H3, which is also represented by two accessions (KP006657, KP006660). It may also be inferred from the network analysis that haplotype represented by H13 (KP006656) is the founder haplotype for H6 (KP006659), H7 (HQ705983), H9

![Fig. 2. Spectrum of frequencies of sites at the ribosomal DNA sequences in Naga King Chili. (a) Intergenic spacer of ribosomal DNA (ITS1), (b) 5.8S gene of ribosomal DNA, (c) intergenic spacer of ribosomal DNA (ITS2) and (d) intergenic spacer of ribosomal DNA (ITS). The solid lines in the spectrum indicate the distributions under neutrality and balance (mutation–drift). The index value of Fu is given.](image-url)
(KP006658), and H4 (KP006661) as basic branches were seen to be connected to this haplotype. Network analysis indicated that haplotype H5 (C. chinense) and closely related haplotype 10 (C. frutescens) are likely to be the ancestral haplotype of Naga King Chili accessions as they were more closely clustered around these haplotypes.

4. Discussions

The ITS sequence region is one of the most popular loci used in molecular phylogenetic studies (Alvarez and Wendel, 2003). It is known to evolve relatively quickly and is used for determining inter-specific (Jorgenson and Cluster, 1988) and intra-specific relationships (Baura et al., 1992). Our results showed that the variability of ITS1 with respect to nucleotide diversity and sequence polymorphism exceeded that of ITS2. Two sites deletion (10 bp and 5 bp) in the ITS1 sequence region of Naga King Chili were also observed in sister species viz., C. chinense, C. frutescens and C. pubescens, which were clustered more closely to Naga King Chili. Within the ITS region, the ITS1 sublocus evolved slightly more rapidly with a more variable length than the ITS2 sublocus (Hillis and Dixon, 1991; Hershkovitz and Lewis, 1996). Much attention has been focused on ITS1 as the more variable sublocus of the two and thereby, presumably, the better species marker (Chen et al., 2001; Narutaki et al., 2002; Hinrikson et al., 2005). Sequence analysis of 5.8S gene revealed a much conserved region in all the accessions of Naga King Chili. Within the ITS region, the ITS1 sublocus evolved slightly more rapidly with a more variable length than the ITS2 sublocus (Hillis and Dixon, 1991; Hershkovitz and Lewis, 1996). Mutual attention has been focused on ITS1 as the more variable sublocus of the two and thereby, presumably, the better species marker (Chen et al., 2001; Narutaki et al., 2002; Hinrikson et al., 2005). Sequence analysis of 5.8S gene revealed a much conserved region in all the accessions of Naga King Chili. As known, the 5.8S rRNA region is highly evolutionary conserved in interspecific and intraspecific levels (Hřibová et al., 2011). However, it is important to note that the 13 bp deletion in the 5.8S gene which discriminated Naga King Chili from the rest of the Capsicum sp. provided strong phylogenetic information of this species. Our findings are in agreement with the earlier report of Purkayastha et al., (2012), thus corroborated with more sequence sources of ITS region and denoted a remarkable genetic difference of Naga King Chili with other Capsicum sp.

In our study to identify sequences which do not fit the neutral theory model at equilibrium between mutation and genetic drift, we used Tajima (Tajima, 1989) and Fu and Li tests (Fu and Li, 1993). Our results showed negative values which were not significant, an indication of neutral variation and population evolving at drift–mutation equilibrium with no evidence of selection. The study was further supported by the assessment of Fu statistics (Fu, 1997). In addition, mismatch analysis of the frequency graph between alleles showing multimodal aspect of the curve indicated a demographic equilibrium and not a recent demographic expansion. Phylogenetic methods may not lead to the desired resolution at the intraspecific level due to lower genetic diversity and non-hierarchical nature of intraspecific data sets (Posada and Crandall, 2001) and complementary network approaches might be valuable alternatives to study phylogenetic structures and haplogroups at the population level. Simulations (Woolley et al., 2008) have shown that MJN (Bandelt et al., 1999) outperforms minimum spanning networks (MSN) because the former is able to infer ancestral haplotypes (Woolley et al., 2008). In the present study, haplotype network analysis showed connections between haplotypes and summaries of all the shortest trees possible. Haplotypes of Naga King Chili accessions were clustered separately suggesting that they are a descendant population derived from a common ancestor. Majority of the Naga King Chili accessions seem to emerge from H3. Haplotype H5, H10 clustering closest to haplotypes of Naga King Chili are likely to be the ancestral haplotype. The absence of star-like cluster of nodes in the network indicated a non-demographic expansion and further supported a demographic equilibrium.

5. Conclusion

The results of this study contributed to the knowledge of the existing genetic status of Capsicum sp. Based on the sequence analysis of the nrDNA ITS region, the phylogenetic relationship of Naga King Chili showed a clear grouping from C. chinense and C. frutescens. This work
not only provided more sequence sources of ITS region of Capsicum species but the basis for clearer discrimination of Naga King Chili from other related chili species. It appeared that Naga King Chili population is evolving at drift--mutation equilibrium and free from directed selection pressure. Furthermore, our findings revealed an ancient evolutionary history of this species.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

MK conceived and designed research. MK and KSD conducted experiments. MK, SK, PT contributed reagents or analytical tools. MK analyzed data. MK, SK, PT wrote the manuscript. All authors read and approved the manuscript.

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