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Research Article

Genetic diversity in king chilli (*Capsicum chinense* Jacq.) genotypes through SDS-PAGE

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Abstract
King Chilli is very popular in North East India and exhibits wide variability. In the present study, sixteen (*Capsicum chinense* Jacq.) genotypes were collected from different areas of North Eastern India and evaluated for genetic diversity using seed protein profiling (SDS-PAGE). The seed protein profiling showed distinct polymorphism in electrophoretic banding patterns and led to the detection of total of 79 polypeptide bands. The genotypes showed considerable variation in protein band number ranging from 15-33. On the basis SDS-PAGE analysis the genotypes were grouped into 2 major clusters. The cluster I was further subdivided to sub-cluster whereas cluster II has only one genotype (CHFKC-14). Based on similarity index genotypes CHFKC-8 (Imphal, Manipur) was most distantly related to CHFKC-14 (Arunachal Pradesh) and could be utilized for crossing programme to create more genetic diversity or segregants of desired characteristics through king chilli breeding programmes.

Keywords
King Chilli, North East India, SDS-PAGE, variabilty

Introduction

Hot chilli (*Capsicum chinense* Jacq.) is most commonly grown and consumed in North Eastern India. The word *chinense* meaning "from China" is a misnomer as this species is originated in the Amazon basin (DeWitt and Bosland, 2009). C. *chinense* is known for the impressive morphological fruit variability, which can be characterized by different shapes, colours, sizes, and pungency levels (Baba *et al*., 2015.) Pods are extremely hot and aromatic, with a persistent heat that can last for hours after the pods are eaten and are one of the hottest chillies with a pungency of 1,000,000 SHUs (Scoville Heat Units).

King chilli has been used conventionally by different ethnic communities of Northeastern India in treating various human ailments. In Nagaland, *Capsicum* spp. including Naga chilli is used to tone up body muscles after heavy workouts whereas hot infusions are used for toothache and muscle pain (Bhagowati and Changkija, 2009). High genetic variability exists in the local landraces of Northeast India. There is a tremendous scope to utilize their genetic variability in planned breeding for further development of elite genotypes. However, very little research towards crop improvement of this crop has been accomplished.

Field evaluation of plant material is often laborious and time consuming, especially when a large number of accessions are to be analyzed. Considering these difficulties, the introduction of biochemical techniques has made possible and a more accurate evaluation of genetic variation, bringing greater precision to measures of genetic diversity. Among the biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Cook, 1995; Das and Mukherjee, 1995; Fufa *et al.*, 2005 and Iqbal *et al*., 2005). Electrophoresis of seed proteins is based on the concept that each genotype/cultivar is distinct and relatively homogenous at the genetic level. Seed protein electrophoresis is frequently employed as an additional approach for species identification and as a useful tool for back-tracking the evolution of various groups of plants (Ladizinski and Hymowitz, 1979). Seed protein profiling is generally used for studying genetic diversity and phylogenetic relationship among the king chilli varieties. As seed proteins have the advantage of being scorable from unviable organs or tissues and the electrophoretic protocol for bulk protein is generally simpler than that for isozymes (Cook, 1984). Furthermore, seed proteins used as genetic marker in the study of genetic variation because they are the primary products of structural genes, any change in the coding sequence of a gene generally reflects the corresponding change in the primary structure of protein (Srivalli *et al*., 1999). Keeping these points into view the study was

conducted to characterize the king chilli genotypes by seed protein profiling (SDS-PAGE).

Materials and Methods

The genotypes were collected from different region of North East India (Table 1). The genotypes were grown in naturally ventilated poly-house in the year 2016 at College of Horticulture and Forestry, Pasighat, Arunachal Pradesh. The chilli seeds were harvested and further analysis was done. The morphological characteristics of different genotypes are presented in Table 1.

Polyacrylamide gel electrophoresis in presence of denaturing agent (SDS) was carried out as per procedure described by Laemmli (1970) with some modifications. Seed sample (1 g) was macerated in mortar and pestle with 5 ml of buffer (0.06 M Tris-HCl, 2.5% Glycerol, 0.5% SDS, 1.25% β-mercaptoethanol, 0.1% TCA, 10 mM urea, 1 mM EDTA) (Yatung *et al*. 2014). The sample was homogenized and kept in water bath at 90^0C for 5 minutes for denaturation of protein. The soluble seed proteins were subjected to SDS-PAGE in gel slabs of 1mm thickness (5% stacking and 12% separating gels). The SDS-PAGE was carried out at a constant current of 25 mA and the gel was kept running until the bromophenol blue reaches the bottom of separating gel. Silver staining was performed as method described by Mortz *et al.* (2001). The gel was first sensitized by 0.02% sodium thiosulphate solution for 5 minutes and washed twice with double distilled water for 1 minute. It was then transferred to staining solution and kept on gel rocker for 20 minutes in dark. After washing with distilled water it was transferred to developing solution and finally the reaction was stopped with 12% acetic acid solution. Gel was washed with double distilled water before visualizing the dark brown band. The electrophorograms were prepared on the basis of protein mobility and the density expressed in Rm values.

The gels were scored as presence (+) or absence (-) of protein polypeptide bands. Depending upon the presence or absence of polypeptide bands, similarity index (SI) (Nei and Li, 1979, Yatung, 2014) between the genotypes was calculated by the following formula:

$$SI = \left(\frac{2Z}{X+Y}\right) X\ 100$$

Where, Z= Number of similar bands between the genotypes, and

X+Y =Total number of bands in the two genotypes compared.

Cluster analysis UPGMA (Unweighted pair group method with arithmetic mean analysis) by using statistical software SPSS for windows package (Version 21).

Result and Discussion

The seed storage profiling showed distinct polymorphism in electrophoretic banding patterns and led to the detection of total of 79 polypeptide bands (Fig. 2). Polymorphism was evident in all storage proteins fraction of the selected king chilli genotypes on the basis of their molecular weight. Genotypes were distinguished on the bases of presence and absence of protein bands at particular Rm value (Supplementary Table). The genotypes showed considerable variation in protein band number ranging from 15-33 with Rm value 0.09 to 0.97. Among the genotypes, CHFKC-15 showed maximum number (33) of protein bands while the minimum number (15) of bands was present in genotypes CHFKC-14. Whereas 21 bands were found in genotype CHFKC-2, CHFKC-8, CHFKC-9 and CHFKC-13. 31 bands in were present in CHFKC-10 and CHFKC-11 while 26 in CHFKC-5 and CHFKC-12.

Band number 1 was present in genotype CHFKC-15 and CHFKC-16 only. Band number 13, 15 and 29 were exclusively present in genotype CHFKC-15 only. Band number 5 in genotype CHFKC-13, band number 9 in genotype CHFKC-10, band number 11 in genotype CHFKC-12, band number 36 band number 44 in CHFKC-6, band number 46 in CHFKC-5, band number 53 in CHFKC-8 and band number 73 in CHFKC-7 were solely present. Band number 23 was present in maximum (11) number of genotypes.

The similarity index coefficient matrix utilizing SDS-PAGE analysis for the sixteen genotypes of king chilli under study has been presented in Table. 2 and the dendrogram generated with matrix value has been presented in Fig. 1. Similarity index generated by SDS-PAGE analysis in the germplasm under study ranged from 0.19 to 1.00. Values of similarity index of coefficient matrix suggested least similarity index of genotype CHFKC-9 with CHFKC-14 (0.11) while genotype CHFKC-8 was found to have maximum similarity index with genotype CHFKC-9 as evident by coefficient value 0.67 (Table 2).

On the basis SDS-PAGE analysis the genotypes were grouped into 2 major clusters (Cluster I and II) (Table 3). The cluster I was further subdivided to sub-cluster whereas cluster II has only one genotype CHFKC-14. Dendrogram based on SDS-PAGE banding pattern grouped genotype CHFKC-10 with CHFKC-3, CHFKC-4, CHFKC-12 and CHFKC-13 while genotypes CHFKC-6 with

CHFKC-5, CHFKC-8, CHFKC-9, CHFKC-11 and CHFKC-15 (Fig.1 and Table 3) which was under sub cluster IA.. CHFKC-1 was grouped with

CHFKC-2 and CHFKC-16 while CHFKC-7 was alone which were placed in one major cluster IB based on the dendrogram (Fig. 1 and Table 3)

The genotypes showed considerable variation in band number of protein in present investigation which ranged from 15-33 which is in concordance with the reports of Kumar and Tata (2010), Kumar *et al.* (2010), Yatung *et al*. (2014) and Peddakasim *et al*. (2015). Among the genotypes CHFKC-15 showed maximum number (33) of protein bands while the minimum numbers (15) of bands were present in genotype CHFKC-14.

Cluster analysis utilizing SDS-PAGE banding patterns produced a dendrogram depicting clear separation of genotypes. Similarity index generated by SDS-PAGE analysis in the germplasm under study ranged from 0.11 to 1.00. Values of similarity index of coefficient matrix suggested least similarity index of genotype CHFKC-9 with CHFKC-14 (0.11) while genotype CHFKC-8 (Imphal ,Manipur) was found to have maximum similarity index with genotype CHFKC-9 (Tseipama, Nagaland) as evident by coefficient value 0.67.

It is evident from the present study that genetic relationship estimated from protein banding pattern enhanced the resolution of diversity and thus provided a better picture of variability as compared to morphological markers. Although SDS-PAGE analysis could show discrete variation among few genotypes of king chilli under study, this protein marker should be applied in future to more number of genotypes to arrive at a reasonable conclusion.

The SDS-PAGE showed considerable variation in protein band numbers in sixteen genotypes. Accessions from different regions seemed to be closely related and accessions from the same region had different genetic back ground. Intra regional diversity could be as a valuable source as inter regional diversity for king chilli improvement. Molecular studies were useful to confirm the genetic diversity based on morphological characters and to characterize these landraces for more detailed examination. Based on similarity index genotypes CHFKC-8 was most distantly related to CHFKC-14 and could be utilized for crossing programme to create more genetic diversity or segregants of desired characteristics through king chilli breeding programmes.

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Table 1. Source of landrace and their morphological characters

S. N.	Genotype	Place of Collection	Stigma exertion	Ripe fruit colour	Fruit shape	Fruit shape at blossom end	Fruit bearing	Fruit position
1.	CHFKC-1	Along (Arunachal Pradesh)	Exerted	Orange red	Triangular	Blunt	Solitary	Pendant
2.	CHFKC-2	Palin (Arunachal Pradesh)	Same level with anther	Dark red	Campanulate	Pointed	Solitary	Pendant
3.	CHFKC-3	Yazali (Arunachal Pradesh)	Exerted	Red	Triangular	Blunt	Solitary	Pendant
4.	CHFKC-4	Kurungkumey (Arunachal Pradesh)	Exerted	Red	Triangular	Blunt	Solitary	Pendant
5.	CHFKC-5	Mebo (Arunachal Pradesh)	Same level	Red	Campanulate	Sunken	Solitary	Pendant
6.	CHFKC-6	Pasighat (Arunachal Pradesh)	Exerted	Red	Campanulate	Pointed	Solitary	Pendant
7.	CHFKC-7	Kiyit (Arunachal Pradesh)	Exerted	Orange red	Elongate	Pointed	Solitary	Pendant
8.	CHFKC-8	Imphal (Manipur)	Exerted	Orange red	Elongate	Sunken & pointed	Solitary	Pendant
9.	CHFKC-9	Tseipama (Nagaland)	Exerted	Dark red	Triangular	Sunken	Solitary	Pendant
10.	CHFKC-10	Daporijo (Arunachal Pradesh)	Exerted	Red	Elongate	Pointed	Solitary	Pendant
11.	CHFKC-11	Mariyang (Arunachal Pradesh)	Exerted	Dark red	Campanulate	Blunt	Solitary	Pendant
12.	CHFKC-12	Pasighat (Arunachal Pradesh)	Exerted	Red	Campanulate	Pointed	Solitary	Pendant
13.	CHFKC-13	Dimapur (Nagaland)	Exerted	Orange red	Triangular	Sunken	Solitary	Pendant
14.	CHFKC-14	Mariyang (Arunachal Pradesh)	Exerted	Red	Triangular	Sunken	Solitary	Pendant
15.	CHFKC-15	Pasighat (Arunachal Pradesh)	Exerted	Red	Campanulate	Sunken	Solitary	Pendant
16.	CHFKC-16	Along (Arunachal Pradesh)	Exerted	Dark red	Campanulate	Blunt	Solitary	Pendant

Table 2. Comparison of scorable protein bands among 16 king chilli genotypes

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CHFKC-1	0.00															
CHFKC-2	0.45	0.00														
CHFKC-3	0.38	0.45	0.00													
CHFKC-4	0.31	0.24	0.42	0.00												
CHFKC-5	0.36	0.38	0.49	0.39	0.00											
CHFKC-6	0.14	0.36	0.34	0.27	0.36	0.00										
CHFKC-7	0.22	0.37	0.20	0.27	0.19	0.15	0.00									
CHFKC-8	0.25	0.43	0.23	0.29	0.51	0.40	0.37	0.00								
CHFKC-9	0.25	0.24	0.45	0.34	0.55	0.4	0.37	0.67	0.00							
CHFKC-10	0.28	0.46	0.52	0.35	0.35	0.29	0.29	0.27	0.35	0.00						
CHFKC-11	0.16	0.27	0.48	0.35	0.35	0.47	0.21	0.35	0.42	0.42	0.00					
CHFKC-12	0.31	0.30	0.45	0.35	0.31	0.44	0.37	0.26	0.51	0.42	0.42	0.00				
CHFKC-13	0.45	0.38	0.36	0.29	0.30	0.31	0.21	0.29	0.29	0.23	0.31	0.60	0.00			
CHFKC-14	0.29	0.17	0.16	0.34	0.29	0.26	0.19	0.28	0.11	0.17	0.35	0.20	0.33	0.00		
CHFKC-15	0.31	0.30	0.32	0.30	0.37	0.35	0.24	0.45	0.37	0.38	0.41	0.31	0.33	0.21	0.00	
CHFKC-16	0.38	0.51	0.29	0.21	0.18	0.29	0.34	0.31	0.15	0.41	0.24	0.27	0.36	0.12	0.35	0.00

Table 3. Major cluster produced by SDS-PAGE analysis in sixteen king chilli genotypes

Cluster	Sub cluster	Sub-sub cluster	Genotypes
I	I A	I A 1	CHFKC-5, CHFKC-6, CHFKC-8, CHFC-9, CHFKC-11 and CHFKC-15
		I A 2	CHFKC-3, CHFKC-4, CHFKC-10, CHFKC-12 and CHFKC-13
	I B	I B 1	CHFKC-7
		I B 2	CHFKC-1, CHFKC-2 and CHFKC-16
II			CHFKC-14

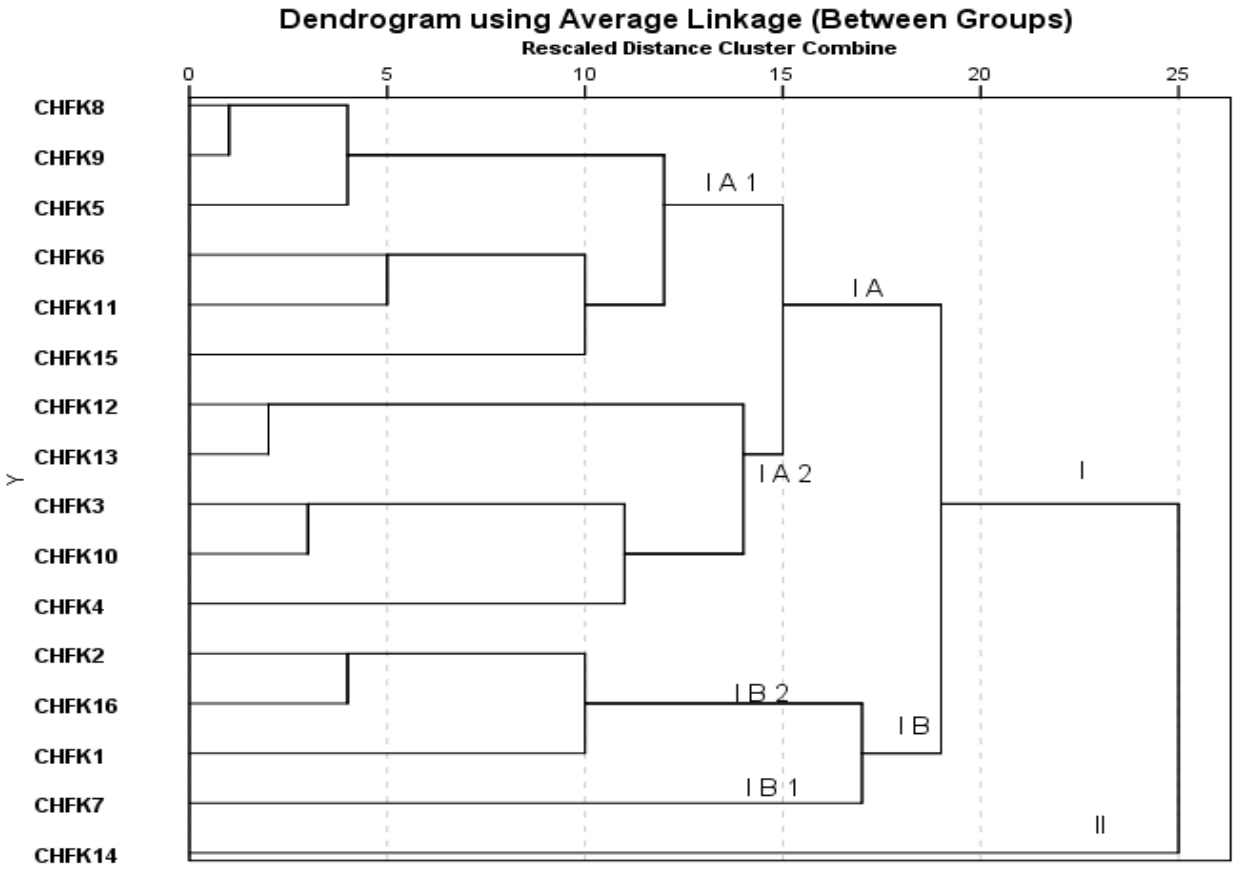

Fig. 1. UPGMA of sixteen king chilli genotypes based on total Seed protein profiles obtained by SDS-PAGE.

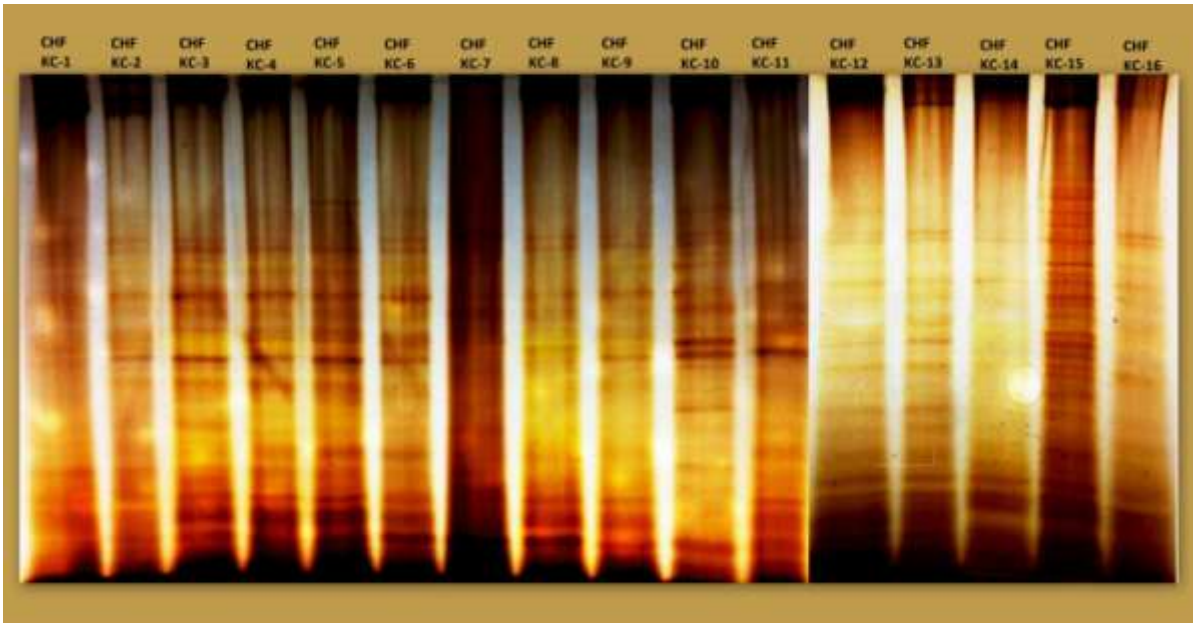

Fig. 2. SDS Banding pattern of sixteen different king chilli genotype


CHFKC-2


CHFKC-6


CHFKC-5


CHFKC-4


CHFKC-8


CHFKC-9

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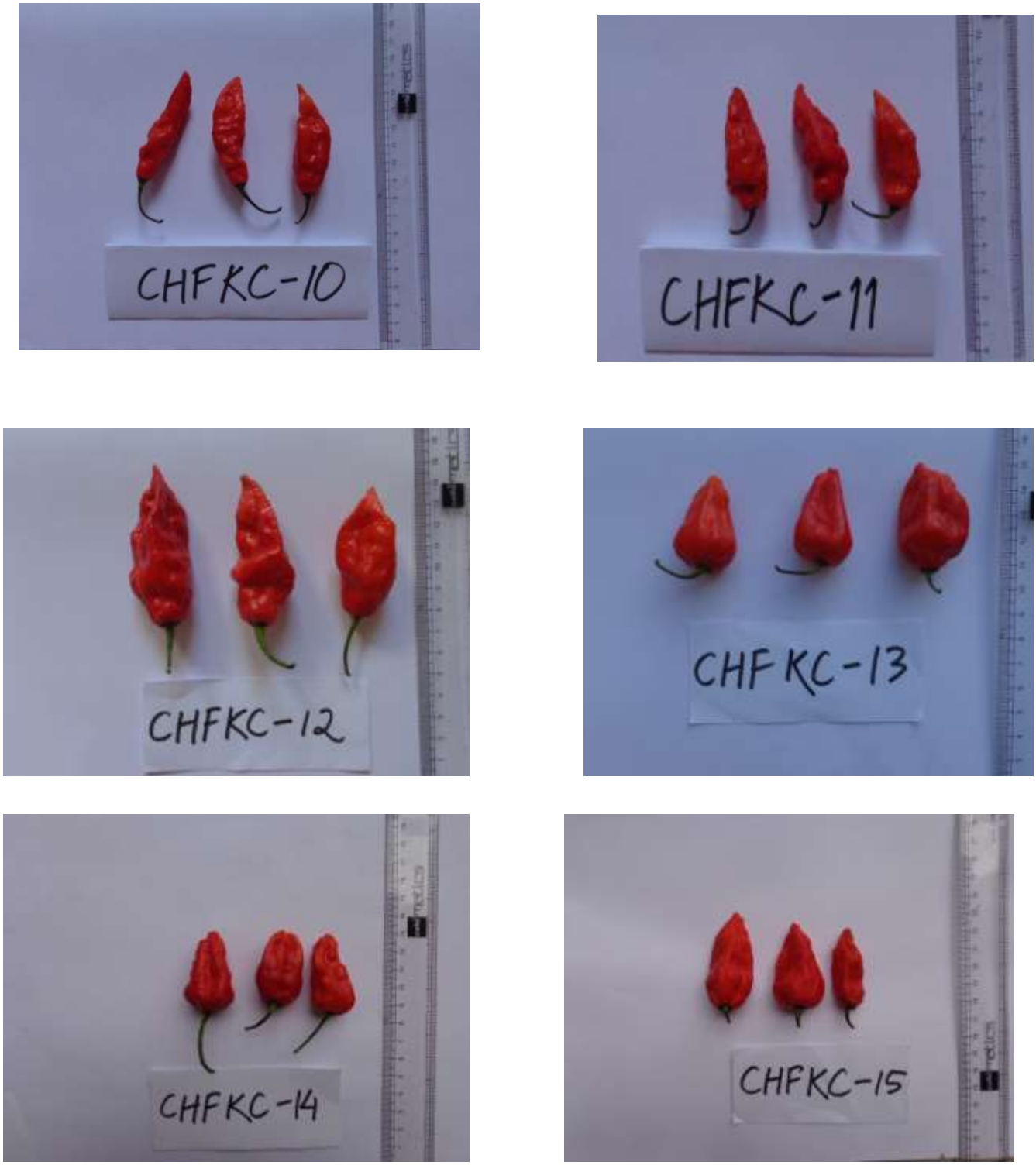

Fig. 3. Diversity of king chilli fruits from North-East India

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