Tropical Agricultural Research Vol. 29 (2): 218 – 224 (2018)
Short Communication

Molecular Diversity Analysis of Conserved *Capsicum chinense* Jacq. Germplasm in Sri Lanka

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ABSTRACT: *Among the Capsicum species, Capsicum chinense has the highest pungency, the highest antioxidant activity and a wide genetic diversity. This study was conducted to evaluate the genetic diversity of C. chinense (Nai miris) germplasm conserved at the Plant Genetic Resources Centre, Gannoruwa, Sri Lanka. Twenty five C .chinense germplasm, two C. annuum, two C. frutescens and one C. baccatum were analysed using 27 Simple Sequence Repeat markers. Total number of amplified alleles was 108 which varied from 1 to 6 per locus. The mean polymorphism information content value was 0.46. Dendogram based on Nei's genetic distances showed three main clusters where 88% of C. chinense germplasm was grouped into one cluster and C. annuum and C. frutescens were separated into two clusters. A high genetic diversity was observed within C. chinense cluster. The genetic diversity identified in this study will be useful for correct identification, conservation and breeding activities of Capsicum species.*

Keywords*: Capsicum chinense, Capsicum species, genetic diversity, molecular characterization*

INTRODUCTION

Chilli (*Capsicum* spp.) is one of the major spice crops all over the world. It is daily consumed by one quarter of the world's population, and the rate of consumption is ever growing. Chilli accounts for 16% of the total spice trade in the world, occupying the second position after black pepper (FAOSTAT, 2013).

Chilli belongs to family *Solanaceae* and consists of 27 species (Onus and Pickersgill, 2004; Ince *et al*., 2009) including five domesticated *Capsicum* species: *Capsicum annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L., and *C. pubescens* Ruiz & Pav. Among the five domesticated *Capsicum* species, *Capsicum annuum* (Common chilli), *C. chinense* (*Nai miris*) and *C. frutescens* (*Kochchi*) are commonly cultivated in Sri Lanka. *C. chinense* and *C. frutescens* species have special characteristics such as resistant to pest and disease incidences, high pungency and resistant to drought conditions (Kannangara, 2013). *C. chinense*, known as hot chilli or hot pepper is the hottest species among the *Capsicum* species which has Scoville Heat Unit (SHU) score over 1.5 million, having the uppermost

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extent of phenolic compounds (Zhang and Hamauzu, 2003). It reveals that *C. chinense* has the highest antioxidant activity and it is known to be an excellent source of phytochemicals, including Vitamins A and C, phenolic compounds, flavonoids and carotenoids (Zhang and Hamauzu, 2003). In addition, *C. chinense* has a wide diversity in traits such as pod colour, pod length, pod size, pod shape, pod weight, plant height, capsaicin content and pungency level (Finger *et al*., 2010). Due to the unique aromatic flavor, *C. chinense* has a high demand as an appetizer, success in breeding (can artificially cross with *C. annuum*) (Costa *et al*., 2009), less germination problems compared to *C. frutescens* and can get a good yield for a long period due to its perennial nature (Kannangara, 2013).

Sri Lanka has a diverse collection of *C. chinense* at Plant Genetic Resources Center (PGRC), Gannoruwa but this diversity has not been properly evaluated (Kannangara, 2013). The evaluation of genetic diversity of *C. chinense* is useful to study the available diversity and for proper species identification and systematic conservation. Further, identification of potential *C. chinense* varieties enhances the *C. chinense* breeding programmes and cultivation in Sri Lanka. Thus, present study was conducted to evaluate the genetic diversity of *C. chinense* accessions conserved at PGRC using SSR markers and clarified misidentified accessions.

MATERIALS AND METHODS

Plant material

A total of 30 *Capsicum* accessions including 25 *C. chinense* accessions, two *C. annuum* accessions, two *C. frutescens* accessions and one *C. baccatum* accession were obtained from PGRC, Gannoruwa, Sri Lanka were used for this study (Table 1). Molecular analysis was conducted at the Biotechnology laboratory of PGRC, Gannoruwa, Sri Lanka.

Table 1. *Capsicum* accessions used for the study

Sample Number	Accession/Collection Number	Common Name	Sample Number	Accession/Collection Number	Common Name
1	C-2014-09-171	*Nai miris*	16	C-2012-3-157	*Nai miris*
2	C-2015-10-90	*Kaha Nai miris*	17	C-2012-2-72	*Nai miris*
3	C-2015-10-91	*Rathu Nai miris*	18	C-2014-6-50	*Nai miris*
4	C-2012-03-206/1	*Nai miris*	19	C-2014-6-51	*Nai miris*
5	C-2012-08-227/1	*Nai miris*	20	C-2014-6-54	*Thakkali miris*
6	C-2015-12-123	*Nai miris*	21	C-2012-3-20	*Nai miris*
7	C-2015-11-106	*Kaha Nai miris*	22	C-2012-3-26	*Nai miris*
8	AC# 13424	*Nai miris*	23	C-2012-3-34	*Nai miris*
9	AC# 13480	*Nai miris*	24	C-2012-3-36	*Nai miris*
10	C-2015-12-119	*Nai miris*	25	C-2012-3-40	*Nai miris*
11	AC# 01058	*C. baccatum*	26	C-2012-8-229	*Nai miris*
12	C-2012-08-240	*Amu miris*	27	C-2012-8-236	*Dam Nai miris*
13	C-2013-06-87	*Maalu miris*	28	C-2012-3-35	*Nai miris*
14	C-2015-08-66	*Kochchi*	29	C-2012-8-258	*Keum Miris*
15	C-2015-08-69	*Kochchi*	30	C-2014-7-115	*Nai miris*

Plant DNA extraction

DNA was extracted from fresh leaves of 30 samples using modified CTAB method (Saghai-Maroof *et al*., 1984) Immature leaf samples were used for DNA extraction based on the population bulk strategy (Rebourg *et al*., 2001; Cota-Sanchez *et al.*, 2006) to represent the alleles in the whole population. Extracted raw DNA samples were quantified by comparing with the band intensity of a known DNA quantity (λ DNA) in on a 0.8% agarose gel to make it compatible for SSR (Simple Sequence Repeats) analysis.

PCR amplification

Amplification was conducted using 27 SSR markers selected from a database (Minamiyama *et al*., 2006) considering the PIC value (Polymorphism Information Content) for *C. annuum* and *Capsicum* relative species, number of alleles and linkage group.

With the total volume of 15 µl PCR reaction mixture was prepared using 15 ng/µl concentrated template DNA of selected *Capsicum* samples, 5x PCR reaction buffer, 25 mM $MgCl_2$,10 Mm dNTP, forward and reverse primers, sterile distilled water and Taq DNA polymerase enzyme (Promega). The amplification was done through a touch down PCR programme of 35 cycles which has 7 touch down cycles and 28 normal cycles. Initial denaturation step at 95 °C for 5 minutes followed by 3 steps namely, denaturation step at 95 °C for 1 minute, annealing step for 2 minutes, extension step at 72 °C for 1 minute and final extension at 72 °C for 10 minutes. Amplification was performed in a 96 well Takara Thermal Cycler. PCR products were analyzed using 8% polyacrylamide gel electrophoresis (PAGE) in 1x TBE buffer. The gels were visualized under UV light using BIO RAD gel documentation.

Data scoring and analysis

The banding pattern for each marker was scored manually as present (1) and absent (0). Genetic diversity was estimated using these scored data obtained from 30 *Capsicum* germplasm for 27 SSR markers through PowerMarker software version 3.25 (Liu and Muse, 2005). Genetic distance was computed using Nei's 1983 (Nei *et al*., 1983) and dendogram was constructed using Unweighted Pair Group Method with Arithmetic averages (UPGMA) algorithm.

RESULTS AND DISCUSSION

Allelic diversity

According to the Summary Statistics computed using PowerMarker software version 3.25, the total number of alleles observed across 27 *C. chinense* populations was 108. Allelic richness was varied from one to six. Six alleles were amplified by CAMS199 and CAMS647 and only one allele was amplified by the marker CAMS236 which was identified as a monomorphic marker in this study. The allelic diversity among the tested *Capsicum* accessions is shown in Table 2. According to the analysis data, it showed high allelic diversity among the tested *C. chinense* accessions.

Table 2. Summary statistics of tested alleles across 25 *C. chinense* germplasm

Marker	Number of amplified alleles	PIC*	Marker	Number of amplified alleles	PIC*
CAMS806	3	0.14	CAMS460	5	0.72
CAMS156	5	0.73	CAMS644	4	0.5
CAMS351	5	0.57	CAMS826	3	0.3
CAMS072	4	0.61	CAMS142	4	0.63
CAMS153	2	0.37	CAMS451	4	0.24
CAMS199	6	0.59	CAMS864	5	0.42
CAMS117	5	0.73	CAMS015	3	0.39
CAMS405	2	0.27	CAMS101	3	0.42
CAMS606	4	0.48	CAMS236	1	0
CAMS162	5	0.44	CAMS340	5	0.44
CAMS855	4	0.42	CAMS844	3	0.46
CAMS647	6	0.72	CAMS679	4	0.42
CAMS885	3	0.39	CAMS336	4	0.59
CAMS684	3	0.5	Mean	4	0.46

** Polymorphic Information Content*

In the study of Dhaliwal *et al.*, 2014 which considered 64 endemic and exotic *Capsicum* species, the number of alleles amplified by these markers was lower than the present study; three alleles by CAMS647, 2 alleles by CAMS117 and three alleles by CAMS072.

The Polymorphism Information Content (PIC) values provide an estimate of discriminating power of a marker by considering not only the number of alleles at a locus but also relative frequencies of these alleles (Dhaliwal *et al*., 2014). Therefore, PIC value is an effective parameter to study the allele diversity. In this study the PIC value ranged from zero to 0.73 with mean the value of 0.46. This value is lower than the mean PIC value recorded in the study of Gonza *et al*., 2014 as 0.66 for 102 *Capsicum* germplasm characterized with 39 SSR markers; Dhaliwal *et al*., 2014 using 50 SSR markers with 64 *Capsicum* accessions found mean PIC value of 0.59; Kwon *et al*., 2005 using 27 SSR markers for 66 chilli varieties, to assess the potential of SSR markers for variety identification by comparing SSR markers and morphological traits in tests of distinctiveness, uniformity, and stability (DUS), the mean PIC value as 0.52. Yumnam *et al.*, 2012 too has recorded the mean PIC value as 0.52, a study which has conducted using 50 SSR marker to identify the genetic diversity of 53 chilli accessions from North Eastern India. This difference might be due to the use of other closely related wild *Capsicum* species and *C. annuum* landraces in those studies and in the present study only domesticated *Capsicum* germplasm was used. Hence, less mean PIC value was obtained.

The frequency based distance values provide a greater confidence for the assessment of genetic diversity and relationship among the concerned species. According to the distance matrix, the genetic distance of the present study ranged from 0.11 to 0.86 within the tested *Capsicum* germplasm. The highest genetic distance value was recorded as 0.86 between sample number 4 (C-2012-03-206/1) and 27 (C-2012-8-236). Both of these samples are *C. chinense* and they are the most distantly related accessions in the selected *Capsicum* germplasm. Most closely related samples are sample number 28 (C-2012-3-35) and 30 (C-

2014-7-115) with lowest genetic distance value as 0.11. It revealed that, there is a high genetic diversity prevailed among *Capsicum* species as well as within the selected *C. chinense* germplasm.

According to the constructed dendogrm (Figure 1), three main clusters can be identified at the distance of 0.05. *C. annuum* species have clustered in Cluster -1 with two *C. chinense* samples, sample number 4 (C-2012-03-206/11) and 5 (C-2012-08-227/1). C. *frutescens* species have clustered separately in Cluster-2 with *C. baccatum* and *C. chinense* sample number 8. But *C. baccatum* showed a higher genetic distance from rest of the accessions in this cluster. This revealed that, *C. baccatum* is more related to *C. frutescens.* Hence, it might be another misidentification or an inter-species cross. The rest, 88% of *C. chinense* have clustered in Cluster-3 with three sub clusters. In the *C. chinense* sub clusters, there was a low genetic distance among the samples.

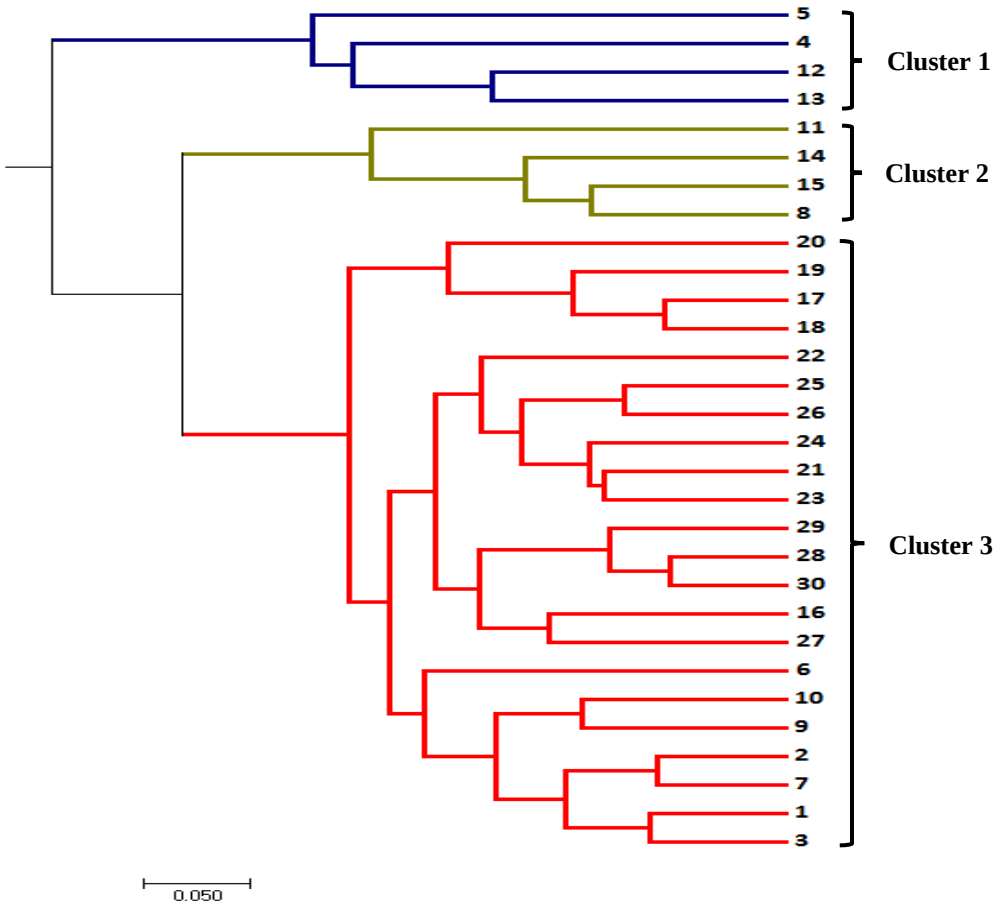

Figure. 1. Dendogram of 30 *Capsicum* germplasm based on Nei's genetic distance

(1- C-2014-09-171, 2- C-2015-10-90, 3- C-2015-10-91, 4- C-2012-03-206/1, 5- C-2012-08-227/1, 6-C-2015-12-123, 7- C-2015-11-106, 8-AC# 013424, 9-AC# 013480, 10- C-2015-12-119, 11-AC# 01058, 12-C-2012-08-240, 13- C-2013-06-87, 14- C-2015-08-66, 15- C-2015-08-69, 16- C-2012-3-157, 17- C-2012-2-72, 18- C-2014-6-50, 19- C-2014-6-51, 20-C-2014-6-54, 21- C-2012-3-20, 22- C-2012-3-26, 23- C-2012-3-34, 24- C-2012-3-36, 25- C-2012-3-40, 26- C-2012-8-229, 27- C-2012-8-236, 28- C-2012-3-35,

CONCLUSIONS

The present study revealed that there is a high genetic diversity among selected *Capsicum* species and within the tested *C. chinense* germplasm. The set of SSR markers used for this study is adequate to characterize most of the *C. chinense* accessions conserved in the Plant Genetic Resources Center Gannoruwa, Sri Lanka. Based on genetic distance, these accessions grouped into 3 main clusters. Some of the *C. chinense* accessions showed similarities with other *Capsicum* species. The genetic diversity identified in this study will be useful in *C. chinense* breeding programs and for conservation activities.

ACKNOWLEDGEMNET

This work was funded by the Department of Agriculture and Integrated Management of Plant Genetic Resources (IMPGR) (Grant no. 285-2-2-4-2502-15-(13)).

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