



## Original article

## Variation of small erect-fruited chili in Thailand

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## ABSTRACT

Single cross varieties of small, erect-fruited chili (*Prik-khee-noo* in Thai) are required because of their high yield and high quality. Production of a single cross needs superior parental lines. This study was conducted to screen for parental lines which could be used for the production of superior single cross. To develop new varieties, high variation of the parental lines is required. Twenty-eight chili accessions (*Capsicum annuum* L.) cultivated in Thailand were collected and evaluated for variation with 343 single nucleotide polymorphic (SNP) markers at the Molecular Marker Laboratory at Hortigenetics Research (S.E. Asia) Ltd., Chiang Mai, Thailand. The results showed low variation among the 28 chili accessions with the polymorphism information content varying from 0.45 to 0.49 with an average of 0.46. The average genetic distance estimated from the SNP markers based on Jaccard's coefficient was 0.29 (ranging from 0.03 to 0.55). However, based on SNP analysis, the unweighted pair-group method with arithmetic averages divided the 28 chili accessions into two main groups and four subgroups. The first group consisted of 23 accessions mostly collected from varieties cultivated in Northeastern Thailand. The second group contained five accessions collected from different locations in other regions of Thailand.

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## Introduction

Chili is an indispensable spice used as a basic ingredient in a great variety of cuisines all over the world being used as a flavoring, a colorant and to add tang and taste to otherwise bland food (Kothari et al., 2010). Chili contains seven times more vitamin C than oranges and the beta-carotenoids (vitamins C and A) in chili are powerful antioxidants that destroy free radicals (Simonne et al., 1997). The small, erect-fruited chili (*Prik-khee-noo* in Thai), is very important economically in Thailand, and extensively used for cooking and processing. Chili is the economic vegetable in Thailand. The total planting area and the quantity of the product for chili production in 2006/2007 were 75,955 ha and 333,672 ton, 8.1 ton/ha, over 60% of which was devoted to small erect-fruited chili. The main production area for small erect-fruited chili is in Northeastern Thailand followed by the North and the East

(Khanobdee, 2009; Department of Agriculture and Cooperation, 2013). Farmers plant both the F<sub>1</sub> hybrid and open-pollinated varieties with a ratio of about 20:80. Market varieties are available from various types—*Capsicum annuum* and *Capsicum frutescens* are the main species of cultivated chili. The commercial varieties, particularly the hybrid varieties are *C. annuum*. Consequently, distinguishing chili varieties, which have narrow genetic variation from each other, becomes more difficult (Sithiwong et al., 2005). Identification of *Capsicum* is traditionally based upon morphological characteristics and hybridization studies (Ince et al., 2010).

However, to classify chili breeding lines using morphological traits is time and labor consuming and is affected by the environment (Rodriguez et al., 1999). Evaluation using DNA marker analysis is not affected by the environment and has been suggested for determination of genetic similarity among genotypes (Tanksley and Orton, 1983). Single nucleotide polymorphic (SNP) markers are particularly useful because of their low cost per data point, high genomic abundance, locus-specificity, co dominance, potential for high throughput analysis and lower genotyping error rates (Rafalski, 2002; Schlotterer, 2004). SNPs have emerged as a

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powerful tool for many genetic applications, including genetic diversity studies, linkage and quantitative trait loci mapping, and marker-assisted breeding (Zhu et al., 2003) and are easily detected using polymerase chain reaction (PCR). Their information value is high compared to that of other markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) according to Testolin and Cipriani (2010). The current research objective was to screen for parental lines which could be used in the production in Thailand of superior, single cross *Prik-khee-noo* using SNP markers and to determine the relationships of these chili lines to assist chili breeders to select parental lines.

## Materials and methods

### Plant material

Twenty-eight accessions of small, erect-fruited chili (*C. annuum* L.) comprising Thai cultivars were sourced from the main production areas of *Prik-khee-noo* in Northeastern (10 accessions), Western (4 accessions), Northern (2 accessions), and Central Thailand (3 accessions), Asian Vegetable Research Development Center (7 accessions) and from a private company (2 inbred lines) and were planted at the Spa Agricultural Farm, Chiang Rai, Thailand. Leaves were sampled and fruit characteristics were recorded Table 1.

### DNA extraction and polymerase chain reaction analysis of for single nucleotide polymorphic markers

Genomic DNA was extracted from the 28 accessions using the modified CTAB method of Doyle and Doyle (1990). The quantity and quality of the genomic DNA pool were determined using a spectrophotometer (NanoDrop 8000; Thermo Scientific, Marietta, OH, USA.). DNA profiling of the 28 accessions was performed in 96-well PCR plates. SNP genotyping was conducted at the Molecular Marker Laboratory at Hortigenetic Research (S.E. Asia) Ltd., Chiang Mai, Thailand. The 390 SNP markers used in this investigation were developed by Dr. Allen Van Deynze from the University of California, Davis, CA, USA.

Single nucleotide polymorphism profiles were obtained using the KASPar™ system (KBioscience; Hertfordshire, UK.) genotyping assay with Douglas™ technology (Douglas Scientific; Alexandria, MN, USA.). One reaction of PCR contained 1.62 µL total volume, consisting of 0.8 µL of 15 ng/µL genomic DNA, 0.8 µL of 1X KASPar master mix and 0.02 µL of primer mix. The cycling started with an initial phase of 10 min at 95 °C, then 10 cycles for 20 s at 94 °C, 60 s at 65 °C for the first cycle and thereafter with a 0.8 °C decrease

each cycle, and finally with constant PCR, 40 cycles for 20 s at 94 °C and 60 s at 57 °C.

### Data analysis for genetic diversity of chili accession

For the SNP marker analysis, the PCR products plates were read in a fluorescent plate reader and the data were plotted and scored using in-house software (East West Seed; Chiang Mai, Thailand) based on the polymorphism of each allele. Genetic similarities among different cultivars were calculated using Jaccard's coefficient (Jackson et al., 1989). The polymorphism information content (PIC) was calculated based on polymorphism scoring from SSR and SNP analysis using Equation (1):

$$PIC = 1 - \sum_{i=1}^k P_i^2 - 2 \sum_{i=1}^{kk} \sum_{j=i+1}^{kk} P_i^2 P_j^2 \quad (1)$$

where  $P_i^2$  is the genotype frequency of the  $i$ th allele and  $P_j^2$  is the genotype frequency of the  $j$ th allele and  $k$  is the number of markers.

Twenty-eight chili accessions (*C. annuum* L.) cultivated in Thailand were characterized with 343 SNP markers at the Molecular Marker Laboratory at Hortigenetic Research (S.E. Asia) Ltd., Chiang Mai, Thailand. Cluster analysis used the unweighted pair-group method with arithmetic averages (UPGMA) and the graphical genotype program version 2.0 software (Van Berloo, 1999). The dendrogram construction test was confirmed using the cophenetic correlation ( $r$ ) following Sokal and Rohlf (1962), which can range from 0 to 1, with  $r = 0.7$ – $0.8$  mean dendrogram construction being fair,  $0.8$ – $0.9$  mean dendrogram construction being good and  $r = 0.90$ – $1$  mean dendrogram construction being very good.

### Parental line selection

Because the two inbred lines included in this study were produced from the leading variety of small, erect-fruited chili in Thailand, the genetic distances (GD) between the two inbred lines (7303F and 7303M) and a subgroup of them were used for parental line selection reference. The chili accessions were selected for new parents if they had a greater genetic distance than either of the two inbred lines. Therefore, the source may relate to the diversity of accession and the additive genetic variance of fruit length is related to the yield component (Singh et al., 2014). The accession source and fruit length were used as additional criteria when the chili accessions had similar GDs.

**Table 1**  
Twenty-eight chili accessions collected in Thailand.

No.	Accession name	Source	No.	Accession name	Source
1	7303F	East West Seed	15	51(Pic-som2)	Srisaket
2	7303M	East West Seed	16	Huarea S.K.13	Ubonratchatane
3	KKU-P11003	KhonKaen	17	51(AFM1081)	Chiang Mai
4	KKU-P11039	KhonKaen	18	Huysiton KS	Kalasin
5	CA 1180	AVRDC	19	51(Srisaket_OP)	Srisaket
6	CA 1131	AVRDC	20	Chinda chumsang	Nakhonsawan
7	CA 860	AVRDC	21	51(CokepotalSBI)	Audtaradit
8	CA 758	AVRDC	22	Mung30 Ct	Ubonratchatane
9	CA 1340	AVRDC	23	Srisawat-18	Kanchanaburi
10	CA 1164	AVRDC	24	51HeatTol-1	Srisaket
11	Chaiprakan OP	Chiang Mai	25	51(Tong Kao CT)	Srisaket
12	Srisawat-48	Kanchanaburi	26	51(Santi_ct)	Srisaket
13	Srisawat-43	Kanchanaburi	27	Chinda OP97302	Nakhonsawan
14	Srisawat-38	Kanchanaburi	28	CA 1828	AVRDC

AVRDC = asian vegetable research development center.

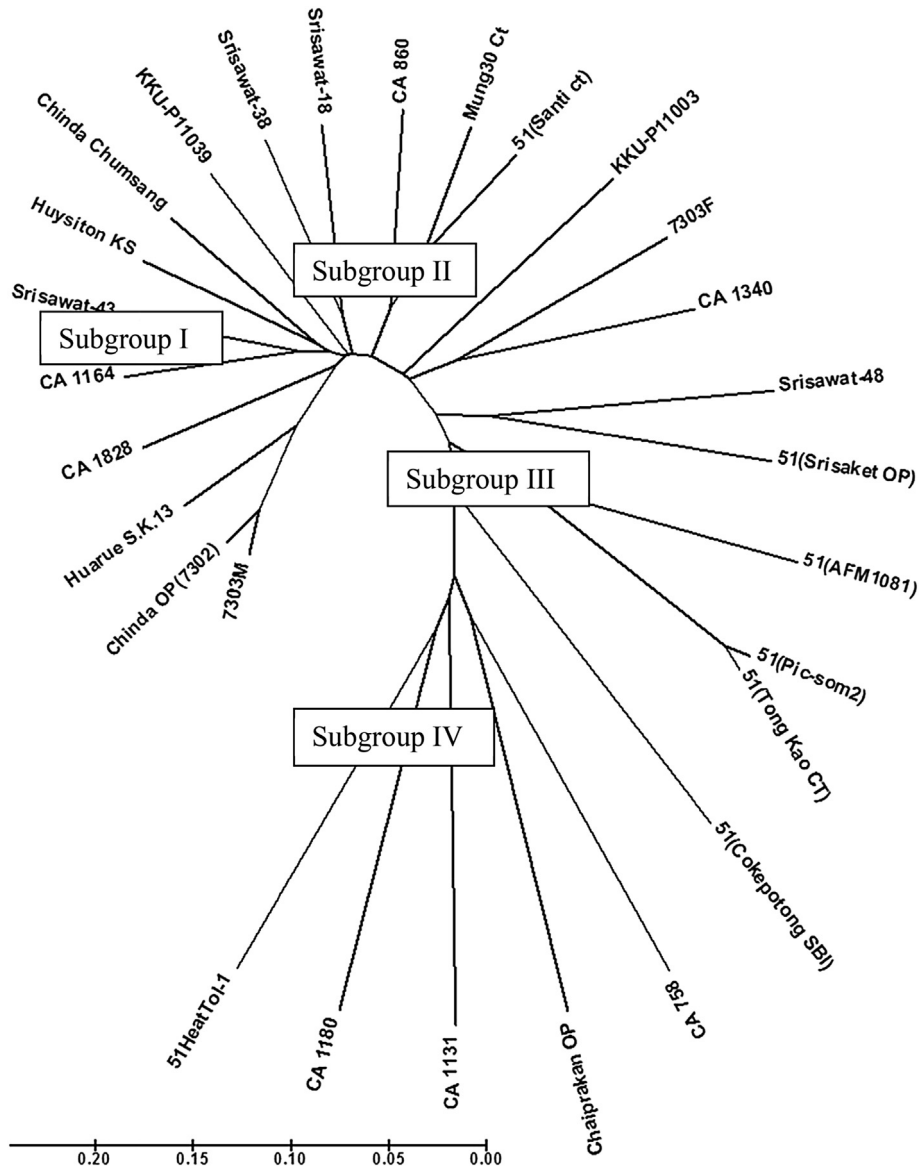


Fig. 1. Dendrogram of 28 small, erect-fruited chili accessions based on Jaccard's coefficient (Jackson et al., 1989) using unweighted pair-group method with arithmetic averages.

## Results

### Single nucleotide polymorphism analysis

Polymorphism was shown in 343 of the 390 SNP markers and this was used to investigate the genetic diversity of the 28 chili accessions. The average SNP polymorphism information content (PIC) was 0.46 (range 0.45–0.49). Based on the polymorphism information, the GD was calculated resulting in a mean for the 28 chili accessions of 0.29 (range 0.03–0.55). The GD between the two inbred lines was 0.30. The widest genetic distance (0.55) was observed between CA 1180 and 51 (Cokepotong SBI).

### Cluster analysis

Based on the GD, cluster analysis using UPGMA classified the 28 chili accessions into two main groups with a cophenetic correlation coefficient of 0.90. The first group, which was further categorized into three subgroups, contained 23 accessions with 61% originating from local cultivars in Northeastern Thailand. The first subgroup

contained 11 accessions—Huarue S.K. 13, CA 1828, CA 1164, Huysiton KS, Chinda Chumsang, KKU-P11039, Chinda OP(7302), Srisawat-43, Srisawat-18, Srisawat-38 and 7303M. The other two subgroups consisted of 12 accessions of which six were collected from the same field and in the same year, with a closer GD of 0.03, namely 51 (Pic-som2) and 51 (Tong kao CT). The second main group comprised five accessions of which three came from AVRDC, namely CA 758, CA 1131 and CA 1180. The other two accessions (Chaiprakarn OP and 51Heat Tol-1) were collected from Northern and Northeastern Thailand Fig. 1.

### Parental line selection

Parental line selection was completed using the genetic distance and subgrouping as reference criteria. Six of the 28 chili accessions—CA 1180, CA 1131, 51 (Cokepotong SBI), CA 758, 51Heat Tol-1 and Chaiprakarn OP—were selected because they had higher GDs than the reference (0.30) and the different subgroup of 7303F. The selected parents were collected from difference sources (AVRDC, Chiang Mai, Uttaradit and Si Sa Ket), and there was variation in the

**Table 2**  
Genetic distance of 28 small, erect-fruited chilis based on Jaccard's coefficient (Jackson et al., 1989).

Code	Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
1	7303F	0																											
2	7303M	0.3																											
3	KKU-P11003	0.3	0.2																										
4	KKU-P11039	0.3	0.2	0.2																									
5	CA 1180	0.4	0.5	0.4	0.5																								
6	CA 1131	0.3	0.4	0.4	0.4	0.4																							
7	CA 860	0.2	0.3	0.3	0.2	0.4	0.3																						
8	CA 758	0.4	0.4	0.4	0.4	0.4	0.4	0.4																					
9	CA 1340	0.2	0.2	0.3	0.2	0.5	0.3	0.3	0.4																				
10	CA 1164	0.3	0.2	0.2	0.2	0.4	0.4	0.2	0.4	0.2																			
11	Chaiprakan OP	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3																		
12	Srisawat-48	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3																	
13	Srisawat-43	0.2	0.2	0.3	0.2	0.4	0.4	0.2	0.4	0.2	0.2	0.3	0.3																
14	Srisawat-38	0.3	0.2	0.3	0.2	0.4	0.4	0.3	0.4	0.3	0.2	0.3	0.3	0.2															
15	51(Pic-som2)	0.3	0.3	0.3	0.3	0.5	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.3														
16	Huarue S.K.13	0.3	0.1	0.2	0.2	0.5	0.4	0.3	0.4	0.2	0.2	0.3	0.3	0.2	0.2	0.3													
17	51(AFM1081)	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.5	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.3												
18	Huysiton KS	0.3	0.2	0.3	0.2	0.5	0.4	0.2	0.4	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.3											
19	51(Srisaket_OP)	0.3	0.2	0.4	0.3	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3										
20	Chinda chumsang	0.3	0.2	0.3	0.2	0.5	0.4	0.2	0.4	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.3									
21	51(Cokepotong SBI)	0.4	0.3	0.4	0.3	0.5	0.4	0.4	0.5	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.4								
22	Mung30 Ct	0.3	0.2	0.3	0.3	0.5	0.3	0.2	0.4	0.2	0.3	0.4	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3							
23	Srisawat-18	0.3	0.2	0.3	0.2	0.4	0.4	0.2	0.4	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.3	0.4					
24	51HeatTol-1	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.5	0.4	0.3					
25	51(Tong Kao CT)	0.3	0.3	0.3	0.3	0.5	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.4				
26	51(Santi_ct)	0.3	0.2	0.3	0.2	0.5	0.3	0.2	0.4	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.1	0.2	0.4	0.3	
27	Chinda OP(7302)	0.3	0.1	0.2	0.2	0.4	0.4	0.3	0.4	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.1	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.2
28	CA 1828	0.3	0.2	0.2	0.2	0.4	0.3	0.2	0.4	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.3	0.2	0.4	0.3	0.2	0.4	0.2

fruit length which ranged from 1.34 to 6.45 cm (data not shown) Table 2.

## Discussion

Two accessions, namely 51(Pic-som2) and 51(Tong kao CT) had the closest GD values of 0.03 and 7303M was very similar to Chinda OP(7302/3M) with a GD value of only 0.05. The results indicated that those lines could have come from the same source and could be sister lines. The information from this study will be useful for breeders studying the genetic diversity of small, erect-fruited chili in Thailand and can be used as a guideline for parental lines selection and for discarding duplicated germplasm. Nevertheless, the low average GD of 0.29 found in these germplasms indicated there was low variation among these accessions because they belonged to the same type (erect fruited) and species (*C. annuum*). These findings were similar to those of Lefebvre et al. (2001) and Heras et al. (1996) who used RFLP and RAPD results, and concluded that pepper cultivars of *C. annuum* had a homogenous genetic background and lacked genetic diversity. Ljun and Xuexiao (2014) also found low genetic polymorphism within *C. annuum* germplasm collections using inter simple sequence repeat.

However, from the current study, six accessions were selected as new parents to develop a single cross hybrid which was expected to be a better hybrid because the parents had higher GDs than the reference, a different subgroup and were sourced from elite lines (7303F and 7303M) with varying fruit length. Further study on genetic diversity in the same type of chili requires more germplasm from outside Thailand to determine the optimum number of markers for genetic diversity detection to provide savings in both cost and time.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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