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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 Hortigenetic 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.



## 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 $F_1$ 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):

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

where $Pi^2$ is the genotype frequency of the ith allele and $Pj^2$ is the genotype frequency of the jth 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 | Ubonratchatanee |
| 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 | Ubonratchatanee |
| 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.