GENETIC UNIFORMITY OF EXOTIC MEDICINAL PLANT GYNURA DIVARICATA IN THAILAND

Ongkarn Vanijajiva and Nuntachai Kundee
Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok 10220

Corresponding author, e-mail: vanijajiva@pnru.ac.th

ABSTRACT

Gynura divaricata (L.) DC., is a traditional medicinal herb used as an important treatment in several diseases such as diabetes, hypertension and inflammation. This study, Sequence related amplified polymorphism (SRAP) markers were used to detect genetic variation among 55 populations of exotic medicinal G. divaricata in Thailand. Twenty-eight SRAP primer combinations produced a total of 180 fragments with an average of 6.43 per primer combination. The genetic uniformity was detected in G. divaricata within Thailand indicating all accessions likely consist of the same genotype. The complete lack of heterozygosity confirms that genetic bottlenecks occurred during the species' spread and cultivation. Its obligate asexual reproduction in introduced ranges which human-mediated may have contributed to its no genetic variation in Thailand.

Keywords

Gynura divaricata, SRAP, Genetic diversity, Asteraceae

INTRODUCTION

Exotic species also termed nonindigenous, nonnative, introduced and alien, are organisms which are not native to a certain area, but instead have been introduced there accidentally or intentionally by anthropogenic activity (Vanijajiva and Kadereit, 2009). Most alien plants introduced and established around the world were introduced for such as food, ornamental

and medicinal purposes. Gynura divaricata (L.) DC., for example, is recently introduced into Thailand because of its medicinal importance in use for treatment of many diseases such as diabetes, hypertension and inflammation (Chen et al., 2009).

Gynura divaricata belongs to the genus Gynura, comprises of 44 species mainly distributed in paleotropical region (Vanijajiva, 2009), of Sunflower family (Asteraceae). It is considered to be native to eastern Asia (Vanijajiva and Kadereit, 2011), which is called “Bai Bei San Qi” in Chinese. In Thailand, the species is popularly known as Juk-Na-Rai or Pae-tum-puong Poeaim and Vanijajiva (2010). Morphologically, G. divaricata is a herbaceous plant about 50-120 cm when flowering with fleshy in its upper parts but woody and procumbent at the base, by having ascending scapose or leafy flowering shoots, and ribbed stems which are usually purple tinged when dried (Vanijajiva and Kadereit, 2011). The G. divaricata was divided into three subspecies subsp. divaricata, subsp. barbareifolia and subsp. formosana (Davies, 1979). Vanijajiva and Kadereit (2011) noticed that the overall appearance of three subspecies of G. divaricata is very similar as they differ in small morphological details. Therefore, the identification of different subspecies of the species is difficult when based only on morphological characteristics.

Despite the importance of investigating the introduction and mechanism of spread, few studies to date has assessed the genetic diversity and structure in introduced populations of G. divaricata in Thailand (Vanijajiva and Kundee, in press). Molecular genetic markers have developed into a powerful tool to analyze genetic characterization and species identification. Sequence-related amplified polymorphism (SRAP), is a novel molecular marker first introduced by Li and Quiros (2001), with advantages such as high polymorphism, repeatability, easy operation, universal, and free combination of positive and negative primers. At present, SRAP technique has been successfully applied in many plant species (Agarwal et al., 2008; Sun et al., 2013).

The objective of the present study is to assess the genetic diversity and population structure among and within populations of G. divaricata from 55 populations in Thailand, and to evaluate the efficiency of SRAP markers in this species. This study will provide useful information for this introduced medicinal herb in Thailand.

MATERIALS AND METHODS

Plant materials

DNA isolations were carried out using fresh leaf of samples from 55 accessions collected across Thailand (Figure 1). All leaf tissue samples were stored in plastic zipper bags containing silica gel for shipping or transport to the laboratory then immediately frozen and stored at -20 $^{\circ}$C until DNA extraction. Voucher specimens of all accessions were deposited in the Phranakhon Rajabhat University Herbarium.

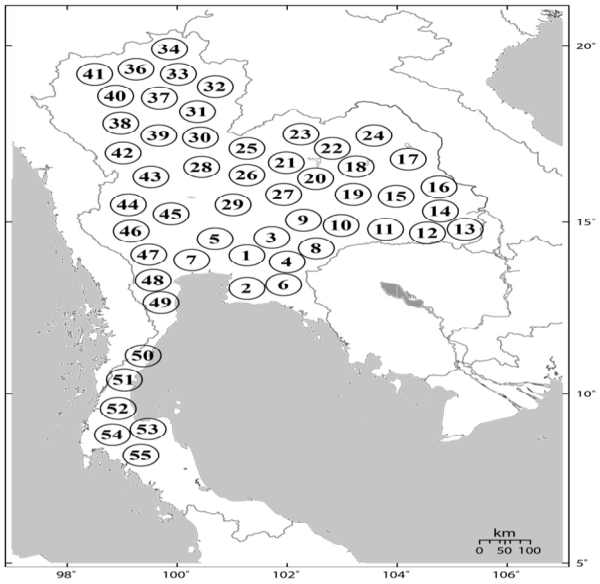

Figure 1 Locations where plants were collected in Thailand.

Genomic DNA extraction

Genomic DNA was extracted from the leaves of 55 accessions using the CTAB method with minor modification (Vanijajiva, 2012). The leaves (500 mg) were ground in a mortar with a pestle. Extraction buffer [(1% (w/v) CTAB, 50 mM Tris–HCl (pH 8), 0.7 M NaCl, 0.1% β-mercaptoethanol)] 500 µl was added and the solution was incubated at 60 C° for 30 min. The homogenate was mixed with 25:24:1 phenol: chloroform: isoamyl alcohol (v/v/v) by gentle inversion. After centrifugation at 13,000 rpm for 15 min, the upper aqueous layer was transferred to a fresh tube. RNA was removed by treating with 2.5 µl of the RNase (10 µg/µl) for 30 min at 37 °C. The extraction of DNA with phenol/chloroform/isoamyl alcohol was repeated one more time. DNA in the solution was precipitated with 0.6 volume of ice-cold isopropanol and washed with 70% ethanol. Following this, the DNA was extracted using CTAB DNA extraction protocol without RNase. The process was repeated until the DNA pellet was free of color (two to three times) and the final pellet was dissolved in sterile deionized water. DNA quality and quantity were determined on 0.8% agarose gel. The DNA was stored at -20 C°, for further use as templates for PCR amplification. The quality of DNA was also evaluated by reading the absorbance at 260 and 280 nm.

SRAP analysis

Primer pairs used in this study were synthesized by Ward Medic Ltd., Part. Thailand (Table 1). The PCR was performed using a Thermohybaid Px2 (Roche Molecular Systems, Inc., USA). The PCR reaction mixtures (25 µl total volume) consisted of 10x Reaction Buffer, 100 ng template DNA, 0.6 mM dNTP mixture, 5 mM $MgCl_2$, 1 unit of Taq polymerase and 0.6 µM of each primer. The SRAP amplification conditions were 5 min initial denaturation at 94 °C and 5 cycles consisting of 1 min denaturation at 94 °C, 1 min primer annealing at 35 °C, and 2 min extension at 72 °C. After the following 30 cycles, the annealing temperature was increased to 50 °C and a final 8 min extension at 72 °C.

Table 1 SRAP primers used in this study

Forward primer	Sequence (5'-3')	Reverse primer	Sequence (5'-3')
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
		Em6	GACTGCGTACGAATTGCA

The SRAP products were all analysed by agarose (1.8% w/v) gel electrophoresis at 150 A for 30 minutes in 0.04 M TAE (Tris–acetate 0.001 M-EDTA) buffer pH 8. The gels were stained with ethidium bromide (10 mg/ml). The gels were viewed and photographed by Bio-Imaging System (Syngene, Genegenuis). To determine SRAP profiles, the size of each DNA band was inferred by comparison with a 100 bp DNA ladder (Promega), used as a molecular weight marker (M). Polymorphisms at all loci were confirmed by three repeating tests for each primer at different times.

RESULTS AND DISCUSSION

DNA isolation

DNA extracted from G. divaricata leaf using a modified Vanijajiva (2012) gave a good and sufficient quality DNA for PCR reaction, and the amount of DNA extracted from the accessions ranged from 150 to 225 µg/g fresh weight leaf material. The ratios of A260/A280 varied from 1.65 to 1.92. The quality of DNA was also tested by PCR, which confirmed that the DNAs were suitable for PCR reaction.

Genetic diversity detected by SRAP analysis

SRAP is a new marker technique based on PCR and it has good repeatability and stability. In this study, it is the first time to analyse the

genetic variation of G. divaricata using SRAP marker. The results showed that SRAP is an economic, efficient, and reliable technique. A total of 30 different primer combinations were employed using five forward and six reverse primers. About 28 of them could produce clear and reproducible bands and were chosen in the following study. A total of 180 bands ranging from 100 to 2000 bp were observed (Figure 2 and 3). All G. divaricata were genetically identical based on the SRAP analysis.

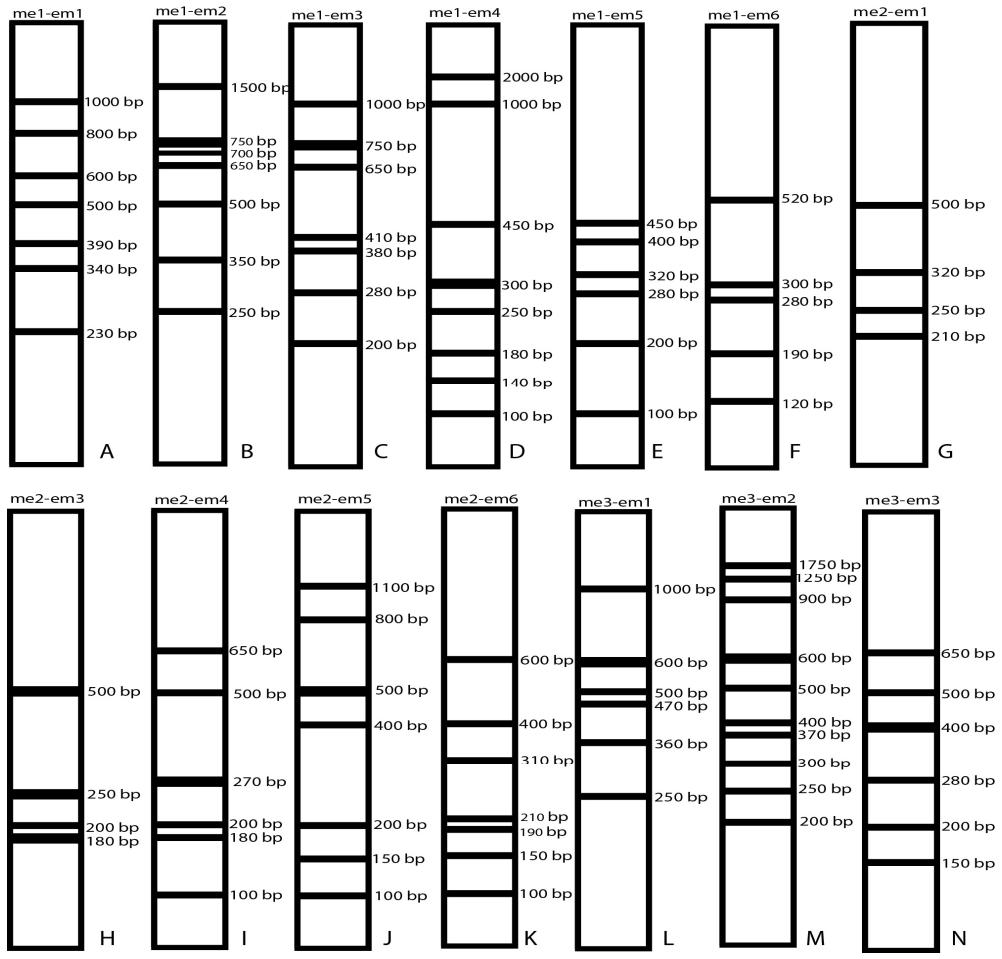

Figure 2 SRAP fingerprints of Gynura divaricata from 14 primer combination: A (me1/em1), B (me1/em2), C (me1/em3), D (me1/em4), F (me1/em5), G (me1/em6), H (me2/em3), I (me2/em4), J (me2/em5), K (me2/em6), L (me3/em1), M (me3/em2), N (me3/em3).

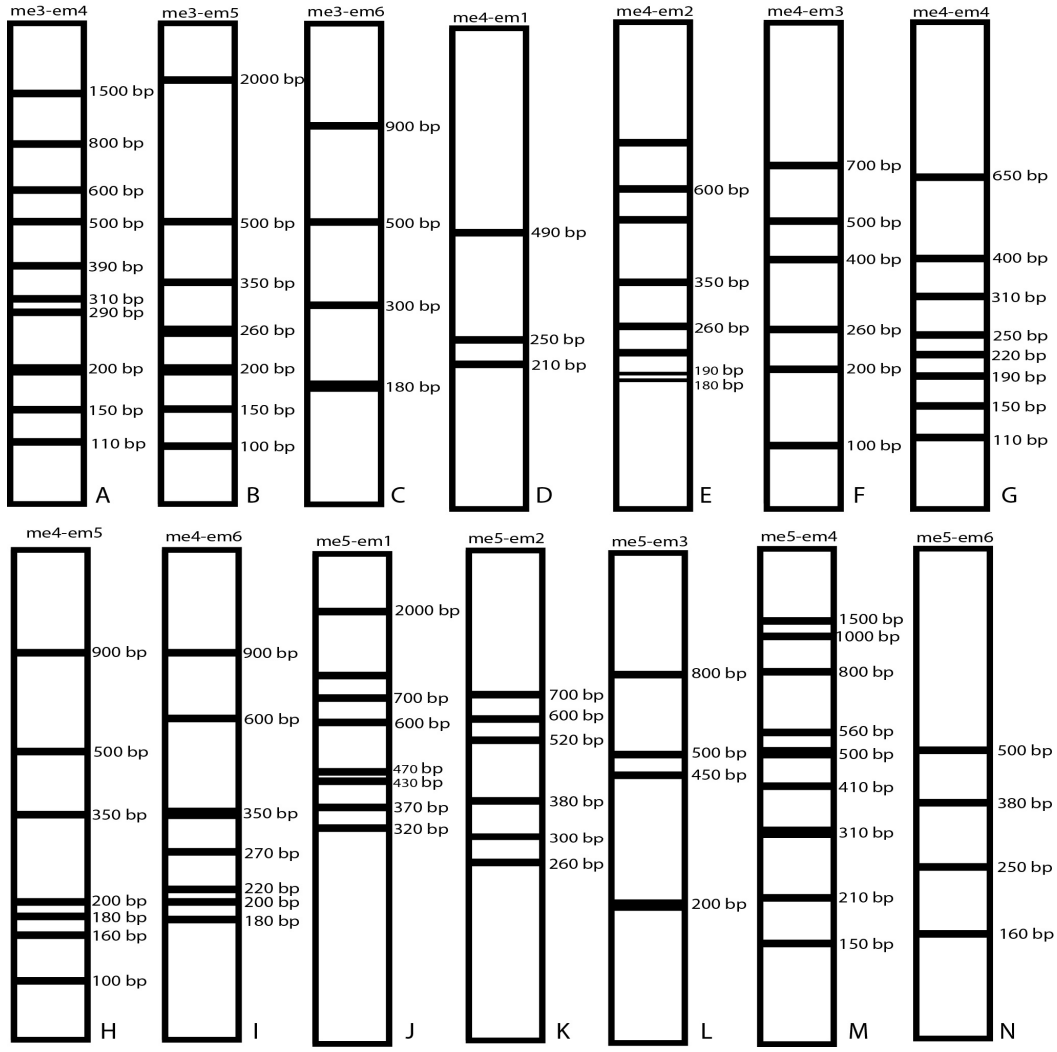

Figure 3 SRAP fingerprints of Gynura divaricata from 14 primer combination: A (me3/em4), B (me3/em5), C (me3/em6), D (me4/em1), F (me4/em2), G (me4/em3), H (me4/em4), I (me4/em5), J (me4/em6), K (me5/em2), L (me5/em3), M (me5/em4), N (me5/em6).

This present study of genetic diversity by SRAP markers confirmed our previous report by RAPD and ISSR marker that there is no genetic variation within and among G. divaricata populations in Thailand (Vanijajiva and Kundee, in press). Novak and Mack (2005) noticed that the low levels of

genetic diversity may be interpreted as lost during a colonization bottleneck, or possibly as the consequence of the possession of certain life-history traits, including asexual reproduction. This agreed with Vanijajiva and Kundee (in press) who suggested that sexual reproduction in this introduced plant might rarely occur and demonstrated that vegetative propagation is the predominant mode of reproduction in Thailand. The results indicated that colonization events including human assisted introductions often involve severe reduction in population size and permanent isolation from a larger parental population. The genetic consequences of the founding event may include rapid alteration and loss of genetic variation.

CONCLUSION

In conclusion, this is the first report of using SRAP markers as a tool for determining genetic variation in Gynura species. Our research indicated that SRAP is a simple, efficient and inexpensive DNA marker technique that is useful for assessing the genetic diversity of exotic medicinal G. divaricata. Lack of genetic variability displayed by this exotic herb could indicate that vegetative reproduction has an important influence on the genetic structure of G. divaricata, and suggest that one introduction or multiple introductions of similar genotypes for the species in Thailand. Although no genetic variation of the species in Thailand is revealed here, comparison of genetic diversity of G. divaricata from different parts of the world, particularly from its origin region, will be needed in order to understand the overall genetic structure of this medicinal herb.

ACKNOWLEDGEMENTS

The authors are grateful to Asst. Prof. S. Poeaim, King Mongkut's Institute of Technology Ladkrabang for supplying initial plant materials. This work was financially supported by the Higher Education Research Promotion and National Research University Project of Thailand (Grant No. 2556A14062001).

REFERENCES

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. **Plant Cell Reports**, 27(4), 617-631.

Chen, L., J.J. Wang, H.T. Song, G.G. Zhang & L.P. Qin. (2009). **New cytotoxic cerebroside from Gynura divaricata.** Chinese: Chemical Letters

Davies, F. G. (1979). The genus Gynura (Compositae) in eastern Asia and the Himalayas. **Kew Bulletin**, 33, 629-640.

Li G. & Quiros C.F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. **Theor. Appl. Genet.** 103, 455–461

Novak S.J. & Mack R.N. (2005). Genetic bottlenecks in alien plant species – influence of mating systems and introduction dynamics. Sinauer: MA.

Poeaim S. & Vanijajiva O. (2010). **Morphological Identification and In Vitro Cytotoxic Evaluation of Crude Extracts from Jak-Na-Rai (Gynura divaricata (L.) DC.).** 16th AAS. and 1st ISAT., pp. 436- 439

Sun, B., Fu, C., Yang, C., Ma, Q., Pan, D., & Nian, H. (2013). Genetic Diversity of Wild Soybeans from Some Regions of Southern China Based on SSR and SRAP Markers. **Am. J. Pl. Sci**. 4, 257-268.

Vanijajiva, O. (2009). The genus Gynura in Thailand. **Thai J. Bot**. 1, 25-36.

Vanijajiva, O. (2012). Assessment of genetic diversity and relationships in pineapple cultivars from Thailand using ISSR marker. **J. Agric. Tech**. 8, 1829-1838.

Vanijajiva, O. & Kadereit, J.W. 2009. Morphological and molecular evidence for interspecific hybridisation in the introduced African genus Crassocephalum (Asteraceae: Senecioneae) in Asia. **Systematics and Biodiversity.** 7, 269-276.

Vanijajiva O. & Kadereit J.W. (2011). A Revision of Gynura. **Asteraceae: Senecioneae**. 49, 285-314.

Vanijajiva, O. & N. Kundee. (2013). Genetic characterization of introduced medicinal plant Gynura divaricata in Thailand International Conference on Interdisciplinary Research and Development in ASEAN Universities. 8-10 August, 2013 Imperial Mea Ping Hotel, Chiang Mai, Thailand.