

## หลักฐานทางพันธุกรรมการแพร่กระจายวัชพืชต่างถิ่น *Crassocephalum rubens* ในเอเชียตะวันออกเฉียงใต้

Genetic Evidence for The Spread of Alien Weed *Crassocephalum rubens* in Southeast Asia

โครงการ วนิชาชีวะ

Ongkarn Vanijajiva

คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏพระนคร, กรุงเทพฯ, 10220

Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok, 10220

Corresponding author: vanijajiva@pnru.ac.th

### บทคัดย่อ

เครื่องหมาย Random amplified polymorphic DNAs (RAPDs) และ inter-simple sequence repeats (ISSR) ได้นำมาใช้ในการวิเคราะห์ลักษณะทางพันธุกรรมของวัชพืชต่างถิ่น *Crassocephalum rubens* ในภูมิภาคเอเชียตะวันออกเฉียงใต้ จากการศึกษาพบว่า *C. rubens* ปัจจุบันมีการแพร่กระจายในประเทศพม่าและประเทศไทยเท่านั้น เมื่อทดสอบโดยใช้ 20 โพรเมอร์ RAPD ให้ 90 แถบ และ 20 โพรเมอร์ ISSR ให้ 43 แถบ จากข้อมูลที่ได้พบว่าประชากร *C. rubens* มีความแตกต่างทางพันธุกรรม อย่างไรก็ตามรูปแบบทางพันธุกรรมไม่สัมพันธ์กับการกระจายตัวทางภูมิศาสตร์ เป็นไปได้ว่าการกระจายพันธุ์ของเมล็ดในระยะไกลที่อาศัยมนุษย์และอื่นๆ อาจเป็นสาเหตุสำคัญที่ทำให้ลักษณะพันธุกรรมไม่สัมพันธ์กับการกระจายของวัชพืชต่างถิ่น *C. rubens* ในเอเชียตะวันออกเฉียงใต้

### ABSTRACT

Random amplified polymorphic DNAs (RAPDs) and inter-simple sequence repeats (ISSRs) markers were used to analyze genetic structure of 30 accessions of alien weed *Crassocephalum rubens* that were sampled from its introduced regions in Southeast Asia. The study found that *C. rubens* currently only spreads in Myanmar and Thailand. Using 20 RAPD primers and 20 ISSR primers, 90 RAPD bands and 43 ISSR bands were produced respectively. Quite considerable genetic variability does exist among different of *C. rubens* populations. However, lack of clear geographical structure of genetic variation among populations suggested that human-mediated, long-distance seed dispersal may have contributed to its weak geographical genetic structure of *C. rubens* in Southeast Asia.

**คำสำคัญ:** วัชพืชต่างถิ่น, ความหลากหลายทางพันธุกรรม, *Crassocephalum rubens*

**Keywords:** alien weed, genetic diversity, *Crassocephalum rubens*

## INTRODUCTION

Alien species are now recognized as a significant factor of global change and often irreversible impacts on biodiversity in many parts of the world. In regions such as Europe, North America and Australia there is a remarkable knowledge of such species particularly in plants. In contrast to this, alien species are poorly studied in Africa and Asia (Vanijajiva & Kadereit, 2009), particularly in Southeast Asia where currently undergoes a rapid economic development and increasing international trade, translating into ecological side effects that are of direct significance for the spread of introduced organisms. In addition, most studies on introduced species have mainly concentrated on their ecology. The genetics structure of alien species has received little attention. It has been suggested that knowledge on genetic evidence could give a better understanding of how colonization occurred or continues to occur, and assist in predicting the potential for populations of alien species to evolve in response to management strategies (Sakai et al., 2001).

*Crassocephalum rubens* (Juss. ex Jacq.) S. Moore, member of the Sunflower family (Asteraceae), is an erect annual herb about 20–50 cm high when flowering (Figure 1). The species is recognized as a new introduced weed species to Asia. *C. rubens* is assumed to have been introduced to Southeast Asia as early as 1986 (Vanijajiva & Kadereit, 2009). However, the locality of its original introduction and its spread from there are still obscure. In present study, RAPD and ISSR technologies were applied to analyse the genetic diversity of *C. rubens* sampled throughout Southeast Asia. The objectives of this study were to detect genetic structure of this alien weed in order to evaluate any historical spread and follow provide references for controlling its expansion in Southeast Asia.



Figure 1 *Crassocephalum rubens* whole plant (A), capitulum (B).

## MATERIALS AND METHODS

DNA isolations were carried out using fresh leaf of samples from 30 accessions collected across Southeast Asia (Figure 2). Voucher specimens of all accessions were deposited in the Phranakhon Rajabhat University Herbarium.

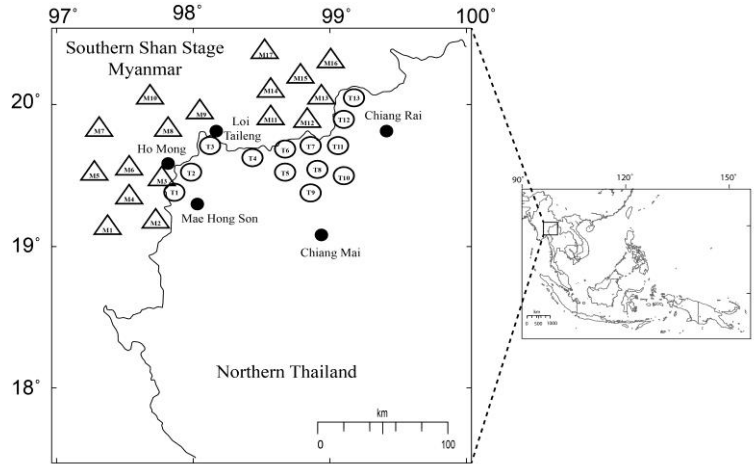


Figure 2 Plot of sampled locations in Southeast Asia.

Genomic DNA was extracted following the CTAB procedure with minor modified (Vanijajiva, 2012). DNA quality and quantity were checked on 1% agarose gels. Twenty random amplified polymorphic DNA (RAPD) primers (Operon Technologies) and twenty inter-simple sequence repeats (ISSR) primers (British Columbia University) were initially screened for analysis. PCR was performed using a Thermohybrid Px2 (Roche Molecular Systems, Inc., USA). RAPD and ISSR PCR reactions were conducted in 25  $\mu$ l volume containing 10x Reaction Buffer, 100 ng template DNA, 0.6 mM dNTP mixture, 5 mM  $MgCl_2$ , 1 unit of Taq polymerase and 0.6  $\mu$ M primers. The RAPD amplification conditions were: 4 min initial denaturation at 94  $^{\circ}C$ ; 45 cycles consisting of 1 min denaturation at 94  $^{\circ}C$ , 1 min primer annealing at 36  $^{\circ}C$ , and 2 min extension at 72  $^{\circ}C$  and a final 4 min extension at 72  $^{\circ}C$ . The ISSR amplification conditions were: 5 min initial denaturation at 94  $^{\circ}C$ ; 42 cycles consisting of 1 min denaturation at 94  $^{\circ}C$ , 1 min primer annealing at 47-55  $^{\circ}C$ , and 2 min extension at 72  $^{\circ}C$  and a final 7 min extension at 72  $^{\circ}C$ .

The RAPD and ISSR 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 RAPD and ISSR 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. Only strong and reproducible RAPD and ISSR bands were scored. Different observed patterns were scored as discrete variables, using 1 to indicate the presence and 0 to indicate the absence of a unique pattern. A principal component analysis (PCA) was conducted using a genetic distance matrix obtained from the binary data set. The SPSS (version 18) data analysis package was used for the statistical analyses.

## RESULTS AND DISCUSSION

This study is the first attempt to investigate the genetic diversity of newly introduced weed *C. rubens* in Southeast Asia using the RAPD and ISSR method. The present study indicated that this weed species mainly distributes in Southern Shan state of Myanmar and Northern Thailand along the edge of forests and abandoned fields. Following an initial screen of 20 RAPD primers and 20 ISSR primers, 15 RAPD primers (OPA02, OPA03, OPA04, OPA10, OPA18, OPAM01, OPAM03, OPAM12, OPAM18, OPB01, OPC01, OPC05, OPD02, OPD03 and OPD08) and 10 ISSR primers (UBC no. 807, 810, 811, 815, 819, 822, 827, 868, and 873) that produced reproducible were selected to amplify DNA samples of all *C. rubens* collections. A total of 90 reproducible fragments of RAPD primers and 43 bands of ISSR primers were scored. Compared the ISSR with the RAPD, the RAPD primers generated greater mean number of bands per primer (mean = 6) than the ISSR primers (mean = 4.8).

The study of RAPD and ISSR demonstrates that the genetic variability does exist among different of *C. rubens* in Southeast Asia. The genetic characteristics of *C. rubens* populations in Southern Shan state of Myanmar show higher levels of genetic diversity than northern Thailand. Sakai et al (2001) noticed that the genetic diversity within the arrived area should be higher than the other areas. This indicated that Shan state of Myanmar probably is the original area when *C. rubens* was arrived. The results agreed with Vanijajiva & Kadereit (2009), who reported that the oldest herbarium sheet of this species was collected from Shan state of Myanmar. However, PCA of genetic distances of *C. rubens* indicates that population clustering is not region specific (Figure 3). This suggests that the spreading route of this alien weed in Southeast Asia cannot be inferred by RAPD and ISSR markers. The unclear geographical structure of genetic variation among populations, together with the distributions of two groups detected in the cluster analysis, indicates that long-distance dispersal through anthropogenic activities might be a major mediator of the dispersal of the species.

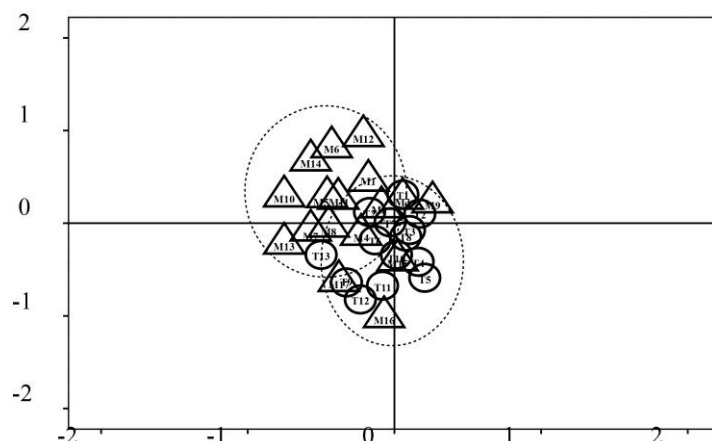


Figure 3 Plot of PCA analysis of genetic distance of 30 *C. rubens* accessions.

## CONCLUSION

The results of this research can be seen as a preliminary study for future researches aimed at defining the level of genetic diversity of *C. rubens* in Southeast Asia. The results demonstrated the potential efficiency of RAPD and ISSR markers for detecting genetic variation of this alien species. However, the historical spreading route of *C. rubens* cannot be inferred by both techniques. Therefore, more research in future is required. Further molecular approaches like SRAP and AFLP markers will be useful in prospect to detect more information of species dispersal and genetic structure of *C. rubens* in Southeast Asia.

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## REFERENCES

- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, et al. The population biology of invasive species. *Annu Rev Ecol Syst.* 2001;32: 305–332.
- Vanijajiva O. Assessment of genetic diversity and relationships in pineapple cultivars from Thailand using ISSR marker. *J. Agri. Tech.* 2012;8: 1829-1828.
- Vanijajiva O, Kadereit JW. Morphological and molecular evidence for interspecific hybridization in the introduced African genus *Crassocephalum* (Asteraceae: Senecioneae) in Asia. *Sys. Biod.* 2009;7: 269-276.