Plants and Ecosystem
ISSN: 2789-7370 (Online)
Volume- 01 (2021)

Journal updates

RESEARCH ARTICLE

https://doi.org/10.54479/pae.v1i01.6664

Genetic Divergence among Populations of Invasive Alien Species *Lantana camara* L. from Selected Areas of Bangladesh

SAILA KABIR | MD ABUL KASHEM | MOHAMMAD ZABED HOSSAIN

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

BSE PRESS

Article History
Received: 05 July 2021
Revised: 28 August 2021
Accepted: 30 August 2021
Published: 10 October 2021

Corresponding Author
Mohammad Zabed Hossain
Contact: zabed@du.ac.bd

ABSTRACT: *Lantana camara* L., a well-known invasive alien species causing invasion and posing threat to native plant species community in different regions of Bangladesh. The present study aimed to investigate the genetic diversity of *L. camara* populations in different regions of Bangladesh. Eight RAPD markers were used in order to probe into its genetic variability. Total number of bands (202), polymorphic loci (104), percentage of polymorphism (97.20%), average Shanon's information index (0.3051±0.115), Nei's gene diversity (0.4733±0.144) was found and in different populations and multiple divergent genetic clustering along with presence of unique alleles (4) for RAPD revealed high genetic diversity among the populations of *L. camara* in different regions of Bangladesh.

Keywords: Invasion, Admixture, Molecular marker, Genetic diversity, Polymorphism.

Invasion of alien plant species in different regions throughout the world is regarded as one of the most serious threat to biodiversity due to their deleterious effect on local plant species diversity on their introduced ranges and serious conservational and ecological impact (1-3). *L. camara* L., native to Central and South America which is known and listed as one of the worst invasive alien species around the world (4,5). This notorious invasive plant is growing aggressively and affecting native species diversity in different regions of Bangladesh (6-9). High genetic diversity can stem from multiple introductions of invasive plant population creating opportunity for admixture, infusion of novel alleles and genetic interactions among invader populations, that elevates genetic variability and leads to invasion success (10-15). Low genetic diversity facilitates reduced capacity to resist control measures while higher degree of genetic diversity could allow adaptation to occur (different ecotypes of the same weed species may rise as an adaptive strategy in different environments of invaded habitat which may require implication of different biological or chemical controlling measures for the same species),

furthermore, herbicide resistance genotype may evolve due to mutation and facilitate higher fitness; potentially reducing the effectiveness of biological and chemical (herbicide) control measures (16-18). Hence, it is important to understand genetic diversity and population structure to determine a population's potentiality to become invasive and to explore their invasion history (introduction, source, spread, gene flow) for better management knowledge (19-23). There are several studies regarding exploration of genetic diversity of invasive species population to investigate invasion history (24-26). Various studies have been used genetic markers (RAPD, AFLP, Microsatellite, SNP, RFLP) and advanced technology (DNA microarray) to identify genotypes responsible for adaptation in new habitats, potentiality for hybridization among weed populations, mutations for herbicide resistance and to develop advanced chemical and biological control in weed management (27). In this paper, we used molecular marker (RAPD) and population genetic tools to focus on genetic variability of *L. camara* L. populations, with a view to aid further studies on its invasion history in order to find out number and patterns of introduction as well as dispersal mechanism across different parts of Bangladesh.

MATERIALS AND METHODS

To analyze genetic diversity of *L. camara* L., leaf samples were collected during the months of March and April in 2017, from nine different locations at least 50 m away from each other under the three administrative divisions Dhaka, Sylhet and Chattogram of Bangladesh (Figure 1, Table 1) based on several reports of invasion by *L. camara* L. (6,9).

Figure 1. Geographic locations from which *Lantana camara* L. specimens were collected for this study (round marked).

Table 1. Geographic information of sampling sites. Here, P= population, Dh= Dhaka, Sy= Sylhet, Ch= Chattogram, CH= Curzon Hall, GH= Gowainghat, SK= Sitakundo, SA= Savar.

P	Site ID	Location	Latitude (°)	Longitude (°)	Sample
Dh	D1	SA	23.87945°	90.2689°	1
	D2	SA	23.87891°	90.268°	1
	D3	SA	23.87375°	90.2653°	1
	D4	SA	23.88608°	90.2678°	1
	D5	CH	23.72705°	90.4016°	1
Sy	S1	GH	24.99725°	91.9365°	1
	S2	GH	24.99493°	91.9322°	1
Ch	C1	SK	22.60285°	91.6758°	1
	C2	SK	22.6024°	91.6424°	1

After collection from the field, leaves were brought to the laboratory, washed, then surface sterilized and stored at -20 °C until DNA extraction. A modified 2% CTAB extraction protocol was followed for faster, and less expensive, high-throughput total genomic DNA extraction (28). The extracted DNA samples were then stored at -20°C after verifying the integrity of the isolated DNA until subsequent PCR amplification. In this study eight random amplified polymorphic DNA (RAPD) were used (Table 2) and PCR was performed using RAPD primers. For each sample, at least two PCR amplifications were performed to evaluate the reproducibility of the bands obtained. For PCR amplification, amplification was carried out in a total volume of 25 µl for each sample containing 50 ng of template DNA, 12.5 µl of PCR mastermix, 1 µl of 100 pico moles of primer and 8.5 µl of sterile de-ionized distilled PCR water. PCR amplification was done in an oil-free thermal cycler (Applied Biosystems 2720 Thermal Cycler). While using RAPD, initial PCR condition was 94 ^{0}C for 4 minute followed by 35 cycles of denaturation at 94 ^{0}C for 1 minute, annealing temperature for 30 seconds then extension at 72 ^{0}C for 2 minutes and final elongation step of 72 ^{0}C for 4 minutes was added. RAPD amplified products were separated electrophoretically on 1.5 % agarose gel prepared using 100 ml 50×TAE buffer and containing 0.5 µg/ml ethidium bromide. Agarose gel electrophoresis was conducted in 50× TAE buffer at 90 V and 300 mA for 45 minute (for RAPD). DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (Cleaver Scientific's MultiSUBTM). The RAPD band profiles were scored visually and recorded as presence (1) or absence (0) of bands. The binary quantitative data matrix obtained were used to determine the total number of bands,

Table 2. Eight arbitrary RAPD primers with, primer sequences, total number of allele (allelic richness) per locus for in *Lantana camara* L. selected for genetic analysis.

Primers	Sequence 5′-3′	Total allele
RAPD 132	AGGGATCTCC	19
RAPD 122	GTAGACGAGC	31
RAPD 104	GGGCAATGAT	12
RAPD 105	CTCGGGTGGG	9
RAPD 106	CGTCTGCCCG	50
RAPD 127	ATCTGGCAGC	27
RAPD 180	GGGCCACGCT	26
RAPD 186	GTGCGTCGCT	28

unique bands, common bands as well as several measures of genetic diversity including number of polymorphic loci, percentage of polymorphism, mean of observed number of allele, effective number of allele, Nei's gene diversity, Nei's genetic distance and Shanon diversity Index by using the computer program ―POPGENE V 1.32 (29-31). Principle Coordinate Analysis (PCoA) was performed to reveal Associations among the individuals from different populations by using the software GenAlex6.5 (32).

RESULTS

In order to investigate genetic diversity of *L. camara* L., we analyzed data obtained from both RAPD and microsatellite band profiling. RAPD data analysis showed that 50-9 bands/alleles were produced per locus with a total of 35 alleles over all loci. RAPD data analysis showed the highest number of total bands (159), common bands (122), polymorphic loci (90) with 84.11% polymorphism within the population of Dhaka and a total of 4 unique bands, Shanon's information index were found 0.5064 and 0.1101 for Dhaka and Sylhet respectively. Nei's gene diversity was found 0.3484 and 0.0794 for Dhaka and Sylhet respectively for all RAPD markers (Table 3). Genetic distance among the populations revealed by RAPD data (Table 4) shows least genetic distance among the samples of Sylhet and Chattogram indicating genetic closeness. Samples from Dhaka population showed diversified pattern of genetic distance. Two of the samples D2 and D3 showed high genetic distance with D1 (0.888) and D4 (0.683), however D2 and D3 anomalously showed low or no genetic distance with samples from Sylhet (0.182-0.242) and Chattogram respectively. Among the samples from Dhaka, D5 showed more or less high distance with samples of Dhaka (0.9593) as well as Chattogram and Sylhet.

Table 3. Number of total bands, common bands, unique bands, polymorphic loci and polymorphism (%) and summary of genetic variation statistics. Here, (Na= observed number of allele; Ne= effective number of allele; I= Shanon's information index; h= Nei's gene diversity) in populations of Dhaka, Sylhet and Chattogram (Chatt.) for RAPD and microsatellite primers.

Population	Dhaka	Sylhet	Chatt.	Total
Total band	159	43	0	202
Common band	122	36	0	158
Unique band	4	0	0	4
Polymorphic loci	90	17	0	104
Polymorphism	84.11 %	15.89 %	0.0 %	97.20 %
Na* (Mean±SE)	1.8411 (0.3673)	1.1589 (0.3673)	1.0000 (0.0000)	1.9720 (0.1659)
Ne* (Mean±SE)	1.6200 (0.3397)	1.1589 (0.3673)	1.0000 (0.0000)	1.4794 (0.2500)
h* (Mean±SE)	0.3484 (0.1685)	0.0794 (0.1836)	0.0000 (0.0000)	0.3051 (0.1155)
I* (Mean±SE)	0.5064 (0.2346)	0.1101 (0.2546)	0.0000 (0.0000)	0.4733 (0.1448)

Table 4. Nei's genetic distance (below diagonal) of RAPD primers in different samples of *L. camara* L. of the different populations from Dhaka, Sylhet and Chattogram regions. Here, P represents population.

P	D1	D2	D3	D4	D5	S1	S2	C1	C2
D1	0.00								
D2	0.8886	0.00							
D3	0.8886	0.0000	0.00						
D4	0.4387	0.6838	0.6838	0.00					
D5	0.9593	0.5457	0.5457	0.4681	0.000				
S1	0.9352	0.1842	0.1842	0.7608	0.5457	0.000			
S2	0.9593	0.2420	0.2420	0.9593	0.7025	0.1730	0.000		
C1	0.8886	0.0000	0.0000	0.6838	0.5457	0.1842	0.2420	0.000	
C2	0.8886	0.0000	0.0000	0.6838	0.5457	0.1842	0.2420	0.0000	0.000

Samples from Chattogram showed no bands at all for any of the RAPD primers, hence shows extreme difference with Dhaka. The mean effective number of allele ranged from 1.6200 to 1.00. Similar results were obtained after principal coordinate analysis of the studied populations for RAPD, which shows the location of all studied populations in a coordinate system. Principal coordinate analysis (PCoA) showed a clear clustering of 6 individuals in which each two individuals were from Dhaka, Sylhet and Chattogram (Figure 2). The rest of the individuals were genetically distant from each other. In this PCoA at the individual level, the first and second coordinate was accountable for 54.29% and 22.84% of genetic variability. These two coordinates together explain 77.12% genetic variation.

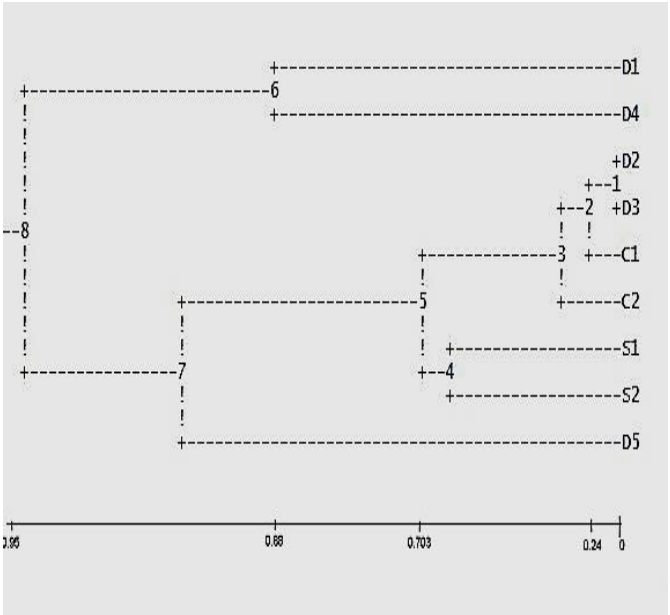

Figure 2. A dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between 9 samples of the populations of *L. camara* L. from Dhaka (D1- D5), Chattogram (C1- C2) and Sylhet (S1-S2) regions according to RAPD analysis.

DISCUSSION

As an invasive species, *L. camara* L. has not been studied at molecular level yet in Bangladesh. This study is the first attempt to investigate the genetic diversity of *L. camara* L. in Bangladesh using the RAPD method. The eight arbitrary RAPD primers produced a total of 202 bands among three different populations, total 104 polymorphic locus showed 97.20 % of polymorphism. Average of other diversity indices like Shanon's information index, Nei's gene diversity was found 0.3051± 0.115 and 0.4733± 0.144 respectively for all RAPD markers, which varied among three different populations of *L. camara* L. Vysniauskiene et al. studied genetic diversity of invasive alien species *Lupinus polyphyllus* in Lithuania using six RAPD markers and found high genetic diversity with an average of 24.17±2.11 RAPD bands, 151 polymorphic bands, an average of 52.6 % polymorphism and Nei's genetic diversity 0.1552±0.860 (18). Furthermore, in our study Nei's genetic distance among the samples from different populations as well as the dendrogram for RAPD showed that samples from Sylhet, Chattogram showed slight region specificity as genetic distances within the samples from Sylhet as well as Chattogram were negligible but samples from Dhaka showed peculiarity, some of the samples showed region specificity while others didn't and the majority of the studied samples grouped regardless of the geographical distances between them. Such result indicates presence of genomic constituents from different allopatric populations, which indicates possibility of separate introduction in Sylhet and Chattogram from genetically distinct native populations as well as in Dhaka lantana populations might be introduced in different times from different source population or may indicate human mediated spread (17) of this invasive.

There are several other research (33,25), used molecular markers to study genetics of the invasive plant and found high genetic diversity. These studies substantiate high genetic diversity of *L. camara* L. found in our study. Furthermore, Ray and Quader found high genetic diversity, private polymorphism and multiple genetic clustering for lantana populations within India (26). In the present study, although genetic distance study and multiple divergent genetic clustering in denrogram for RAPD marker showed anomaly in genetic distance regardless of geographic distance at some extent but other various diversity metrices, polymorphism, presence of unique bands altogether showed high genetic diversity among the populations of *L. camara* L. and indicates that population clustering has some region specificity. Genetic variability of weed population poses significant challenges for proper control and management of weed populations.

As several studies demonstrated invasive status of *L. camara* L. in Bangladesh and our research clarifies the high genetic diversification of *L. camara* L. populations; therefore, caution should be exercised during further introduction of genetically distinct

group of lantana from divergent sources to prevent subsequent increase of its genetic variability (6,8,9). Higher genetic divergence as well as population differentiation will require a wider range of possible controlling measures to stop the spread of this invasive species.

CONCLUSION

The present study summarizes high genetic diversity among populations of *L. camara* L. in different regions of Bangladesh, and it also supports the idea of introduction of genetically distinct groups of *L. camara* L. from divergent founders from different geographic sources in Bangladesh. This study will help knowing the dispersal patterns, adaptive strategies and the control measures and proper management approaches of this notorious invasive plant *L. camara* L. in different regions of Bangladesh.

CONFLICT OF INTEREST

This article has no conflict of interest.

REFERENCES

1. Pyšek P, Richardson DM. Invasive species, environmental change and management, and health. Annu. Rev Environ Resour. 2010;35:25–55.
2. Vila M, Hejda M, Hulme PE, et al. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett. 2011;14:702–8.
3. Shrestha BB. Invasive alien plant species in Nepal. Frontiers of Botany. 2016; pp:269-284.
4. Raghubanshi AS, Tripathi A. Effect of disturbance, habitat fragmentation and alien invasive plants on floral diversity in dry tropical forests of Vindhyan highland: a review. Trop Ecol. 2009;50:57-69.
5. Richardson DM, Rejmanek M. Trees and shrubs as invasive alien species– a global review. Divers Distrib. 2011;17:788–809.
6. Aktar A, Zuberi MI. Invasive alien species in Northern Bangladesh: Identification, inventory and impacts. Int J Biodivers Conserv. 2009;1:129-134.
7. Hossain MK, Alam MN. Allelopathic effects of *Lantana camara* leaf extract on germination and growth behaviour of some agricultural and forest crops in Bangladesh. Pak J Weed Sci Res. 2010;16:217–26.
8. Rahman MH, Roy B. Population structure and curative uses of invasive plants in and around the protected forests of Bangladesh: A means of utilization of potential invasive species. Journal of Ecosystems 2014;1-14.
9. Dutta S, Hossain MK, Hossain MA, et al. Exotic plants and their usage by local communities in the Sitakunda Botanical Garden and Eco- Park, Chittagong, Bangladesh. Forest Res. 2015;4:1-9.
10. Roman J, Darling JA. Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol. 2007;22:454–464.
11. Suarez A, Tsutsui N. The evolutionary consequences of biological invasions. Mol Ecol. 2008;17:351–60.
12. Henry P, Lay GL, Goudet J, et al. Reduced genetic diversity, increased isolation and multiple introductions of invasive giant hogweed in the western Swiss Alps. Mol Ecol. 2009;18:2819–2831.
13. Uller T, Leimu R. Founder events predict changes in genetic diversity during human-mediated range expansions. Global Change Biology. 2011;17:3478–85.
14. Verhoeven KJF, Macel M, Wolfe LM, Biere A. Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. Proc R Soc B. 2011;278:2–8.
15. Rius M, Darling JA. How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol Evol. 2014;29:233–242.
16. Sterling TM, Thompson DC, Abbott LB. Implications of invasive plant variation for weed management. Weed Tech. 2004;18:1319–24.
17. Yang J, Tang L, Guan YL, Sun WB. Genetic diversity of an alien invasive plant Mexican Sunflower (*Tithonia diversifolia*) in China. Weed Science. 2012;60:552–7.
18. Vysniauskien R, Ranceliene V, Zvingila D, Patamsyte J. Genetic diversity of invasive alien species *Lupinus polyphyllus* populations in Lithuania. Agriculture. 2011;98:383-390.
19. Lavergne S, Molofsky J. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Nat Acad Sci USA. 2007;104:3883–8.
20. Rosenthal DM, Ramakrishnan AP, Cruzan MB. Evidence for multiple sources of invasion and intraspecific hybridization in *Brachypodium sylvaticum* (Hudson) Beauv. in North America. Mol Ecol. 2008;17:4657–69.
21. Crawford KM, Whitney KD. Population genetic diversity influences colonization success. Mol Ecol. 2010;19:1253-1263.
22. Guillemaud T, Ciosi M, Lombaert E, Estoup A. Biological invasions in agricultural settings: insights from evolutionary biology and population genetics. Comptes Rendus Biologies. 2011;334:237-46.
23. Hagenblad J, Hülskötter J, Acharya KP, Brunet J, et al. Low genetic diversity despite multiple introductions of the invasive plant species *Impatiens glandulifera* in Europe. BMC Genetics. 2015;16:103.
24. Zini E, Clamer M, Passerotti S, et al. Eight novel microsatellite DNA markers in *Rhodiola rosea* L. Conserv Genet. 2009;10:1397-9.
25. György Z, Szabó M, Bacharov D, Pedryc A. Genetic diversity within and among populations of Roseroot (*Rhodiola rosea* L.) based on molecular markers. Not Bot Horti Agrobo. 2012;40:266-273.
26. Ray A, Quader S. Genetic diversity and population structure of *Lantana camara* in India indicates multiple introductions and gene flow. Plant Biol. 2013;16:651–8.
27. Slotta TAB. What we know about weeds: insights from genetic markers. Weed Sci. 2008;56:322–6.

28. Allen GC, Flores-Vergara MA, Krasynanski S, et al. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat Proto. 2006;1:2320-2325.
29. Nei M. Genetic distance between populations. Am Nat. 1972;106:283-92.
30. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 1973;70:3321-3.
31. Lewontin RC. Testing the theory of natural selection. Nature. 1972;236:181-2.
32. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 2012;28:2537–9.
33. Liang Y, Liu J, Zhang SP, et al. Genetic diversity of the invasive plant *Coreopsis grandiflora* at different altitudes in Laoshan Mountain, China. Can J Plant Sci. 2008;88:831-7.

Acknowledgements
The authors gratefully acknowledge the financial support to this research from the Ministry of Education of the Government of the People's Republic of Bangladesh under the Grants for Advanced Research.

Author's contributions
All three authors contributed equally.

To cite the article
Kabir S, Kashem MA, Hossain MZ. Genetic divergence among populations of invasive alien species Lantana camara L. from selected areas of Bangladesh. Plants and Ecosystem. 2021;1:9-14.