Macedonian pharmaceutical bulletin, 68 (Suppl 2) 37 - 38 (2022)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.013
Short communication

AFLP vs SSR markers in population genetic studies: Genetic diversity of Dalmatian sage (*Salvia officinalis* L.)

Zlatko Šatović*[1,2], Filip Varga[1,2], Marija Jug-Dujaković[3], Martina Grdiša[1,2], Tonka Ninčević[3], Klaudija Carović-Stanko[1,2], Zlatko Liber[2,4]

[1]*University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb*
[2]*Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Svetošimunska cesta 25, 10000 Zagreb, Croatia*
[3]*Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia*
[4]*University of Zagreb, Faculty of Science, Marulićev trg 9a, 10000 Zagreb*

Introduction

AFLP (Amplified Fragment Length Polymorphism) and SSR (Single Sequence Repeats; microsatellites) markers are widely used in plant genetic studies and it is fair to say that the fundamental insights into plant population dynamics and structure have been gained from the analysis of either AFLP or SSR data, or both. The AFLP technique requires no prior knowledge of the genetic make-up of a species and produces a large number of polymorphic fragments that are generally scored as dominant markers. SSRs, on the other hand, are species-specific, codominant, multiallelic markers.

In this study, we wanted to compare some basic population genetic parameters calculated on the basis of both marker systems.

Materials and methods (or other sections)

A total of 531 specimens of Dalmatian sage (*Salvia officinalis* L.; Lamiaceae) from 25 localities along the eastern Adriatic were analysed. The collected populations are kept as part of the Collection of medicinal and aromatic plants in Zagreb (http://cpgrd.hapih.hr).

Total genomic DNA was extracted from fresh leaves. AFLP analysis was performed with four selective primer combinations as described in Jug-Dujaković et al. (2020).

SSR analysis was performed using eight SSR markers isolated in Dalmatian sage (Radosavljević et al. 2010; 2011), as described in Rešetnik et al. (2016).

Genetic diversity of Dalmatian sage populations based on AFLP and SSR markers was described by calculating the expected heterozygosity (H_E) of each population, population differentiation (F_{ST}) between populations and Nei's standard genetic distances between populations. For AFLP data, the inbreeding coefficient (F_{IS}) was estimated using the Bayesian approach. For SSR data, observed heterozygosity (H_O) was calculated and tested for deviations from Hardy-Weinberg equilibrium (HWE). Analysis of molecular variance (AMOVA) was used to partition total AFLP diversity among and within Dalmatian sage populations.

Pearson's correlation coefficients and Mantel tests were used to calculate and test the correlation between matrices obtained by AFLP and SSR markers.

Results and discussion

Four AFLP primer combinations yielded 559 polymorphic markers, while eight SSR markers detected a total of 150 alleles. The average expected heterozygosity (H_E) based on AFLP markers ($H_{E\text{-}AFLP}$ = 0.176) was considerably lower than that based on SSRs ($H_{E\text{-}SSR}$ = 0.756). Moreover, the correlation between the H_E values was negative (r = -0.189) and non-significant (P = 0.364).

The inbreeding coefficient ($F_{IS\text{-}AFLP}$ = 0.024) estimated using the Bayesian approach based on the AFLP data was similar to the multilocus estimate of the inbreeding coefficient based on the SSR data ($F_{IS\text{-}SSR}$ = 0.034). After applying the sequential Bonferroni corrections, no

*zsatovic@agr.hr

significant deviations from the HWE were found at any locus in any population.

The overall F_{ST} value estimated based on the AFLP data ($F_{ST\text{-}AFLP}$ = 0.046) was half that estimated based on the SSR data ($F_{ST\text{-}SSR}$ = 0.088). The correlation between the matrices of FST values based on both marker data was significant but weak (r = 0.339; P_{Mantel} < 0.001).

AMOVA analysis showed that a similar percentage of the total genetic diversity was attributable to differences among individuals within populations based on the AFLP (91.53%) and SSR (91.14%) data. Thus, the overall φ_{ST} value based on AFLP ($\varphi_{ST\text{-}AFLP}$ = 0.085) was similar to that based on SSR ($\varphi_{ST\text{-}SSR}$ = 0.089), both being highly significant (P < 0.0001). However, the correlation between the matrices of φ_{ST} values based on both marker data was weak (r = 0.393; P_{Mantel} < 0.001).

Finally, the average pairwise Nei's genetic distance between populations based on AFLP data ($D_{Nei\text{-}AFLP}$ = 0.010) was much lower than that based on SSR data ($D_{Nei\text{-}SSR}$ = 0.431), while the correlation between matrices was weak (r = 0.315; P_{Mantel} < 0.001).

Conclusion

The essential information on the dynamics within and among population of Dalmatian sage obtained from the AFLP and SSR data was congruent: (a) the inbreeding coefficient tends to be close to zero, justified by its outcrossing nature, (b) most of the genetic diversity is due to variability within populations, and (c) genetic differentiation among populations is low, indicating strong gene flow among the populations.

However, the correlation of the matrices of F_{ST}, φ_{ST} and genetic distance obtained by AFLP and SSR markers was generally low, although highly significant. Therefore, caution should be exercised when interpreting the results of different studies conducted with different marker systems.

Acknowledgements

This research was funded by project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia.

References

Jug-Dujaković, M., Ninčević, T., Liber, Z., Grdiša, M., Šatović, Z., 2020. *Salvia officinalis* survived in situ Pleistocene glaciation in 'refugia within refugia' as inferred from AFLP markers. Plant Syst. Evol. 306, 38. https://doi.org/10.1007/s00606-020-01665-9.

Radosavljević, I., Jakše, J., Javornik, B., Šatović, Z., Liber, Z., 2011. New microsatellite markers for *Salvia officinalis* L. (Lamiaceae) and cross-amplification in closely related species. Am. J. Bot. 98(11), e316–e318. https://doi.org/10.3732/ajb.1000462.

Radosavljević, I., Jakše, J., Šatović, Z., Javornik, B., Liber, Z., 2010. Isolation and characterization of polymorphic microsatellites loci from common sage (*Salvia officinalis* L.; Lamiaceae). Mol. Ecol. Resour. 10, 404–408. https://doi.org/10.1111/j.1755-0998.2009.02827.x.

Rešetnik, I., Baričević, D., Batîr Rusu, D., Carović-Stanko, K., Chatzopoulou, P., Dajić-Stevanović, Z., Gonceariuc, M., Grdiša, M., Greguraš, D., Ibraliu, A., Jug- Dujaković, M., Krasniqi, E., Liber, Z., Murtić, S., Pećanac, D., Radosavljević, I., Stefkov, G., Stešević, D., Šoštarić, I., Šatović, Z., 2016. Genetic Diversity and Demographic History of Wild and Cultivated/Naturalised Plant Populations: Evidence from Dalmatian Sage (*Salvia officinalis* L., Lamiaceae). PLoS One. 11(7), e0159545. https://doi.org/10.1371/journal.pone.0159545..