DETECTION OF DNA POLYMORPHISMS IN GLASSY-WINGED SHARPSHOOTERS (*HOMALODISCA COAGULATA*) BY PCR-BASED DNA FINGERPRINTING METHODS

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INTRODUCTION

The glassy-winged sharpshooter *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is a xylem feeding leafhopper that is a serious pest because it vectors a strain of *Xylella fastidiosa*, a bacterium that causes Pierce's disease of grapevines (Turner and Pollard 1959; Nielsen 1968). DNA markers have proved to be valuable tools for population genetic studies. DNA fingerprinting methods that do not require prior knowledge of genome sequences include ISSR-PCR (Inter-Simple Sequence Repeat-Polymerase Chain Reaction), RAMP (Randomly Amplified Microsatellite Polymorphisms), SAMPL (Selective Amplification of Microsatellite Polymorphic Loci) and RAPD (Random Amplification of Polymorphic DNA). RAPDs produce dominant markers, whereas ISSR-PCR, RAMP, and SAMPL incorporate Simple Sequence Repeats (SSR) and are capable of identifying co-dominant markers if utilizing 5'-anchored or compound ISSR primers (reviewed in Karp and Edwards 1997), but without known family relationships (segregation/backcrosses) these markers are scored as dominant.

OBJECTIVES

Develop molecular genetic markers for the glassy-winged sharpshooter by the following methods ISSR-PCR, RAMP, SAMPL, and RAPD to estimate the most sensitive and efficient procedure. Screening of the methods was initiated with a small number of insects (3). Identification of DNA polymorphisms (POPGENE software) in natural populations was determined with 10-30 insects with the various DNA fingerprinting methods.

RESULTS AND CONCLUSIONS

Initially, one insect was utilized to screen with the four DNA fingerprinting methods, than three insects (Weslaco, TX) per primer or primer pair (pp) (46 total) were used to estimate the sensitivity and efficiency of each method. The results of this small scale screening procedure are presented in Table 1. A total of 205 polymorphic markers were generated with the four methods, with ISSR-PCR, pp-ISSR-PCR, RAMP, SAMPL, and pp-RAPD producing 34, 41, 58, 32, and 40 polymorphic markers, respectively. The Efficiency Ratio (number of polymorphic markers/number of primers amplified) of each method was as follows: 6.83 (pp-ISSR-PCR), 6.80 (ISSR-PCR), 4.83 (RAMP), 3.33 (pp-RAPD), and 2.91 (SAMPL). The Screening Efficiency (number of polymorphic markers/number of primers screened) indicated that both pp-ISSR-PCR (2.41) and ISSR-PCR (2.27) were the most efficient methods. To test the utility of some of these DNA fingerprinting methods on identifying DNA polymorphisms in a natural population of glassy-winged sharpshooters (Weslaco, TX), 10-30 insects were employed (Table 2). Depending on the sample size, the number of polymorphic loci ranged from 5 (pp-RAPD, reaction #6) to 32 [ISSR compound primer 13, $A(CA)_7(TA)_2T$] and percentage polymorphic loci was 100% for most primers or primer pairs. Gene diversity ranged from 0.095 to 0.263 for ISSR compound primer 10, $G(TG)_4(AG)_4A$ and pp-RAPD reaction #6, respectively. A small-scale geographic or multi-populations analysis was conducted with ten insects each from Weslaco, TX and Bakersfield and Riverside, California and RAMP (reaction #54). A dendrogram based on Nei's genetic distance by the method of UPGMA and the multi-populations genetic variation statistics are demonstrated on Figure 1. The two California cities, Bakersfield and Riverside formed a cluster that was separated from Weslaco, Texas. The Weslaco population demonstrated the greatest genetic diversity (0.20). Geographic specific markers may also be an indication of subdivided populations. The present results confirmed the utility of the DNA fingerprinting screening procedure and demonstrated extensive genetic variation in natural populations of glassy-winged sharpshooters by the four PCR-based DNA fingerprinting methods.

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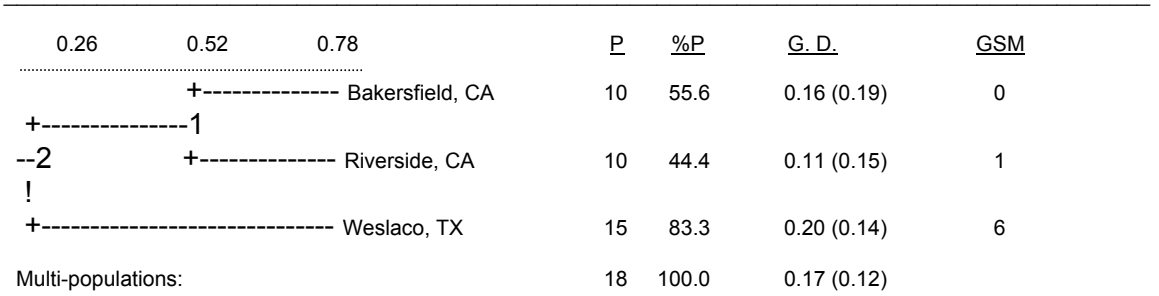
Table 1. Summary of the DNA fingerprinting methods screening procedure. pp, methods incorporating primer pairs.

Method	No. Primers Screened	No. Primers Amplified	No. Polym. Markers	Efficiency Ratio	Screening Efficiency
ISSR-PCR	15	5	34	6.80	2.27
pp-ISSR-PCR	17	6	41	6.83	2.41
RAMP	93	12	58	4.83	0.62
SAMPL	40	11	32	2.91	0.80
pp-RAPD	45	12	40	3.33	0.88
Total	210	46	205		

Table 2. Summary of selected results from the various DNA fingerprinting methods. P, polymorphic loci; %P, percentage polymorphic loci; G. D., gene diversity.

Method	Reaction (#) or primer (p)	Primer(s)	Sample Tm	Size	Loci	P	%P	G. D. (SD)
ISSR-PCR	p-9	$CCAG(GT)_7$	52°	30	28	28	100	0.147 (0.124)
ISSR-PCR	p-10	$G(TG)_4(AG)_4A$	41°	30	25	25	100	0.095 (0.097)
ISSR-PCR	p-13	$A(CA)_7(TA)_2T$	54°	30	32	32	100	0.121 (0.091)
pp-ISSR-PCR	#7	$KKVRVRV(TG)_6$ $C(CT)_4(GT)_4G$	47°	10	15	14	93.3	0.171 (0.116)
RAMP	#54	$G(TG)_4(AG)_4A$ OPM-02	43°	10	15	15	100	0.231 (0.117)
RAMP	#75	$C(AC)_4(AG)_4A$ OPV-14	41°	30	21	21	100	0.197 (0.153)
SAMPL	#34	E + AGC $C(AC)_4(AG)_4A$	58°	30	14	14	100	0.102 (0.074)
pp-RAPD	#1	OPA-03/A-10	36°	10	11	10	90.9	0.194 (0.165)
pp-RAPD	#6	OPA-03/M-02	36°	30	5	5	100	0.263 (0.155)
pp-RAPD	#17	OPA-10/V-14	36°	30	15	15	100	0.165 (0.158)

Figure 1. Small-scale geographic populations genetic analysis. High molecular weight genomic DNA from ten insects from each location was amplified by RAMP with reaction #54. GSM, number of geographic specific markers.



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Abstracts