GENETIC DIVERSITY OF *RHOPALOSIPHUM PADI* L. (HOM.: APHIDIDAE) USING MICROSATELLITE MARKERS

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ABSTRACT: The aphid *Rhopalosiphum padi* is an important pest of cereal crops worldwide. Because of having both sexual and asexual populations, it has been the target of extensive ecological and genetic studies. In this paper we report on genetic variation of *R. padi* in Iran based on microsatellite markers. Genetic diversity of thirty five *R. padi* clones, collected from various cultivated and wild host plants in different regions in Iran, was studied using eight microsatellite markers. Out of eight markers, five showed 16 polymorphic bands, varying from 2 to 5, with an average of 3.2. Phylogenetic analyses revealed that Iranian aphid clones collected from northern, north-eastern, northwestern, southern, south-western and central region of Iran were clustered into four distinct groups. A significant correlation was observed between aphid clones and geographical regions. According to our results, geographic populations differed significantly and their genetic distance could be mainly explained by geographic separation. Apparently geographic barriers hinder gene flow and genetic variation among Iranian *R. padi* clones.

KEY WORDS: Cereal, aphid clones, phylogenetic analyses, geographic populations.

There are about 4400 species of aphids in the world of which about 250 species are major plant pests. They inflict economic damage directly by feeding and indirectly by transmitting plant viruses (Blackman & Eastop, 2000). Aphids are highly specialized plant feeders and their life cycle are unusual among arthropods, as it includes obligate shifting between unrelated host taxa, elaborate polyphenisms and variation in reproductive strategy within a single species (Moran,1992). Despite the two- fold cost of sex, the vast majority of multicellular eukaryotic species reproduce sexually and over evolutionary time scale, asexual lineages have generally appeared, flourished and disappeared (Maynard Smith, 1978). There are many organisms that reproduce asexually or by cyclical parthenogenesis like aphids, rotifers, lake snails etc. Many of these organisms are geographically widespread and temporally persistent. This makes aphids powerful models to study the evolutionary consequences of the different ways in which genetic variation is generated and organized (Sunnucks et al., 1996, 1997).

Among aphids, *R. padi* has been the subject of extensive biological and molecular studies that have allowed the characterization of two major reproductive lineages: (1) sexual lineages that alternate sexual and asexual reproduction and complete their annual life cycle on two different hosts. (2)

asexual lineages that live year round on cereals although most of them have retained the ability to produce males in autumn (Simon & Hebert, 1996).

R. padi is one of the most important economical aphids, especially on maize, barley, oats and wheat in Iran. Although *R. padi* specimens have been collected on *Prunus* spp., In the Caspian sea region, Sexual form of the aphid has not been reported yet from Iran. *R. padi* not only causes the leaves of gramineae to roll and form a spiral but also transmits several cereal virus disease and being the principal vector of barley yellow dwarf virus (BYDV) (Aleosfoor et al., 2007).

Knowledge of the patterns of genetic diversity of an insect pest population can aid in the understanding of its biology and ultimate control in an integrated pest management system. However, information regarding the genetic diversity of *R. padi* is lacking and few molecular studies have been conducted on this destructive pest (Delmotte et al., 2001, 2002). The objective of the study was to characterize the genetic structure of *R. padi* populations in Iran.

Among different molecular markers, microsatellites are becoming increasingly popular in gene mapping and population studies have been used in a number of aphid studies (eg. Sunnucks et al., 1996, 1997; Simon et al., 1999a; Fuller et al., 1999; Wilson et al., 1999, 2002; Haack et al., 2000; Sloane et al., 2001; Delmotte et al., 2001, 2002; Leewellyn et al., 2003; Khouadja et al., 2003; Papura et al., 2003; Miller et al., 2003; Vorburger et al., 2003; Figueroa et al., 2005; Dolatti et al., 2005; Yong Mo et al., 2007; Andrew et al., 2009). How ever, it is the first time they are applied to Iranian populations of *R. padi*.

MATERIAL AND METHODS

1- Sample collection

Samples of *R.padi* were collected from wheat, barley and wild plants in 35 different regions of Iran In March to April 2007-2009 (Fig. 1). Individual females were allowed to reproduce on a barley seedling, following a standard procedure in order to establish a parthenogenetic line (Caillaud et al., 1995). Great care was taken to avoid cross contamination of the aphids. Individuals from each parthenogenetic line were removed two months after clone initiation and stored at -70 □C prior to DNA extraction and PCR analysis.

2- DNA extraction, PCR amplification and electrophoresis

DNA was extracted from individual wingless aphids using the salting out procedure (Sunnucks & Hales, 1996). The cd DNA extract was checked and roughly quantified by electrophoresis on 8% agarose gel and dilutions were made correspondingly in order to obtain a DNA concentration of 5-7 ng/ul. Genotypes of individual *R. padi* were examined at eight microsatellite loci (Simon et al., 2001).

Polymerase chain reactions (PCR) were performed with the following programme: a denaturation step at 94 ˚C for 2 min followed by 35 cycles of denaturation at 94 ˚C for 20 s, 20 s at a locus specific annealing temperature (Table 1) and an elongation step at 72 °C for 20 s, then an extension cycle at 72 °C for 2 min . Amplification products were resolved on a 6% polyacrylamide urea electrophoresis gel and visualized after silver nitrate staining (Simon et al., 2001).

3- Data Analyses

Genetic diversity was measured by evaluating the differences in allele number per locus, polymorphism information content and similarity values. The degree of polymorphism was quantified using the polymorphic information content (PIC)

$$PIC = 1 - \sum_{i}^{n} p^2 g$$

Where pg is the frequency of an individual genotype.
Probability identity (PI) and Discriminating power (D) was measured as follows:

$$PI = \sum pi^4 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} (2 p_i p_j)^2$$

$$D = 1 - PI$$

Presence or absence of each band was coded as 1 or 0, respectively, in a binary matrix (BDM). Similarity analyses were conducted using a complete BDM comprising data with the NTSYS 2.02 program using the Jaccard association coefficient of similarity. The resulting pairwise similarities were expressed as triangular similarity matrices. Cluster analysis were conducted by applying the SAHN option on similarity estimates using the unweighted pair-group method arithmetic average (UPGMA) and the resulting clusters were expressed as dendrogram using the option TREE PLOT (Sneath & Sokal, 1973).

RESULTS AND DISCUSSION

1- Polymorphism of microsatellite markers

Of the eight microsatellite loci screened, only R6.3, R3.171, R5.29b, R5.50 and R2.73were polymorphic. Two alleles were observed at R6.3 and R5.50 and three, four and five alleles at R5.29b, R2.73 and R3.171 respectively (table 2). R5.50 was fixed for a single allele in all populations surveyed and PCR amplification failed for loci R1.35 and R5.138.

A total of 16 alleles were detected among the 35 aphid genotypes with an average of 3.2 alleles per locus. The number of alleles per locus ranged from 2 (in R6.3 and R5.50) to 5 (in R3171). The PIC values for the microsatellite loci ranged from 0.1028 to 0.338 with an average of 0.19. The low PIC value was observed for the R5.29b and the high PIC value was observed for R3.171. the low PI value belonged to R3.171 and the high PI value was observed in R5.29b. The high discriminating power (D) was observed in R3.171 (table 2). The results show that among these five primers, R3.171with the highest PIC and D values is the best primer for separating the genotypes of Iranian *R. padi* populations.

2- Cluster analysis of DNA polymorphism

The genetic relationships among aphid genotypes are presented in a dendrogram based on informative microsatellite alleles (Fig. 2).

All genotypes were clearly grouped into four major branches in the dendrogram with less than 25% similarity based on Jaccard similarity index. Branches 1, 2 and 4 represented aphid subpopulations from northeastern, northern and central of Iran, respectively, while branch 3 consisted of a mixed group from northwestern, western, southern and southwestern regions of the country.

35 clones of Iranian *R. padi* showed that they were heterozygous at most loci with study of clonal diversity. Such a pattern of diversity in obligatory parthenogenetic aphid populations have been reported before (Sunnuck, et al., 1996; Papura et al., 2003; Simon et al., 1996; Wilson et al., 1999; Dolati et al., 2005; Figueroa et al., 2005).

According to our results, a significant correlation was observed between aphid clones and geographical regions in most cases. Indeed, geographic populations differed significantly and their genetic distance could be mainly explained by geographic separations. This finding is similar to the results of Simon et al. (1996) and Dolati et al. (2005) but in contrast with the result of Miller et al. (2003).

The analysis of the cluster dendrogram at the point of 10% similarity (based on Jaccard similarity) grouped all genotypes in two groups. Besides few exceptions, the first group represented sub populations from north and north- eastern and the second group represented subpopulations from north- western, centeral and southern of Iran. It is probable that populations from north- western Iran migrated to the south and central regions , this phenomenon has been reported about the obligatory parthenogenetic aphid *R. maidis* and *Sitobion avenae* (Dong et al., 1987; Wiktelius et al., 1984). According to the dendrogram, it seems that aphid populations in centeral Iran (Isfahan, Yazd and Shahrekord) have a longer history compared to the populations in Fars and Khuzistan provinces.

It is surprising that despite their few clonal variation, populations of *R. padi* were able to successfully colonize different cultivated and wild host plants throughout different agroclimatic zones along hundreds of kilometers in Iran. However, aphids, by their asexual mode of reproduction, their high capacity through direct flights or trade exchanges and the existence of "super clones" seem to be particularly well suited to rapid conquest of new habitats. According to the cluster dendrogram, the samples from north of Iran were grouped into two different branches. It's maybe related to both winter severity and the availability of *Prunus* trees. Although the existence of sexual forms of *R. padi* in Iran has not been reported yet. So additional work is needed to get a clearer understanding on the existence of sexual forms, which is also a requirement to design appropriate management strategies. In this study *R. padi* populations were not genetically differentiated according to their host plants. Detailed studies should be conducted to identify the interplay among mode of reproduction, weather climate and gene flow that may affect local adaptation of the aphids.

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