Host chemistry and genotypic variation of aphid populations

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Abstract

Different experimental approaches have shown that host-plant chemistry, particularly hydroxamic acids (Hx) in cereals, has a strong and non-random modulating effect on phenotypic as well as genotypic variations in aphids, both at the individual and population levels. The RAPD-PCR phenotypic composition of *Sitobion avenae* populations living on wheat cultivars differing in their Hx levels showed changes during development of the crops, with a preferential flux of RAPD-PCR phenotypes from the low-Hx to the high-Hx wheat. Additionally, the study showed the appearance of new RAPD-PCR phenotypes, albeit only in the high-Hx cultivar. Simultaneously, the RAPD-PCR stability of three monoclonal colonies feeding on young seedlings of the same wheat cultivars and of oat, a Hx-lacking cereal, were analysed during ten generations in greenhouse experiments. Two new RAPD-PCR phenotypes appeared on the high-Hx wheat, one on the low-Hx wheat, and none in oat. The appearance of new RAPD-PCR phenotypes is discussed in terms of the mutagenic properties of Hx, while the disappearance of RAPD-PCR phenotypes was attributed to antibiosis and antifeeding effects of DIMBOA.

Introduction

Sitobion avenae is one of the most abundant aphid species in wheat crops in Chile. *S. avenae* has shown a strong and non-random genetic variation closely related to the host-plant (Caillaud *et al.*, 1995; De Barro *et al.*, 1995a,b). However, genotypic (or phenotypic) variation of *S. avenae* populations among and within cultivars of the same host-plant species differing in their chemical defence levels, has not been addressed.

A group of secondary metabolites involved in cereal resistance against aphids are hydroxamic acids (Hx) (Niemeyer & Pérez, 1995), where DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), the main Hx aglucone in wheat extracts (Niemeyer, 1988), exhibits a moderately strong mutagenic effect through direct interaction with DNA

(Hashimoto & Shudo, 1996), and has also been proposed to produce mutagenic oxygen species after its metabolisation within the aphid (Figueroa *et al.*, 1999b; Loayza-Muro *et al.*, 2000).

We hypothesize that aphid populations will show differential changes in their RAPD-PCR (Random Amplified Polymorphic DNA-Polymerase Chain Reaction) profiles over time, in relation to the chemistry of the host-plant. We explored the RAPD-PCR phenotype composition of *S. avenae* aphid populations on two wheat cultivars differing in their Hx levels, throughout the development of the crops, and during several aphid generations in greenhouse experiments.

Materials and methods

Sitobion aphids were collected in the field, quarantined for parasitoids, and identified as *S. avenae* (Fabricius) and *S. fragariae* (Walker) using molecular and morphological criteria (Figueroa *et al.*, 1999a). The collections were performed on two wheat cultivars (cv): cv Huayún (*Triticum aestivum* L.) (low-Hx) and cv. Chagual (*T. durum* L.) (high-Hx) (0.667 ± 0.907 and 3.595 ± 0.257mmol DIMBOA/kg fresh weight, respectively). Three groups of data were obtained: at the start of the season (SS: September 3, 1999), at mid season (MS: October 29, 1999), and at the end of the season (ES: December 3, 1999). DNA was extracted from one single adult wingless individual (about 800µg weight) using the "salting out" method (Sunnucks & Hales, 1996), and resuspended in 40µl of sterile ultrapure water. DNA dilutions from 1/10 to 1/500 were tested for RAPD-PCR, and 1/100 was used as the best ratio in terms of reproducibility. All the RAPD-PCR reactions for each individual were repeated twice, using amplification conditions according to Figueroa *et al.* (1999a). The non-parasitised individuals, corresponding to 109 aphids from cv Huayún and 58 from cv Chagual, were screened using five random decamer primers for RAPD-PCR. One of them was used to evaluate the stability of a RAPD-PCR profile in successive generations in the laboratory (see below). Under the assumption that each band represents a single locus, the presence or absence of polymorphic fragments was used as alleles (Lynch & Milligan, 1994). The genotypic frequencies were computed with RAPDistance v. 1.04 (Armstrong *et al.*, 1994). Population genetic structure was analysed using POPGENE v. 1.31 (Yeh *et al.*, 1999). Cavalli-Sforza's distances were computed using RAPDDIST v. 1.0 (Black IV & Antolin, 1997). An AMOVA was applied to assess the statistical significance of the divergences between and within populations using WINAMOVA v. 1.55 (Excoffier, 1995).

To evaluate the appearance of new phenotypes in aphid colonies growing on different hosts, a glasshouse experiment was performed. One hundred clonal lineages individually caged were established from single aphids from a multiclonal colony. After one parthenogenetic generation, the clones were screened for RAPD-PCR phenotype using one single random primer. Ninety-six clones exhibited the same RAPD-PCR profile (named as phenotype A), and were pooled and multiplied on the same two hosts of the field experiment, i.e. wheat cv Huayún and cv Chagual, and oat (*Avena sativa* L.), a Hx-lacking cereal. Synchronised adult wingless aphids were placed on two-leaf seedlings of each host individually caged in an acrylic cylinder with a netted lid, and considered as the first generation. Aphids reproduced for six days and the total progeny was left on the plant. To screen the RAPD-PCR profiles, a sample of 50 fourth-instar aphid nymphs was randomly chosen from each of the three groups. The nymphs were individually reared on oat seedlings

(to avoid Hx) until their progeny reached adulthood. Five wingless adults of each of the 150 clonal lineages were used for RAPD-PCR screening. The remaining aphids (5-10 nymphs) were transferred to new two-leaf seedlings of each host until they became adults, initiating the second generation. This procedure was repeated for the next seven generations. Each generation thus lasted eleven days.

Results and discussion

Only seven bands (13.7%) were observed as polymorphic in field-collected aphids with the five primers used. Hence, the RAPD-PCR profiles were obtained with combinations of them, allowing the identification of 31 different RAPD-PCR phenotypes: 14 from cv Huayún, 10 from cv Chagual and 7 from both cultivars. The analysis revealed the presence of host-specific bands as well as ubiquitous bands. As the season progressed, on cv Huayún no band showed a significant decrease over time, and a single band showed a significant increase ($p<<0.0001$). On cv Chagual two bands showed a significant decrease over time ($p<0.005$), and one band showed a significant increase ($p<0.02$). Between aphid populations at each collection date, two bands [band CFa8-750 bp, (ES: $p<<0.0001$; MS: $p<<0.0001$; LS: $p<0.029$) and band CFa9-690 bp, (ES: $p<0.0001$; MS: $p<0.008$; LS: $p<0.0002$)], were observed to be significantly different at all collecting dates, while band [CFa9-1150 bp] was significantly different only during the last collection [$p<0.002$].

Table 1: Distribution and abundance of the 31 RAPD-PCR phenotypes found among aphid populations living on cv Huayún (H) and cv Chagual (Ch) along one season.

Date	Host	RAPD-PCR phenotype															
		Sa1	Sa2	Sa3	Sa4	Sa5	Sa6	Sa7	Sa8	Sa9	Sa10	Sa11	Sa12	Sa13	Sa14	Sa15	Sa16
ES	H (59)	0	0	9	1	2	16	13	2	1	2	1	1	1	1	1	1
	Ch (9)	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MS	H (35)	0	0	0	0	1	7	12	1	1	7	1	2	0	0	0	0
	Ch (22)	7	2	1	2	0	1	1	0	0	0	0	0	0	0	0	0
LS	H (15)	0	0	0	0	2	12	0	0	0	0	0	0	0	0	0	0
	Ch (27)	2	1	9	1	1	1	4	0	0	0	0	0	0	1	0	0

		Sa17	Sa18	Sa19	Sa20	Sa21	Sa22	Sa23	Sa24	Sa25	Sa26	Sa27	Sa28	Sa29	Sa30	Sa31	Nb of clones
ES	H (59)	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0	18
	Ch (9)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3
MS	H (35)	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	11
	Ch (22)	0	0	0	0	0	0	0	0	4	1	1	2	0	0	0	10
LS	H (15)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3
	Ch (27)	0	0	0	0	1	0	0	0	2	0	0	1	1	1	1	14

ES: Early season; MS: Mid season; LS: Late season.
In parenthesis, the number of individuals collected by host-plant and season.

The RAPD-PCR profiles were found to vary significantly depending on the wheat cultivar and the time of the collection. Most RAPD-PCR phenotypes appeared to be cultivar-specific since they seldom appeared simultaneously or along time in both cultivars (Table 1).

Since both cultivars did not differ in sowing time, developmental stage, or exposure to winds, the differential distribution of RAPD-PCR phenotypes on wheat cultivars differing in Hx levels, suggests that plant chemistry may act as a mechanism generating interpopulation differences. The overall phenotypic richness diminished as the season progressed, as previously reported (De Barro *et al.*, 1995a). Seven phenotypes (Sa1-Sa7) were found to be present in all three collections, two of them, Sa1 and Sa2, being exclusive to cv Chagual. The other 24 RAPD-PCR phenotypes appeared in different collections but never more than twice (Table 1). Three RAPD-PCR phenotypes that disappeared from cv Huayún were later found on cv Chagual suggesting a migration from cv Huayún to cv Chagual, but 13 disappeared entirely. A Cavalli-Sforza phenotypic distances analysis revealed a phenotypic structure changing at interpopulation and intrapopulation levels, because of increased phenotypic divergence (φ_{ST}=0.307; significantly different from zero, $p< 0.001$) as the season progressed. Interestingly, the aphid population from cv Huayún changed over time to become more similar in phenotypic structure to cv Chagual at the start of the season, and vice-versa. A hierarchical analysis also showed that 69.3% of the variance was due to differences within the populations at each sampling date.

Several RAPD-PCR phenotypes appeared (8 on cv Chagual and 5 on cv Huayun) during the growth of wheats in the field, while others permanently disappeared. A possible explanation relies on migration between wheat cultivars and surrounding wild grasses. However, since *S. avenae* exhibits a low genetic variability in Chile, with a prevalence of only a few genotypes (Figueroa *et al.*, 1999a), a migration does not entirely explain the differential appearance/extinction of several RAPD-PCR phenotypes between cv Chagual and cv Huayún. Since the highest increment in RAPD-PCR phenotypes on cv Chagual was observed after the first collection, when cultivars have highest Hx levels (Argandoña *et al.*, 1981), the effect of cultivar chemistry on the stability of a single RAPD-PCR phenotype over nine generations (the approximate duration of wheat crops), was tested under greenhouse conditions. Using wheat plants at a development stage where Hx levels are near their maxima (Niemeyer, 1988), this study allowed the detection of new aphid RAPD-PCR phenotypes with a characteristic banding profile different from that of the initial phenotype (Table 2). The predominant and most recurrent RAPD-PCR phenotype observed was phenotype A. Two other RAPD-PCR phenotypes were detected after generation IV (Table 2). They differed from the original phenotype by the presence of one extra band of 720 bp (phenotype B), or two extra bands of 720 and 680 bp (phenotype C). Although rarely observed, these bands were only detected in aphids collected from cv Chagual fields. It seems to be likely that phenotype C arose from phenotype B rather than from phenotype A. Interestingly, RAPD-PCR phenotype B arose independently in two maternal lineages. The appearance of two new RAPD-PCR phenotypes in aphids reared on wheat cultivars, and the absence of new phenotypes in oat, a cereal lacking Hx, suggests a putative participation of Hx as a selection/inducing factor of genetic variability.

The mechanism by which Hx could promote the appearance of new RAPD-PCR phenotypes may be hypothesised as follows: DIMBOA may be produced through the action of β-glucosidases present in aphid saliva (Miles, 1999) on DIMBOA-glucoside ingested by aphids from wheat plants (Givovich *et al.*, 1994), and further accumulated in the aphid body (Niemeyer *et al.*, 1989). Chemical and biochemical arguments would explain promotion of mutations by DIMBOA. Thus, the aldehyde group of the aldol (Niemeyer *et al.*, 1982), and the electrophilic nitrogen atom (Pérez & Niemeyer, 1985), may covalently bond to nucleophilic sites at the DNA molecule (Hashimoto & Shudo, 1996). Additionally, there is evidence for an induction of the detoxification and oxidative metabolisms of *S. avenae* aphids

exposed to DIMBOA (Figueroa *et al.*, 1999b; Loayza-Muro *et al.*, 2000), suggesting an increased occurrence of reactive oxygen species which could cause mutations at DNA.

Table 2: RAPD-PCR phenotypes in samples from oat, and wheat cultivars, along nine generations.

Generations	Oat	cv. Huayún	cv. Chagual
I-II-III	A	A	A
IV	A	A, B (1)[a]	A
V	A	A, B (1)	A, B (1), C (1)
VI	A	A, B (1)	A, C (2)
VII	A	A, B (1)	A
VIII-IX	A	A	A

[a] In parenthesis, the number of new RAPD-PCR phenotypes detected in samples of 50 individuals per plant treatment.

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