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Short Communication

PCR-Based Detection of Aphids in the Gut Contents of Arthropod Predators

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Abstract

Fodders (maize, sorghum, chari, shaftal and alfalfa), wheat and mustard crops were sampled round the year in the cropland of Punjab. The most abundant pest species of the cropland were aphids namely *Aphis maidis, Schizaphis graminum,* and *Diuraphis noxia*, which were subjected to gut analysis. Species-specific primers of these aphid species were applied on the selected predators. *Coccinella septumpuctata* and *Oxyopes javanus* were positive for the consumption of *A. maidis* (corn aphid), *Hippodemia convergens* for *S. graminum* (wheat aphid), while *Neoscona theisi* was positive for *Diuraphis* noxia (wheat aphid). Such findings seemed to be helpful for implementation of species specific biological control against a specific pest in our agro-ecosystems. © 2013 Friends Science Publishers

Keywords: Predation; Arthropods; Gut contents; *Aphis maidis; Schizaphis graminum; Macrosiphum miscanthi;* PCR

Introduction

Arthropods are the most diverse group of organism in the agro-ecosystem. Ecosystem based arthropod fauna are integral to evaluate existing cropping practice and aid in redesigning of farming systems to make them economically viable and environment sustainable. The potential of arthropod predators to suppress pest populations has been thoroughly established by recent literature reviews (Chang and Kareiva, 1999; Symondson, 2002). Arthropods, including insects, spiders and mites are important with regard to crop loss because they include beneficial as well as pest species. Both the groups play a key role in the stability of agro-ecosystem (Olfert *et al.,* 2002).

Serological assays were considered to be important for the study of predation by a species (Ruberson and Greenstone, 1998). ELISA is one of them but the production of antibodies is a complex and time taking process in these assays. It is said that the situation is not same for different quantifiable comparison. An interesting alternative to this approach is the detection of prey DNA in predator's gut. Many advantages to this technique includes already available sequences of many insect species, accessibility of molecular probes in the form of commercial kits and few prey species-specific primers, which can easily be manufactured and used in reproducible protocols (Zaidi *et al*., 1999; Chen *et al*., 2000).

The cytochrome oxidase II (COII) a mitochondrial gene is available in multiple copies per cell which increases the chances of good amplification from the extract of predator's gut (Zhang *et al.,* 2007).

Analysis of insect predator gut contents is very useful in providing information on trophic interactions and predator-prey dynamics. Direct field observations are not very helpful in this regard, due to the movement of animal among different available habitats. Whereas, molecular experiments tracking trophic interactions in food-webs through polymerase chain reaction (PCR) provides the mean for amplification and thus visualizing the DNA (Sheppard and Harwood, 2005). Here we are presenting the results of our hypothesis that PCR assay confirms the presence of different aphid species in the guts of beetles and spiders.

Materials and Methods

Seven generalist predator and three prey species abundant in the field sample were selected for molecular analysis. Zoophagous species comprised of *Coccinella septumpunctata, Cheilomenes sexmaculata, Hippodemia variegata, H. convergens, Chrysoperla carnea, Neoscona theisi* and *Oxyopes javanus,* while phytophagous species comprised of *Aphis maidis, Schizaphis graminum,* and *Diuraphis noxia.*

The collected specimens were immediately stored in 100% ethanol separately in 5 mL glass vials and genomic DNA was extracted with few modifications as devised by Zhu and Greenstone (1999). Total genomic DNA concentration was measured by spectrophotometer (AARI

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2010, USA) at 260 nm wavelength. Quality of DNA was checked by running 5 µL of extracted DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving smear in the gel were rejected.

Analysis

Early morning and late evening was suspected to be the peak time period of feeding for these predators. At that time they were captured, brought alive to the laboratory, dissected and their guts were removed and preserved by freezing at -40^{o}C. Protocols for species specific mitochondrial cytochrome oxidase II primers for major aphid species were used as given in Chen *et al.* (2000) with few modifications. Sequences, annealing temperature and size of the fragment of species-specific mitochondrial COII primer for selected aphid species used were shown in Table 1 (earlier reported by Chen *et al.*, 2000). PCR reactions, using aphid primers (Table 1) were performed as denaturation of DNA at 94^{o}C for 3 min followed by 35 amplification cycles with 30s denaturation at 94^{o}C, 30s annealing at 57^{o}C, and 1 min extension at 72^{o}C. DNA was finally extended for 2 min at 72^{o}C after amplification. PCR products were separated on 1.5% agarose gel, stained with ethidium bromide and photographed under UV light.

Results and Discussion

The predators guts were considered as a specific site of digestion and were isolated after dissection. On this basis, we could identify DNA of three aphid species viz. *A. maidis, S. graminum* and *D. noxia* in the gut of selected predator species. PCR reactions were performed with few modifications according to the requirement. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide and observed under UV light.

All the seven predators viz. *C. septumpunctata, C. sexmaculata, H. convergens, H. variegata, O. javanus, N. theisi* and Araneae nymph were examined for the consumption of three common aphid species viz. *A. maidis, S. graminum* and *D. noxia. C. septumpuctata* and *O. javanus* captured from the field early in the morning or late evening are expected to feed at that time, were positive for the consumption of *A. maidis* while the unfed (control) were negative. A fragment of approximately 200bp was present in the predator's DNA sample of *C. septumpunctata* and *O. javanus* (Fig. 1). The negative results by control specimens demonstrated that the animals also consumed alternate available preys in the crop field.

Similarly the fed *H. convergens* were positive for the consumption of *S. graminum*. A fragment of approximately 111bp was present in the DNA samples of selected predator (Fig. 2). Following this the fed *N. theisi* was positive for the consumption of *D. noxia*. A fragment of 100bp was present in DNA sample of predator (Fig. 3). The results showed that we could identify the DNA of specific aphid species in the guts of arthropod predators for ecologically relevant intervals following ingestion. The detection of DNA in the gut decays with the passage of time but there is no finite detectability period. However, it was noted that after four to five hours, 94% to 97% of the meals could have been detected (Hoogendoorn and Heimpel, 2001). Also the larger fragments are difficult to detect rapidly in the gut than smaller ones. These results would be helpful in studying predator's feeding cycles (Agusti *et al.* 2000).

By using species specific primer of *A. maidis* (a known pest of the cropland) the consumption rate was confirmed by gut analysis of *C. septumpunctata* and *O. javanus.* Few other

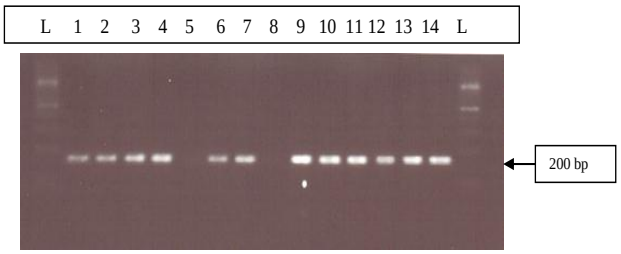

Fig. 1: PCR amplification of *C. septumpunctata* and *O. javanus* fed *A. maidis.* L is 100 bp DNA ladder on both margins, Lane 5: Unfed *C. septumpunctata*, Lane 8: Unfed *O. javanus*, Lane 6: *A. maidis* DNA (captured from sugarcane), Lane 7: *A. maidis* DNA (captured from fodder), Lane 9: *A. maidis* DNA (captured from wheat), Lane 10: *A. maidis* DNA (captured from mustard), Lane 1: *C. septumpuncatata* fed (captured from sugarcane), Lane 2: *C. septumpuncatata* fed (captured from fodder), Lane 3: *C. septumpuncatata* fed (captured from wheat), Lane 4: *C. septumpuncatata* fed (captured from mustard), Lane 11: *O. javanus* fed (captured from sugarcane), Lane 12: *O. javanus* fed (captured from fodder), Lane 13: *O. javanus* fed (captured from wheat), Lane 14: *O. javanus* fed (captured from mustard)

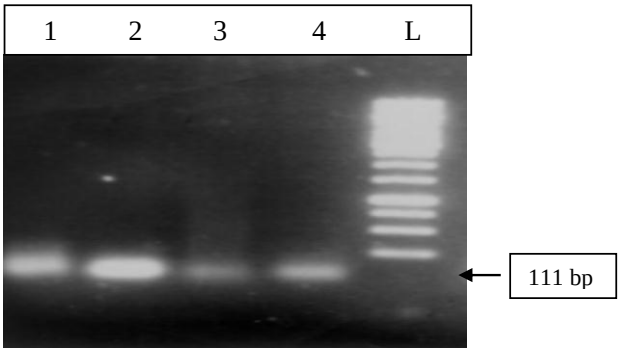

Fig. 2: PCR amplification of *H. convergens* fed *S.graminum*. L is 1kb DNA ladder on right margins, Lane 1: Fed *H. variegate* (captured from fodder), Lane 2: Fed *H. variegate* (captured from wheat), Lane 3: Fed *H. variegate* (captured from mustard), Lane 4: *S. graminum* DNA (captured from wheat)

Table 1: Details of different species specific primers of *Aphis maidis, Schizaphis graminum* and *Diuraphis noxia* (Chen *et al.* 2000)

Accession	Primer sequence	Species	Fragment size
ClaCOIIF	CCAATTCTAACAATTAAAATTTTTGGA	*Aphis maidis*	198
ClaCOIIR1	GAATAACATCATCTGATGAAATTAAA		
GbCOIIF2	GATGTTATTCACTCATGAACA	*Schizaphis graminum*	111
GbCOIIR1	GTCCAAAATATATTCCTGGG		
RwaCOIIF3	TGAACTATCCCAAGATTAGC	*Diuraphis noxia*	100
RwaCOIIR1	CATTGTCCAAAATATAATCCA		

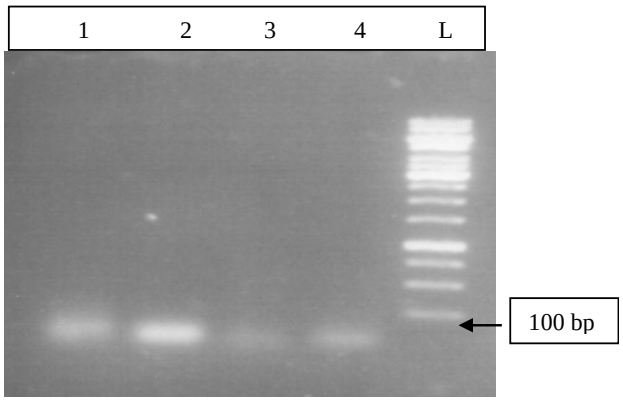

Fig. 3: PCR amplification of *N. theisi* fed *D. noxia*. L is 1kb DNA ladder on right margins, Lane 1: Fed *N. theisi* (captured from fodder), Lane 2: Fed *N. theisi* (captured from wheat), Lane 3: Fed *N. theisi* (captured from mustard), Lane 4: *D. noxia* DNA (captured from fodder)

pest species like *S. graminum* and *D. noxia* were found in the gut of *H. convergens* and *N.* theisi. Similar kind of approach was used for the detection of various aphid species in the gut of *O. salticus* spiderlings (Greenstone and Shufran, 2003; Inayat *et al.,* 2011, 2012). Gut analysis through PCR is useful because of less time consuming, less expensive, provides more logical results and if a specific prey fragment is present, valuable results could be achieved as compared to ELISA (Zaidi *et al.*, 1999).

Gut analysis of predators provide information on trophic interactions and predator-prey relationship. Predation data is very useful in formulating functional response curves and pest life tables. Major requirement for this estimation is the number of preys present in the gut of a predator and only a positive assay can provide this record (Greenstone, 1996). Furthermore, one must be aware of the routes other than predation by which insect remains can come to reside in the gut of another arthropod (Sunderland, 1996).

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