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Research Note

Sequencing of internal transcribed spacer 2 (ITS2) of *Ceratovacuna lanigera* Zehntner (Homoptera: Aphididae)

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ABSTRACT: The woolly aphid, *Ceratovacuna lanigera* Zehntner (Homoptera: Aphididae), assumed an epidemic proportion in Maharashtra and Karnataka in the year 2002. Presently this pest is prevalent in India in major sugarcane growing areas and it is important to find out the population differences to assess their epidemic potential. Molecular markers such as internal transcribed spacer (ITS) play an important role to bring out the population differences at the molecular level. Hence, ITS 2 region of *C. lanigera* was sequenced using specific primers (product size 448 bp) and the sequence was submitted to GenBank (DQ 825651).

KEY WORDS: Bangalore population, *Ceratovacuna lanigera*, ITS-2, sequencing

The sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner, was first recorded in South East Asia (Arakaki, 1992) and later found to occur in parts of northeastern India (Basu *et al.*, 1958). These aphids live in large colonies, sucking the sap from the phloem of sugarcane leaves and excrete copious honeydew on the foliage leading to the development of sooty mould. Heavy infestation by *C. lanigera* has been reported to cause significant reduction in the yield of cane and up to 15% reduction in sugar content (Gupta *et al.*, 1995). The sugarcane woolly aphid was reported in pest form from Western India and has subsequently spread to other parts of the country (Rabindra *et al.*, 2002). The spread of the pest to different regions has thrown up the challenge of determining the location from which the aphid has spread to other regions. The internal non-coding transcribed spacer region between 18s and 28s rRNA usually has higher degree of polymorphism than the coding region (Hoy, 1994). The ITS region has been used intensively to infer phylogenetic relationships. In the present study, ITS2 region of Bangalore population was sequenced to enable the future study of population variation in the country.

Collection of samples

Ceratovacuna lanigera was collected from the sugarcane plants maintained at PDBC, Bangalore. Prior to DNA isolation, the wax layer on the aphid was removed by rinsing in sterile water repeatedly.

DNA extraction

Total DNA from individual aphids (single

adults) was isolated according to Sappal *et al.* (1995). Individual aphids were homogenized in 100il lysis buffer (200 mM Tris-HCl, pH8.0; 70 mM EDTA; 2M sodium chloride; 20 mM $Na_2S_2O_5$) and to the above, 35µl of sodium lauryl sarcosine (5%) was added and incubated at 55°C for 2hr. The homogenate was spun at 15000rpm for 15min and to the supernatant 10 M ammonium acetate (13.5il) and isopropanol (135il) were added and incubated at –20°C overnight.

DNA was pelleted at 20000 rpm for 20min at 4 °C and washed with ice-cold 70% ethanol and resuspended in TE buffer (Tris 10mM, EDTA 1mM, pH 8.0).

PCR amplification and Sequencing of ITS-2 region

PCR reaction was performed in 50 il reaction volume containing 1unit of Taq, 0.2 mM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 1X PCR buffer, 10 picomoles of forward and reverse primers and 5-50 ng of DNA template. The primers used to amplify the ITS2 region were 5'-TGTGAACTGCAGGACACATG-3' (forward) and 5'-GTCTTGCCTGCTCTGAG-3'(reverse). The following were the cycling parameters used in this study: initial denaturation 95°C for 5min followed by 30 cycles of denaturation at 95°C for 1min., annealing at 55°C for 1 min., extension at 72°C for 2 min and a final extension at 72°C for 10 min. The PCR amplified product was resolved in 1.2% agarose gel and stained with ethidium bromide (10 µg/ ml). The stained PCR band was eluted using gel extraction kit and sequenced using ITS2 specific primers both in forward and reverse directions in an ABI PRISM 377 automated DNA sequencer.

Agarose gel electrophoresis showed expected product size of approximately 500 bp (Fig. 1) obtained in this study. Direct sequencing of the above PCR amplified product resulted in 448 bp fragment. Sequences from 5 individuals were analysed in a sequence alignment editor Clustal W to find out errors in sequencing. Out of these five sequences only one sequence (Table 1) was submitted to NCBI (DQ 825651). Therefore this sequence can be used to compare sequences from other populations of *C. lanigera* to construct phylogeny and other ecological studies.

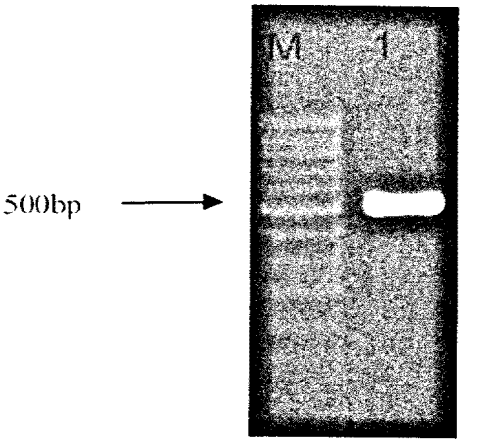

Fig. 1. PCR amplification of ITS-2 region of *C. lanigera* (Lane M- 50bp ladder 1 - *C. lanigera*)

Table 1. ITS-2 sequence of *Ceratovacuna lanigera* Zehntner – 448bp

catttcaagcacattgttgcccacggagatgtctcccggacca cgcctggctgagggtcgttcaaacaa acaaaaatcagact gctcgtattttatgcga gcgaatttactgagcg ttcgctttggttaatg ctcttctctgagcattgcctctcgtcgctttaaatga tagaaacaatttttatttttttttatt caaatttttgaaagataaaa ataaatatcaagggtctgc tatcgaaagttttgagaattaaaaag aaaaattccaaagctctttgatcgtcagtcgagtcccg gagcctatttgcacgccgatcgaagatcacagt gcaccg ggcggaccgatcgtacgaaaa tgagagtt ttttttttttttctctca gcatga tcaattttgaaagtattaatattta tttaaatgcgaaata gaaaagaataaaatta

Ribosomal DNA sequences are suitable for phylogenetic studies at many taxonomic levels from major lineages of life to intraspecific levels (Hillis *et al.*, 1991). At the species and intraspecific levels, the internal transcribed spacers (ITS1 and ITS2) are often used as taxonomic tools in many groups such as fungi (Carbone *et al.*, 1993), plants (Hsiao *et al.*, 1994), and animals (Bowles *et al.*, 1993) including insects (Cambell *et al.*, 1993; Kuprus *et al.*, 1994).

In aphids also, the ITS region showed variability (Von Dohlen, unpublished data). Sequence variation in ITS2 region of aphids collected from different parts of India will help us understand their phylogenetic relationship and

their origin. ITS2 sequence of SWA, Bangalore is a key that can be used in such phylogenetic studies.

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