

Original Research Article

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Molecular Characterization and their Phylogenetic Relationship Based on Mitochondrial Cytochrome Oxidase I of Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* (Guenée) (Lepidoptera: Pyralidae)

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ABSTRACT

The shoot and fruit borer, *Leucinodes orbonalis* has a countrywide distribution and has been categorized as the most destructive and most serious pest causing huge losses in brinjal. In the present study total of 21 populations of *L. orbonalis* collected mainly from Karnataka and also from Andhra Pradesh, New Delhi and Uttar Pradesh were molecularly characterized. The sequencing resulted in 600-636 bp product of CO I gene and amino acid composition of nucleotide, which varied from 195-206 in different populations. The certain amino acids were either present nor absent in different populations indicating their diversity. The pair-wise genetic distance of *L. orbonalis* populations ranged from 0.00 to 0.051, suggesting that there was variation in population of *L. orbonalis* from different locations. The highest genetic distance was observed from the population of Udupi district i.e. 0.051, followed by Koppal district is 0.047, Raichur, Chitoor, Vijayapura, Bagalkot and Guntur population having genetic distance of about 0.039 and other populations have different genetic distances which are related to each other. Therefore, it is inferred that there was no significant molecular diversity within *L. orbonalis* of different geographical locations of India with respect to COI but there is difference in the amino acid composition and genetic distance among the populations.

Keywords

Brinjal,
L. orbonalis,
Genetic distance,
Population, COI.

Article Info

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Introduction

The brinjal, *Solanum melongena* L. (Solanaceae), often referred as an eggplant or an aubergine in other regions of the world and is grown for its fleshy fruits. It is one of the widely used vegetable crops by most of the people and is popular in many countries, viz., Central, South and South East Asia, some parts of Africa and Central America (Gurubban, 1977). Brinjal is grown throughout the country, all round the year (Choudhary, 1970) and is believed to be cultivated in India for the last 4,000 years.

South East Asian countries account for almost 50 per cent of world's area under brinjal cultivation. In India, the shoot and fruit borer, *Leucinodes orbonalis* has a countrywide distribution and has been categorized as the most destructive and most serious pest causing huge losses in brinjal (Patil, 1990). The yield loss could be as high as 20-90 per cent in certain conditions in different states (Dhandapani *et al.*, 2003; Kalloo, 1988; Raja *et al.*, 1999; Sasikala *et al.*, 1999; Jhala *et al.*, 2003; Lal *et al.*, 1976). The yield loss due to

the pest is to an extent of 70-92 per cent (Eswara Reddy and Srinivas, 2004; Chakraborti and Sarkar, 2011; Jagginavar *et al.*, 2009). It is also reported that there will be reduction in vitamin C content to an extent of 68 per cent in the infested fruits (Hemi, 1955).

Genetic characterization of populations of insect pests plays a pivotal role in determining management strategies of the insect pest (Guirao *et al.*, 1997). Use of isozymes as biochemical marker to study the insect populations began in the late 1950s and continues to be widely applied (Black *et al.*, 1992). Within the last few years, techniques using the polymerase chain reaction (PCR) to amplify specific genomic regions have become widespread (Saiki *et al.*, 1988; Mullis and Faloona, 1987). Williams *et al.*, (1990) developed the technique that uses a single decamer primer to amplify arbitrary regions of a genome. They demonstrated that RAPD-PCR process reveals polymorphisms in the genomes of a wide variety of insect species. Molecular characterization of insects has been frequently conducted on the basis of existence of polymorphic DNA fragments amplified by PCR. Williams *et al.*, (1994) have reported geographical distribution of Argentine stem weevils, *Listronotus bonariensis* (Kuschel), by RAPD-PCR using genomic DNAs. Haymer and McInnis (1994) have also pointed out a distinctive variation in PCR-amplified DNA patterns between laboratory cultured and wild populations of Mediterranean fruit flies, *Ceratitidis capitata* (Weidemann). Molecular marker technology is a powerful tool in analyzing the phylogeny, evolution and population dynamics in insects (Symondson and Liddell, 1996; Smith and Wayne, 1996).

The number of polymorphic markers was higher indicating the diversity among the *Leucinodes* populations (Lynch and Milligan, 1994; Williams *et al.*, 1990). Similar results

were recorded in other pests like screwworm flies (Barum *et al.*, 1995) and Mediterranean fruit fly (Haymer *et al.*, 1997; Infante Malachias *et al.*, 1999; Boulding, 1998). Genetic similarities among geographical populations from the data were within the similarity coefficients ranging from 0.41 to 0.77. Host mediated variation has also been reported (John *et al.*, 2002). Karthikeyan *et al.*, (2005) studied six different populations of *L. orbonalis* were collected and subjected to analysis of genetic variability in terms of carboxylesterase isozyme pattern and DNA polymorphism using RAPDPCR. Pattern of carboxylesterase revealed a similar isozyme cluster in the populations namely, Sivaganga (population-3), Dindigal (population-4), Virudhunagar (population-5) and Coimbatore (population-6). Similarly, the populations of *L. orbonalis* recorded 3 distinct randomly amplified polymorphic DNA markers in all populations grouped above. This pattern of genetic variability in the populations was also supported by the analysis of the similarity indices and UPGMA dendrogram. Mitochondrial genes are commonly used for evolutionary studies as they have a number of characteristics such as (i) maternal inheritance with little or no recombination, (ii) general conserved gene and composition, (iii) small size of gene and (iv) lack of introns (Gissi *et al.*, 2008). In the present study, an attempt was made to use cytochrome oxidase subunit 1 (COI) gene for understanding the genetic variations among *Leucinodes* species bred on brinjal.

Materials and Methods

Collection of shoot and fruit borer

Twenty one different populations of shoot and fruit borer larvae were collected from damaged brinjal shoots and fruits from different places of Karnataka and other states like Andhra Pradesh, New Delhi and Uttar Pradesh (Table 1).

Molecular characterization

DNA was extracted from the larvae using Qiagen D Neasy® kit/ CTAB method, following the protocols of manufacturers. The DNA obtained by either of the methods mentioned above was checked for its quality by agarose gel electrophoresis.

The gel was prepared by dissolving 0.8 per cent agarose in 1x TBE buffer and boiled. About 2 µl of ethidium bromide (EtBr) was added to 50 ml of the agarose and poured in the electrophoresis template placed with comb and allowed to solidify for around 40 min.

Thereafter, it was placed in the gel electrophoresis unit with running buffer. The DNA samples were loaded into the wells and electrophoresed at 80 mA at 150 volts for 30 min. It was observed under UV illuminator and documented in DNA bioimaging systems using gel capture software.

The DNA with high quality was taken for the amplification of the particular sequences using CO1 primers. The CO1 amplification was carried out for the DNA samples with the master mix and temperature conditions as follows: Template DNA (2 µl), Taq buffer (3 µl), DNTPs (1 µl), Forward primer (2 µl), Reverse primer (2 µl), Taq polymerase (1 µl), Water (19 µl) and total reaction volume (30 µl). The temperature profile having initial denaturation (94 °C for 5 min), Denaturation (94 °C for 1 min), Annealing (45 °C for 30 min), Extension (72 °C for 1 min), Repeat step 2 to step 4 for 35 cycles, Final extension (72 °C for 10 min) and hold at 40°C for 5 min.

The primer sequence of CO1 used was Forward primer: 5' GGTCAACAAATCATAAAGATATTGG 3' and Reverse primer: 5' TAAACTTCAGGCTGACCAAAAAATCA 3'. The master mix was mixed with DNA template and Taq polymerase was added at

last separately. It should be noted that the whole reaction mix should be prepared by keeping it on ice. As soon as the reaction mix is prepared and dispensed, the PCR tubes are kept in the wells of the thermal cycler and the temperature conditions are set and run.

Agarose gel of 1.5 per cent was used to observe the amplified products which was loaded and run at 80 mA at 150 volts for 30 min. The amplified sequences were sent for sequencing to M/s Chromous Biotech Pvt. Ltd., Bengaluru, for sequencing and the nucleotides were retrieved from the trace files provided. The sequences were analysed in BLAST (Basic Local Alignment Search Tool) to check the homology of the partial sequences and annotated. The sequences were then submitted to GenBank and the accession numbers were received.

Results and Discussion

DNA isolation from *Leucinodes* species breeding on brinjal crop

The DNA was isolated from matured larvae of *Leucinodes* species collected on brinjal crop from diverse regions of Karnataka and other states. The sequences obtained from the 21 populations were deposited in GenBank and through blast similarity test; all specimens were confirmed to be *L. Orbonalis* (Table 1).

Mitochondrial cytochrome oxidase (mtCOI) gene analyses of *Leucinodes* species breeding on brinjal

PCR analysis of mtCOI region of 21 individuals of *Leucinodes* species breeding on brinjal collected from 21 locations were amplified (Fig. 1). The sequencing of PCR products by using custom services revealed various sequences of test isolates which ranged from 600-636 bp.

Computation of amino acids composition of *Leucinodes* specimen on brinjal nucleotide sequences

The data concerning amino acids composition of *L. orbonalis* specimens collected on brinjal, from different locations is given in table 2. The total number of amino acids among 21 *Leucinodes* specimens varied from 195 to 206. The highest value recorded for number of amino acids was 206, collected on brinjal from Chitradurga, while in other populations it ranged from 195 to 203 and in the out group population of *L. orbonalis* from South Africa, 200 aminoacids were recorded (Table 2).

The 21 *L. orbonalis* specimens collected from different places, showed variation in their amino acid composition. Populations collected from Koppal showed presence of cysteine amino acid, but others showed absence of that amino acid. The population collected from Udupi district showed absence of glutamine amino acid but others showed presence of that amino acid in all the populations. The population collected from Mandya showed the presence of tryptophan and all others showed absence of that amino acid. None of the populations showed the presence of methionine amino acid. The average of amino acid composition was highest in isoleucine (17.94), followed by leucine (14.76) (Table 2).

Pair-wise genetic distances between *Leucinodes* species breeding on brinjal

The pair-wise genetic distance of *L. orbonalis* populations ranged from 0.00 to 0.051 (Table 3), suggesting variation in population of *L. orbonalis* from different locations. The highest genetic distance was observed from the population of Udupi district (0.051) followed by Koppal district (0.047), Raichur, Chittoor, Vijayapura, Bagalkot and Guntur population having genetic distance of 0.039

and other populations had different genetic distances which are related to each other. The lowest genetic distance between populations was evident in populations of Kolar, Bagalkot, Vijayapura, Chittoor, Raichur, Kalaburgi, and out group from South Africa (0.00) (Table 3).

Phylogenetic analysis of aligned mtCOI gene sequences for genetic relatedness amongst *Leucinodes* species breeding on brinjal

Based on the bootstrap consensus Neighbouring joining tree, genetic differences were observed between populations of different locations having geographical distribution with two major clades (Fig. 2; Table 3). The first major clade can be divided into two sub clades. The first subclade can be again divided into two clusters.

The population from Guntur and Bagalkot were clustered together and their genetic distances varied from 0.00-0.03 and showed genetic variation and were separated from other populations. The population from Vijayapura separated from above cluster was having a genetic distance of about 0.03 (Fig. 2). The population from Chittoor and Raichur were clustered together and showed genetic distance of 0.00-0.039 from percent pairwise genetic analysis.

The population from Belagavi separated from the above cluster was having a genetic distance of about 0.018. The Bengaluru and Yadgiri population was clustered together having a genetic distance which varied from 0.004-0.029 and showed lowest level of genetic distance. The Shivamogga and Doddaballapur population was clustered together and having genetic distance which varied from 0.013-0.037 (Fig. 2; Table 3).

Table.1 Details of GenBank accession numbers of COI region different population of *Leucinodes orbonalis*

Sl. No.	Strain Name	Code Name	Population name	Total base pairs	Bankit id	Accession number
1	LO-HASSAN-C1	HSN	HASSAN	624	1790928	KP641172
2	LO-VARANASI-C1	VRN	VARANASI	618	1790936	KP641173
3	LO-BELAGAVI-C1	BGM	BELAGAVI	624	1790940	KP641174
4	LO-DHARWAD-C1	DWD	DHARWAD	623	1791489	KP659261
5	LO-SHIVAMOGGA-C1	SMG	SHIVAMOGGA	624	1791490	KP659262
6	LO-DODDABALLAPUR-C1	DBP	DODDABALLAPUR	624	1791491	KP659263
7	LO-ANANTAPUR (AP)-C1	ANT	ANANTAPUR (AP)	624	1792096	KP662094
8	LO-MANDYA-C1	MDA	MANDYA	621	1792100	KP662095
9	LO-GUNTUR(AP)-C1	GTR	GUNTUR(AP)	624	1792102	KP662096
10	LO- NEW DELHI-C1	NDL	NEW DELHI	624	1792626	KP676169
11	LO-KOLAR-C1	KLR	KOLAR	624	1792633	KP676170
12	LO-CHITRADURGA-C1	CDG	CHITRADURGA	636	1792637	KP676171
13	LO-BAGALKOT-C1	BKT	BAGALKOT	606	1792640	KP676172
14	LO-VIJAYAPURA-C1	BJR	VIJAYAPURA	600	1792644	KP676173
15	LO-BENGALURU-C1	BNG	BENGALURU	606	1793380	KP687242
16	LO-CHITTOOR (AP)-C1	CTR	CHITTOOR (AP)	600	1793388	KP687243
17	LO-RAICHUR-C1	RCR	RAICHUR	600	1793398	KP687244
18	LO-KALABURGI-C1	GBG	KALABURGI	600	1793402	KP687245
19	LO-KOPPAL -C1	KPL	KOPPAL	615	1793903	KP693449
20	LO-UDUPI-C1	UDP	UDUPI	615	1793906	KP693450
21	LO-YADGIRI-C1	YDG	YADGIRI	603	1793910	KP693451
22	LN- OUT GROUP-C1	-	SOUTH AFRICA	618	-	LN624707.1

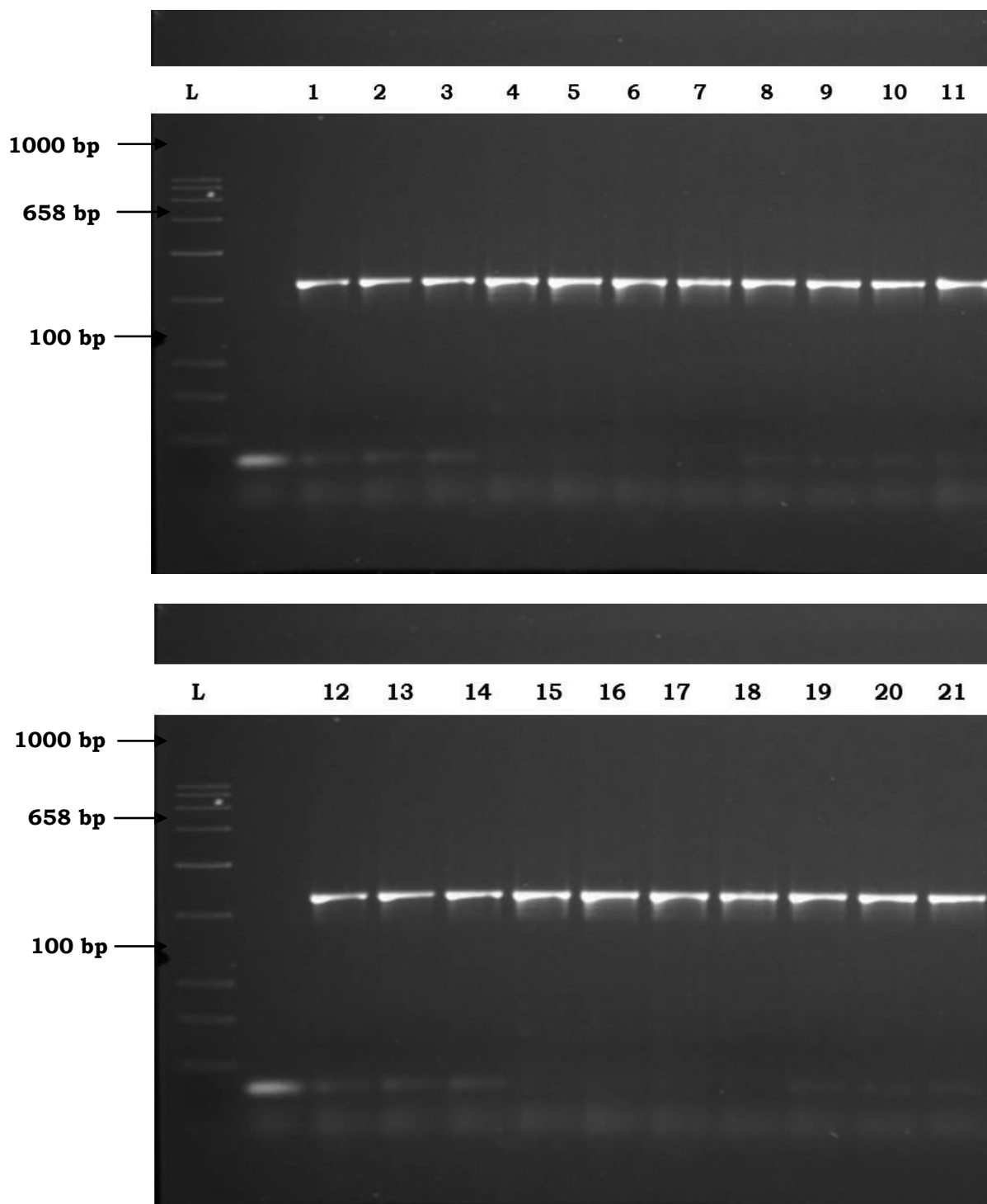
Table.2 Computation of amino acids composition of different populations of *Leucinodes* recorded from brinjal

Populations	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	Total
HSN	7.39	0.00	3.45	0.49	6.40	10.34	1.48	17.73	0.99	14.78	0.00	6.40	6.40	0.49	3.45	7.88	6.40	4.43	0.00	1.48	203.00
VRN	7.46	0.00	2.99	0.50	5.97	10.45	1.99	17.41	1.00	14.93	0.00	6.47	6.97	0.50	2.99	7.96	6.47	4.48	0.00	1.49	201.00
BGM	7.39	0.00	3.45	0.99	6.40	10.34	1.48	17.24	0.49	14.78	0.00	6.40	6.40	0.49	3.45	8.37	6.40	4.43	0.00	1.48	203.00
DWD	7.39	0.00	3.45	0.49	6.40	10.34	1.48	17.73	0.99	14.78	0.00	5.91	6.40	0.49	3.45	8.37	6.40	4.43	0.00	1.48	203.00
SMG	7.39	0.00	3.45	0.49	6.40	10.34	1.48	17.24	0.99	14.78	0.00	6.40	6.40	0.49	3.45	8.37	6.40	4.43	0.00	1.48	203.00
DBP	7.35	0.00	2.94	0.49	6.37	10.29	1.96	17.16	0.98	14.22	0.00	6.86	5.88	0.49	3.92	8.33	7.35	4.41	0.00	0.98	204.00
ANT	7.39	0.00	3.45	0.49	6.40	10.34	1.48	17.73	0.99	14.78	0.00	6.40	6.40	0.49	3.45	7.88	6.40	4.43	0.00	1.48	203.00
MDA	7.39	0.00	3.45	0.49	6.40	10.34	1.48	17.24	0.99	14.78	0.00	6.40	6.40	0.49	3.45	7.88	6.40	4.43	0.49	1.48	203.00
GTR	7.39	0.00	3.45	0.99	6.40	9.36	1.48	17.73	0.49	14.78	0.00	6.40	6.40	0.49	4.43	7.88	6.40	4.43	0.00	1.48	203.00
NDL	7.39	0.00	3.45	0.49	6.40	10.34	1.48	17.73	0.99	14.78	0.00	6.40	6.40	0.49	3.45	7.88	6.40	4.43	0.00	1.48	203.00
KLR	7.39	0.00	3.45	0.49	6.40	9.85	1.48	17.73	0.99	14.78	0.00	6.40	6.40	0.49	3.94	7.88	6.40	4.43	0.00	1.48	203.00
CDG	7.77	0.00	3.40	0.97	6.31	10.68	1.46	17.96	0.49	14.56	0.00	6.31	6.31	0.49	3.40	7.77	6.31	4.37	0.00	1.46	206.00
BKT	7.61	0.00	3.05	1.02	6.60	10.15	1.52	18.78	0.51	14.72	0.00	6.60	6.09	0.51	3.55	7.61	6.09	4.57	0.00	1.02	197.00
BJR	7.69	0.00	2.56	1.03	6.67	10.26	1.54	18.97	0.51	14.87	0.00	6.67	5.64	0.51	3.59	7.69	6.15	4.62	0.00	1.03	195.00
BNG	7.61	0.00	3.05	1.02	6.60	10.15	1.52	18.78	0.51	14.72	0.00	6.60	6.09	0.51	3.55	7.61	6.09	4.57	0.00	1.02	197.00
CTR	7.69	0.00	2.56	1.03	6.67	10.26	1.54	18.97	0.51	14.87	0.00	6.67	5.64	0.51	3.59	7.69	6.15	4.62	0.00	1.03	195.00
RCR	7.69	0.00	2.56	1.03	6.67	10.26	1.54	18.97	0.51	14.87	0.00	6.67	5.64	0.51	3.59	7.69	6.15	4.62	0.00	1.03	195.00
GBG	7.69	0.00	2.56	1.03	6.67	10.26	1.54	18.97	0.51	14.87	0.00	6.67	5.64	0.51	3.59	7.69	6.15	4.62	0.00	1.03	195.00
KPL	8.96	0.50	2.49	1.00	5.47	10.45	1.49	17.41	0.50	14.93	0.00	6.47	5.97	0.50	3.98	7.96	5.47	4.98	0.00	1.49	201.00
UDP	7.96	0.00	2.49	1.49	5.97	10.45	1.49	17.41	0.50	14.93	0.00	5.97	5.97	0.00	4.98	7.96	6.47	4.98	0.00	1.00	201.00
YDG	7.65	0.00	3.57	1.02	6.63	10.20	1.53	17.86	0.51	14.80	0.00	6.63	6.63	0.51	3.57	7.14	6.12	4.08	0.00	1.53	196.00
LN624707.1 (Out group)	8.00	0.00	3.50	1.00	6.00	11.50	1.50	18.00	0.50	14.50	0.00	6.50	6.00	0.50	3.50	7.50	6.00	4.50	0.00	1.00	200.00
Avg.	7.62	0.02	3.13	0.82	6.37	10.32	1.54	17.94	0.70	14.76	0.00	6.46	6.19	0.48	3.65	7.87	6.30	4.51	0.02	1.29	200.45

Table.3 Pair wise genetic distance based on mt.COI gene sequences of *Leucinodes* spp. recorded from brinjal

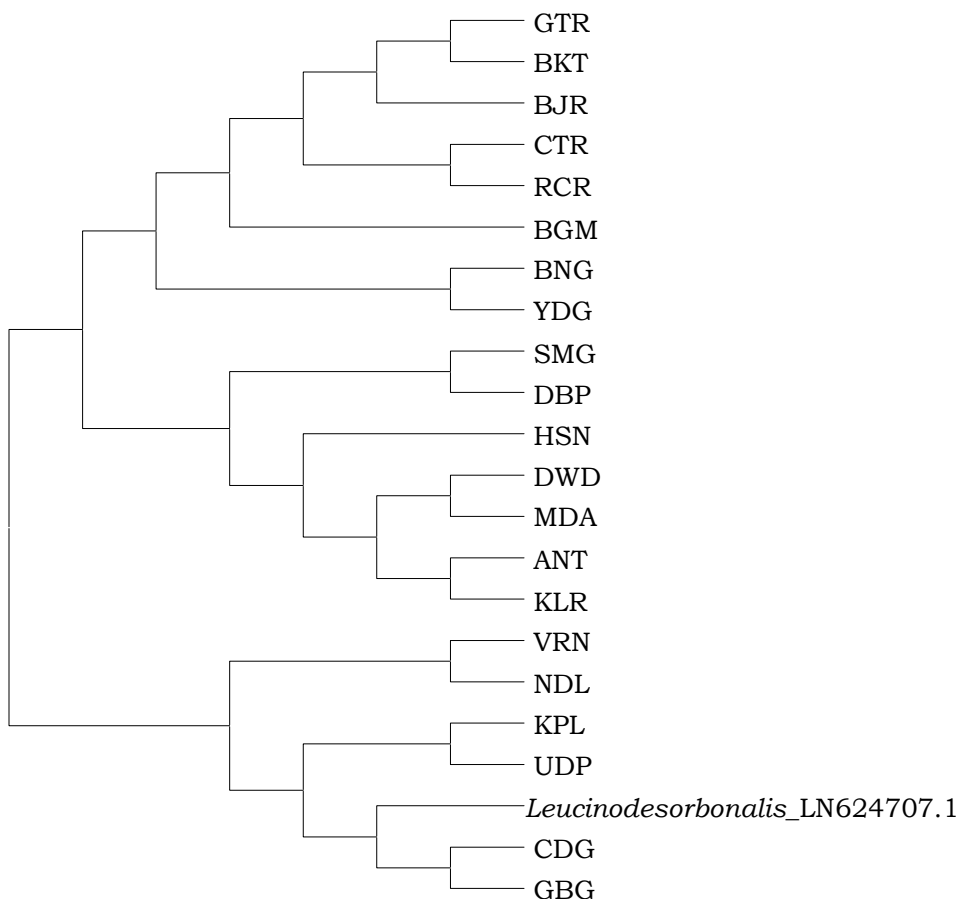
Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
HSN		0.004	0.006	0.005	0.005	0.007	0.003	0.004	0.006	0.004	0.003	0.004	0.006	0.006	0.004	0.006	0.006	0.004	0.007	0.008	0.004	0.004
VRN	0.011		0.006	0.005	0.005	0.008	0.003	0.004	0.006	0.002	0.003	0.003	0.006	0.006	0.004	0.006	0.006	0.003	0.007	0.007	0.004	0.003
BGM	0.018	0.018		0.007	0.005	0.009	0.005	0.006	0.002	0.005	0.005	0.005	0.002	0.002	0.005	0.002	0.002	0.005	0.008	0.008	0.004	0.005
DWD	0.015	0.015	0.026		0.006	0.008	0.004	0.004	0.007	0.004	0.004	0.005	0.007	0.007	0.005	0.007	0.007	0.005	0.008	0.008	0.005	0.005
SMG	0.016	0.013	0.016	0.020		0.008	0.004	0.005	0.006	0.004	0.004	0.004	0.006	0.006	0.005	0.006	0.006	0.004	0.008	0.008	0.005	0.004
DBP	0.029	0.030	0.037	0.033	0.031		0.006	0.007	0.009	0.007	0.006	0.007	0.009	0.009	0.007	0.009	0.009	0.007	0.010	0.011	0.007	0.007
ANT	0.005	0.005	0.016	0.009	0.011	0.024		0.002	0.005	0.002	0.000	0.002	0.005	0.005	0.002	0.005	0.005	0.002	0.007	0.007	0.003	0.002
MDA	0.009	0.009	0.020	0.013	0.015	0.028	0.004		0.006	0.003	0.002	0.004	0.006	0.006	0.003	0.006	0.006	0.004	0.007	0.007	0.004	0.004
GTR	0.016	0.016	0.002	0.024	0.018	0.039	0.014	0.018		0.005	0.005	0.004	0.000	0.000	0.004	0.000	0.000	0.004	0.008	0.007	0.004	0.004
NDL	0.007	0.004	0.015	0.011	0.009	0.026	0.002	0.005	0.013		0.002	0.002	0.005	0.005	0.003	0.005	0.005	0.002	0.006	0.007	0.003	0.002
KLR	0.005	0.005	0.016	0.009	0.011	0.024	0.000	0.004	0.014	0.002		0.002	0.005	0.005	0.002	0.005	0.005	0.002	0.007	0.007	0.003	0.002
CDG	0.009	0.005	0.013	0.013	0.011	0.028	0.004	0.007	0.011	0.002	0.004		0.004	0.004	0.003	0.004	0.004	0.000	0.006	0.006	0.003	0.000
BKT	0.016	0.016	0.002	0.024	0.018	0.039	0.014	0.018	0.000	0.013	0.014	0.011		0.000	0.004	0.000	0.000	0.004	0.008	0.007	0.004	0.004
BJR	0.016	0.016	0.002	0.024	0.018	0.039	0.014	0.018	0.000	0.013	0.014	0.011	0.000		0.004	0.000	0.000	0.004	0.008	0.007	0.004	0.004
BNG	0.009	0.009	0.013	0.013	0.015	0.028	0.004	0.007	0.011	0.005	0.004	0.004	0.011	0.011		0.004	0.004	0.003	0.007	0.007	0.003	0.003
CTR	0.016	0.016	0.002	0.024	0.018	0.039	0.014	0.018	0.000	0.013	0.014	0.011	0.000	0.000	0.011		0.000	0.004	0.008	0.007	0.004	0.004
RCR	0.016	0.016	0.002	0.024	0.018	0.039	0.014	0.018	0.000	0.013	0.014	0.011	0.000	0.000	0.011	0.000		0.004	0.008	0.007	0.004	0.004
GBG	0.009	0.005	0.013	0.013	0.011	0.028	0.004	0.007	0.011	0.002	0.004	0.000	0.011	0.011	0.004	0.011	0.011		0.006	0.006	0.003	0.000
KPL	0.028	0.024	0.032	0.032	0.030	0.047	0.022	0.026	0.030	0.020	0.022	0.018	0.030	0.030	0.022	0.030	0.030	0.018		0.008	0.007	0.006
UDP	0.032	0.028	0.032	0.035	0.032	0.051	0.026	0.030	0.030	0.024	0.026	0.022	0.030	0.030	0.024	0.030	0.030	0.022	0.030		0.007	0.006
YDG	0.011	0.011	0.011	0.015	0.013	0.029	0.005	0.009	0.009	0.007	0.005	0.005	0.009	0.009	0.005	0.009	0.009	0.005	0.024	0.026		0.003
LN624707.1 Out group	0.009	0.005	0.013	0.013	0.011	0.028	0.004	0.007	0.011	0.002	0.004	0.000	0.011	0.011	0.004	0.011	0.011	0.000	0.018	0.022	0.005	

Fig.1 PCR amplification of mtCOI region with *L. orbonalis* population collected from Different locations with universal Folmer primers



Note: 1. Hassan 2. Varanasi 3. Dharwad 4. Shimoga 5. Belgaum 6. Koppal 7. Bangalore rural 8. Ananthapur 9. Mandya 10. Guntur 11. Udupi 12. New Delhi 13. Kolar 14. Chitradurga 15. Bagalkot 16. Bijapur (Vjayapur) 17. Raichur 18. Gulberga (Kalaburgi) 19. Yadagiri 20. Bangalore urban 21. Chittoor

Fig.2 Original tree based on Tamura 3-parameter model for CO1 DNA sequences for different *L. orbonalis* populations based on Maximum likelihood bootstrap method for 2000 replications (Bootstrap consensus tree). CO1 sequence from *L. orbonalis* (accession #LN624707.1) was Used as out group sequence



The population from second clade is again subdivided into two subclades, the population from Varanasi and New Delhi was clustered together which showed the population having lowest variation and the genetic distance varied from 0.002-0.010. The population from Koppal and Udupi was clustered together which showed the population having lowest variation and the genetic distance varied from 0.018-0.051. The other cluster was again divided into three subclusters, the population from Chitradurga and Kalaburgi were clustered together and were having the genetic distance that varied from 0.000-0.028 and showed genetic differences.

The population from South Africa as out group showed relatedness towards the populations from Chitradurga and Kalaburgi and showed similarity of species having the lowest genetic distance that varied from 0.000-0.028 (Fig. 2; Table 3).

Genetic characterization of populations of insect pests plays a pivotal role in determining management strategies of the insect pest (Guirao *et al.*, 1997). Use of isozymes as biochemical marker to study the insect populations began in the late 1950s and continues to be widely applied (Black *et al.*, 1992). Within the last few years, techniques

using the polymerase chain reaction (PCR) to amplify specific genomic regions have become widespread (Saiki *et al.*, 1988; Mullis and Faloona, 1987). Williams *et al.*, (1990) developed the technique that uses a single decamer primer to amplify arbitrary regions of a genome. They demonstrated that RAPD-PCR process reveals polymorphisms in the genomes of a wide variety of insect species.

It was important to distinguish *Leucinodes* species breeding on brinjal crop using mitochondrial DNA markers as a standard identification protocol. Molecular diversity studies in various insects of importance in agriculture helped in identifying new species (Ball and Armstrong, 2006), biotypes (Perring, 2001) cryptic species (Hebert *et al.*, 2004) pheromone and voltinism races (Marcon *et al.*, 1999) and haplotypes (Toda and Murai, 2007), which are difficult to identify through morphological characters. The extent of genetic variation between geographical populations depends on several factors, including gene flow between populations, host range, environment and time since separation (Baker and Stebbins, 1965; Hartl, 1980). In view of this, understanding the genetic differences among the populations of *L. orbonalis* using COI nucleotide sequence will provide better understating of genetic diversity. Studies of genetic variability could help to decide whether the entire *L. orbonalis* is in fact a single interbreeding population in which strong gene flow can occur. Assessing the genetic variability could help to design the management strategies of the insect pests (Marimuthu *et al.*, 2009).

Mitochondrial genes are commonly used for evolutionary studies as they have a number of characteristics such as (i) maternal inheritance with little or no recombination, (ii) general conserved gene and composition, (iii) small size of gene and (iv) lack of introns (Gissi *et*

al., 2008). In the present study, an attempt was made to use cytochrome oxidase subunit 1 (COI) gene for understanding the genetic variations among *Leucinodes* species bred on brinjal. During the study, COI genes for 21 populations of brinjal were obtained from 21 different locations using cytochrome oxidase subunit 1 (COI) gene showed that all population has identity as *L. orbonalis*.

In the present investigation the results from Cytochrome C Oxidase I (COI) gene showed that nucleotide analysis of genetic diversity and phylogenetic analysis of the COI indicate that, the *L. orbonalis* from different geographical regions are homogenous and the populations showed having overall mean distance (0.015 ± 0.003), which is in agreement with reports by Shashank *et al.*, (2015). Topologies of neighbour-joining (NJ) trees indicate all the populations belong to single major clade. Therefore, it is inferred that there was no significant molecular diversity within *L. orbonalis* of different geographical locations of India with respect to COI.

The present analysis showed that average nucleotide composition from the populations was 31.95 % (A), 39.89 % (T/U), 14.36 % (C), and 13.80 % (G). Similar study has reported by Shashank *et al.*, (2015), the average nucleotide composition across all *L. orbonalis* was A = 31.8 %, T = 40.0 %, C = 13.3 % and G = 14.9 %. The pair-wise genetic distance of *L. orbonalis* populations ranged from 0.00 to 0.051, suggesting that there is not much variation in population of *L. orbonalis* from different locations, which corroborates with findings of (Shashank *et al.*, 2015). Marimuthu *et al.*, (2009) investigated that, random-amplified polymorphic DNA markers were used to assess the genetic diversity of *L. orbonalis* population collected from different field locations of Tamil Nadu and reported that out of 17 random-amplified polymorphic DNA primers screened, only 11

primers generated polymorphic bands (up to 14 bands). According to their level of similarities, only two major clusters with no variation among population were deduced.

The present finding suggests that there was no wider difference in the case of existing population of *L. orbonalis*, which is similar to findings of Geetha Rajalakshmi *et al.*, (2006), who worked out the mean similarity for 10 geographic populations of *L. orbonalis* and found that all these populations were closely related in their study in Tamil Nadu. Further, our results indicated that there is a stable inherited gene flow among the present population of *L. orbonalis* lessening genetic difference which may be endorsed to passive and active dispersal of the insect besides nonexistence of host-induced disparity among the population. As molecular variability of *L. orbonalis* population is an important consideration for brinjal, constant monitoring is essential to study the possible development of resistance.

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