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Manjunath J
Scientist (Entomology), Regional Agricultural Research Station, Nandyal, Kurnool, Andhra Pradesh, India

Manjula K
Professor (Entomology), S.V Agricultural College, Tirupati, Chittoor, Andhra Pradesh, India

Muralikrishna T
Principal Scientist (Entomology), Institute of Frontier Technologies, Regional Agricultural Research Station, Tirupati, Chittoor, Andhra Pradesh, India

Hariprasad KV
Associate Professor (Entomology), Department of Entomology, S.V Agricultural College, Tirupati, Chittoor, Andhra Pradesh, India

Prasad TNVKV
Principal Scientist (Soil Physics), Institute of Frontier Technologies, Regional Agricultural Research Station, Tirupati, Chittoor, Andhra Pradesh, India

Ravindra Reddy B
Associate Professor (Statistics), S.V Agricultural College, Tirupati, Chittoor, Andhra Pradesh, India

Corresponding Author:
Manjunath J
Scientist (Entomology), Regional Agricultural Research Station, Nandyal, Kurnool, Andhra Pradesh, India

Genotypic variation of groundnut bruchid *Caryedon* spp from different locations of Rayalaseema zone of Andhra Pradesh in India

Manjunath J, Manjula K, Muralikrishna T, Hariprasad KV, Prasad TNVKV and Ravindra Reddy B

Abstract
Study on genotypic diversity in groundnut bruchid *Caryedon* spp collected from various 24 different locations of Rayalaseema region of Andhra Pradesh in India. A molecular PCR-CTAB based test was used for molecular identification of *Caryedon* spp with using of the specific species primers of *Caryedon serratus* and *Caryedon gonagra.* Although genetic diversity was different between agro-ecological zones tests show no significant differences in structuring according to agro-ecological zone. These tests, as well as the phylogenetic relationships that our results imply, indicate that there is a genetic differentiation between individuals from groundnut storing areas compared to those from areas where the stored groundnuts is absent or low.

Keywords: PCR-CTAB, genetic differentiation, grain insect pest, geographical strain, molecular technique, groundnut bruchid, *Caryedon serratus* and *Caryedon gonagra*

1. Introduction
Groundnut (*Arachis hypogaea,* Linnaeus) is a leguminous oilseed crop and its native is South America. Groundnut is called as the 'King' of oilseeds. It is one of the most important food and cash crops of our country. While being a valuable source of all the nutrients, it is a low priced commodity. Groundnut is also called as wonder nut and poor men's cashew nut. Groundnut is one of the most important cash crops of our country. It is a low-priced commodity but a valuable source of all the nutrients. The groundnut has a distinct position among the oilseeds as it can be consumed and utilized in diverse ways. The chemical composition of groundnut compares favorably with that of dry fruits. Some of the nutrients like protein and thiamine are available in higher quantities in groundnut than any other dry fruits. Groundnut oil is considered as stable and nutritive as it contains just the right proportion of Oleic acid (40-50%) and Linoleic acids (25-35%) [11]. It is a rich source of edible oil (47-5%) and high quality of protein (30%) and hence groundnut is valued both for edible oil and confectionery purposes. The groundnut kernels are consumed as raw, boiled, roasted or fried products and also used in varieties of culinary preparations like peanut butter, peanut milk, chocolates [5].

Groundnut is grown on 26.4 million ha worldwide with a total production of 37.1 million metric tons and an average productivity of 1.4 metric t/ha. Over 100 countries worldwide grow groundnut. Developing countries constitute 97% of the global area and 94% of the global production of this crop. It is the most important commercial oilseed crop mostly grown in the semi-arid tropical region like India. In India, the groundnut production was 7180.5 thousand tons in 2015-16. There were nine States having groundnut production of more than 100 thousand tons *viz.*, Gujarat, Rajasthan, Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, West Bengal and Telangana in 2015-16 [9]. Out of 100 species of insect pests attacking the stored groundnut, pod bruchid *Caryedon serratus* (Olivier) is a major cosmopolitan pest of economic importance [14]. The groundnut bruchid, *C.serratus* is the primary storage pest of unshelled groundnuts in many parts of Asia and throughout West and Central Africa [3]. Generally the harvested produce is stored by farmers, processors, seed agencies and other oil extraction units for about 6-9 months before final use [1]. In India, storage losses of groundnut ranges between 10 and 15 per cent [12].

Then a particular study with the same molecular tools was performed for the differentiation between geographical strains in the *Caryedon* spp. complex, with a special attention paid to

C .serratus strains originating from different continents (Africa, South America and Europe). Extracted DNA from individuals of the same species and strain. The differences observed in band profiles of the different species were highly significant and enabled the easy differentiation between them. It was shown frequent dissimilarities between the band profiles of geographical strains of *C. serratus*. The potential for RAPD-PCR technique to provide useful genetic data for the discrimination up to the population level of insects found in stored products in international trade is discussed.

Morphological study a more thorough molecular analysis, through which recognized that *Caryedon serratus* and *C. gonagra* are sister species and they revived in the last as a valid species. With that information, the purpose it serves this research is to determine which of the two species accurate determination [4].

2. Materials and Methods

2.1 Survey and sample collection

To study the molecular characterization of groundnut bruchid *Caryedon* spp, a survey was conducted in different locations of four districts of Rayalaseema region of Andhra Pradesh state, selected four districts *viz.*, Ananthapuramu, Chittoor, Kurnool and Y S R Kadapa. The survey was carried out during 2014-15 and 2015-16. At each survey, representative samples of 1 kg groundnut pods were drawn from the surveyed storage places. Groundnut pods containing the eggs, grubs and pupae of *Caryedon* spp were collected from twenty four (24) locations and transferred to clean plastic jars (20 × 12 × 10 cm) and were kept separately. The jars were covered with rearing cloth and maintained in the laboratory. These cultures were multiplied separately continuously for utilization of adult bruchids for genotypic experimentation studies

The study on genotypic diversity in groundnut pod borer *Caryedon* spp collected from various 24 different locations of Rayalaseema region of A.P was carried out at Department of Entomology in Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, and A.P India. (Table 1).

Table 1: Codes fixed for the *Caryedon* spp populations collected from different locations of Rayalaseema region of A.P.

S. No.	Location	Population code
2014-15		
Ananthapuramu Dist		
1	Market yard, Uravakonda(M)	UMY
2	Farmers Godown, Raketla, Uravakonda(M)	RF
3	Seed godown, ARS, Kadiri (M)	KARS
Chittoor Dist		
4	Seed godown, Kalikiri (M)	KKVK
5	DCMS, Seed godown, Chittoor (M)	DCMSC
6	Seed godown, RARS, Tirupati (M)	TRARS
Kurnool Dist		
7	Seed godown, NSC, Nandyal (M)	NNSC
8	M/s Neelakanteswara farmers seed godown, Ayyalurmetta (M)	NAPSSC
9	Farmers godown, Yallur (V), Gospadu (M)	YFG
Dr YSR Kadapa Dist		
10	Seed godown of Krishi Vigyan Kendra (KVK), Utukur (V), and Chinnathakommedinne (M).	UKVK
11	M/s Sri Lakshmi Narasimha Oil mill, Kadapa (M)	KLO
12	Seed godown APSSDC, Kadapa (M)	APSSCK
2015-16		
Ananthapuramu Dist		
13	Farmer seed godown, Tharimela, Pamidi (M)	FST
14	Seed godown, APSSDC, Prasannayapalli (V), Ananthapuramu (M)	BAPSSC
15	Seed godown Krishi Vigyan Kendra, Reddipalli, Bukkarayasamuduram (M)	RKVK
Chittoor Dist		
16	Seed godown, APSSDC, Kalahasthi (M)	KAPSSC
17	M/S Sri Balaji seed godown, Pileru (M)	PBG
18	Farmers godown, Bangarupalem (M)	MVO
Kurnool Dist		
19	Farmers seed godown, Madikerra (M)	MFG
20	Seed Godown, Dhone (M)	DSG
21	M/s Little Krishna seed processing plant, Chabolu, Nandyal (M)	CLK
Dr YSR Kadapa Dist		
22	Seed godown, Chapadu (M)	CSG
23	Seed Godown, ARS, Utukur, Chinnathakommedinne (M)	POM
24	Mydukur cold storage seed godown, Mydukur (M)	MCSG

2.2 *Caryedon* spp sampling

Adult bruchids of *Caryedon* spp obtained from the pod sample cultures of surveyed areas were used for studying the genetic in all, a total of 400 individuals of both sexes corresponding to 24 different populations with 20 individuals per population were studied for their genetic variation. Adult bruchids were transferred to absolute alcohol and were stored in deep freezer (-20 °C).

2.3 Procedure for extraction of DNA from groundnut bruchid *Caryedon* spp

The groundnut bruchid populations collected from 24 locations of Rayalaseema region were chosen for molecular characterization after identifying through morphological and taxonomic characters as *C. serratus* and *C. gonagra*. Total genomic DNA was extracted from prothorax of individual bruchid. The abdomen, elytra and antennae were kept apart to

avoid contamination by fungi, nematodes and the specimens were preserved in absolute alcohol till further use at -20 °C [16].

The prothorax of the adult groundnut bruchid was homogenized by using pre-sterilized pestle and mortar in 1 ml absolute alcohol and transferred to sterile eppendorf tubes and suspended in a pre-heated (65 °C) 900 µl of extraction buffer containing 2% CTAB, 100Mm Tris, 20m MEDTA, 1.4 M Nacl, 1% PVP and 1% β-mercapto ethanol [7]. The homogenates were mixed by vortexing for few minutes and incubated for one hour at 65 °C in water bath and then the samples were centrifuged for 10 minutes at 10,000 rpm. Supernatant was collected into separate tubes and equal volumes of phenol : chloroform (1:1) were added and again the samples were kept for centrifugation for 10 minutes at 10000 rpm, to the supernatant, 0.6 volume (Eg: 300 µl then 0.6 × 300 =180 µl) of ice cold iso-proponal were added and incubated at -20ºC for overnight for precipitation of DNA. After incubation, the tubes were spun at 13,000 rpm for 20 minutes a 4 °C and the supernatant was discarded. Later the pellet was washed with 70 per cent ethanol (100 µl) and centrifuged at 13,000 rpm for 10 minutes at 4 °C. The pellet was allowed to air dry. The dried pellet was dissolved in 100 µl sterile distilled water and RNase A (10 ng/ml) was added and tubes were stored at -20 °C for further studies.

2.4 Determining DNA concentration and quality

The concentration and quality of DNA was estimated using nanodrop spectrophotometer at 260 nm (ND-1000, USA). Nanodrop spectrophotometric method was based on measuring the amount of ultraviolet (UV) irradiation that is absorbed by the bases. The ratio of OD 260/280 was determined to assess the purity of the sample. If the ratio is 1.8 : 2.0, the absorption is due to nucleic acids. The quantity of DNA was calculated using the formula.

1-OD = 50 mg DNA and taking the dilution factor

DNA mg/ml= A260 × dilution factor

It was verified by running DNA sample (2 ml) from each isolate mixed with 2 ml of 1 per cent loading dye on 1.0 per cent agarose along with 1 and 1.5 KB marker.

2.5 PCR amplification of DNA of groundnut bruchid *Caryedon* spp by using specific primers

Composition of PCR reaction was optimized. The standardized amplification assay was as follows: 75 ng Template DNA- 2.5 µL, Taq DNA polymerase (Fermentas)- 3 Units - 1.5 µL; 2.5 mM $MgCl_2$ - 1.5 µL; 0.2 mM dNTPs mix - 1.0 µL; 10 pmole forward primer (sigma) - 2.0 µL, 10 pmole reverse primer (sigma) - 2.0 µL, 10 × assay buffer (Fermentas) - 2.5 µL in a reaction volume of 25 µL.

Reagents	Volume/tube (µL)
Taq assay buffer (10×)	2.5
dNTP's (2 mM)	1.0
Forward primer (10 pmole)	2.0
Reverse primer (10 pmole)	2.0
$MgCl_2$ (2.0 mM)	1.5
Taq DNA polymerase (3 U µL-1)	1.5
Template DNA (75 ng)	2.5
Sterile distilled water	12.0
Total	25.0

2.6 List of specific primers used for *Caryedon serratus* and *Caryedon gonagra*

The primers CB1 /CB2 (Cytochrome B (Cyt. B) end region) and CIL / CIU (ITS1 ribosomal DNA) of *Caryedon serratus* were used for identification of *C. serratus* [13].

The primers A3CytBF / A3CytBR, CsF / CsR, A2CyF / A2CyR and 47CMOsF / 47CMOsR of *C. gonagra* sequences retrieved from GenBank [2]. Duplicated sequences removed and 7 unique sequences were used for primer design (gi|739058235, gi|739058231, gi|739058210, gi|739058206, gi|739058202, gi|45000074 and gi|739058224). Primers were designed using Primer3 web tool using default parameters [8] [15].

A total of 6 primer sets were obtained from Sigma Company and used for studying the genetic constitution (Table 2).

Table 2: List of Primers used for *Caryedon serratus* and *Caryedon gonagra*

S. No	Primer	Sequence (5' → 3')
1	CB1	TATGTACTACCATGAGGACAAATATC
	CB2	ATTACACCTCCTAATTTATTAGGAAT
2	CIL	GCGTTCGAARTGCGATGATCAA
	CIU	GTAGGTGAACCTGCAGAAGG
3	A3CytBF	TCCTTTTGAGGCGCTACTGT
	A3CytBR	TGCTGCTACAATAAAGGGGAA
4	CsF	CTATTGTTACTGCCCACGCC
	CsR	GGCGGATAAACAGTTCACCC
5	A2CyF	CTATTGTTACTGCCCACGCC
	A2CyR	GGCGGATAAACAGTTCACCC
6	47CMOsF	TCCTTTTGAGGCGCTACTGT
	47CMOsR	TGCTGCTACAATAAAGGGGAA

2.7 PCR conditions for amplification of DNA of *C. serratus* and *C. gonagra*

The Polymerase Chain Reaction (PCR) was performed by using Bio rad (Thermal cycler), with following temperature profile (Table 3). The initial denaturation at 92 °C for 3 min followed by 35 cycles of denaturation at 92 °C for 1 min; annealing at 48 °C for 30 sec; extension at 72 °C for 1 min with final elongation at 72 °C for 15 min. for *Caryedon* spp primers (Table 4).

Table 3: PCR conditions maintained in the amplification of DNA of groundnut bruchid *C. serratus* and *C. gonagra*

S. No.	Step	Temperature (°C)	Duration (Min)	No. of cycles
1	Initial denaturation	92.0	3	1
2	Denaturation	92.0	1	35
3	Annealing	48.0	0.5	35
4	Extension	72.0	2	35
5	Final Extension	72.0	15	1
6	Hold	4.0	∞	-

Table 4: Annealing temperatures for different primers of *C. serratus* and *C. gonagra*

S. No	Primer	Annealing Temperature(ºC)
1	CB1/ CB2	48 for 1 min
2	CIL/ CIU	48 for 1 min
3	A3CytBF/ A3CytBR	47 for 1 min
4	CsF/ CsR	48 for 1 min
5	A2CyF/ A2CyR	47 for 1 min
6	47CMOsF/ 47CMOsR	48 for 1 min

The amplified PCR products were separated by electrophoresis in 1.8% agarose gel run in 1 × TAE buffer at 90V for 1.5 h till amplified fragments were separated. Amplified patterns were documented by gel document (Gel

doc, Bio-Rad). Based on size of the amplicon, the species was confirmed as both *C. serratus* and *C. gonagra.*

3. Results and Discussions

3.1 DNA extraction from adult groundnut bruchid *Caryedon* spp

DNA was extracted from *Caryedon* spp from 24 locations of Rayalaseema region in the procedure described [16]. The resultant DNA was checked through agarose gel electrophoresis. After getting DNA band, quantification of DNA was done with nanodrop spectrophotometer. The amount of DNA in the sample is directly proportional to the amount of UV radiation absorbed. The absorbance was measured at 260 nm (A_{260}) for DNA and at 280 nm (A_{280}) for proteins. The nitrogen bases in nucleotides have an absorption maximum at about 260 nm. In contrast to nucleic acids, proteins have UV absorption maximum of 280 nm, typically due to the tryptophan residues. The absorbance of DNA sample at 280 nm gives an estimate of the protein contamination of the sample. The ratio of A_{260} to A_{280} (>1.5) was determined to establish purity of the DNA (Brown, 1998). $A_{260/280}$ ratio which is a direct reflection of amount of DNA compared to protein. DNA quantity of adult groundnut beetle was standardized to 75ng/µL.

3.2 Optimization of PCR amplification conditions for DNA amplification

In the present study, 6 sets of primers related to cytochrome B, ITS1 region of groundnut beetle were employed to identify the groundnut beetle population collected from 24 locations of Rayalaseema region of Andhra Pradesh. The PCR conditions were optimized in terms of concentration of template DNA, Taq DNA polymerase and $MgCl_2$ concentration. Varying concentration of template DNA from 100 ng to 125 ng in a reaction volume of 25 µl, 75 ng DNA gave specific bands. A titration of different concentration of Taq DNA polymerase and $MgCl_2$ showed that 3.0 unit of Taq DNA polymerase and 2.0 mM $MgCl_2$ in a final reaction mixture gave optimum, reproducible and well resolved results. A higher or lower concentration resulted in either sub optimal or lack of complete amplifications. The final reaction mixture contain 2.5 µl of Taq assay buffer (10X), 1.0 µl of dNTP's (2mM), 2.0 µl of Forward primer (10 pmole),2.5 µl Reverse primer (10 pmole), 2.0 µl Template DNA (75 ng), 1.5 µl of $MgCl_2$ (2.0 mM) and 1.5 µl of Taq DNA polymerase (3U/µl) in a PCR reaction volume of 25 µl. The standardization of annealing temperature for each primer pair was done by gradient PCR as given below.

3.3 Molecular Characterization of Groundnut bruchid Population Using Specific Primers

A total of 24 populations (UMY, RF, KARS, KKVK, DCMSC, TRARS, NNSC, NAPSSC, YFG, UKVK, KLO, APSSCK, FST, BAPSSC, RKVK, KAPSSC, PBG, MVO, MFG, DSG, CLK, CSG, POM and MCSG) representing 24 locations in Rayalaseema region was characterized by using six primers CB1/CB2, CIL/CIU, 47F/47R, A3Cyt BF/ A3Cyt B R, CsF/CsR and A2CytF/ A2Cyt. Among the above six primers, two primers *i.e.* CB1/CB2 and CIL/CIU were targeted for *C. serratus* and the remaining four primers A3CytBF/ A3CytBR, CsF/CsR, A2CyF/A2CyR and 47CMOsF/47CMOsR were designed for *C. gonagra* based on the sequences available in Gen bank database.

3.4 Molecular Analysis of groundnut bruchid using CB1/CB2 primer

Among the 24 populations, all the samples were amplified with CB1/CB2 primer at 518 bp which was confirmed as *C. serratus* in accordance with [13]. (Fig 1 and 2).

3.5 Molecular Analysis of groundnut bruchid using CIL/CIU primer

In case of CIL/CIU primer, twenty samples were positive and resultant amplification was observed at 874bp and it was confirmed as *C. serratus*. The amplification was absent in four samples collected from KLO, FST, BAPSSC and RKVK. Besides, target band, non-target amplification was observed at ~190-200bp with CIL/CIU primers of all the positive samples except KLO (Fig 3 and 4).

3.6 Molecular Analysis of groundnut bruchid using A3CytBF/A3CytBR primer

Among the 24 samples, all the 24 samples were amplified with A3CytBF/ A3CytB Rat 197 bp. These samples were indeed to *C. gonagra* (Fig 5 and 6).

3.7 Molecular Analysis of groundnut bruchid using CsF/CsR primer

Out of 24 samples, 22 samples were amplified at 1500 bp with CsF/CsR and in two samples, BAPSSC and POM the amplification was not observed. Non- target amplification was also noticed in the positive samples at ~200 bp. Based on these the samples were confirmed as *C. gonagra* (Fig 7 and 8).

3.8 Molecular Analysis of groundnut bruchid using A2CytF/A2CytR primer

Among the 24 samples, 12 samples were amplified with A2CytF/A2Cyt primer at 690 bp *viz*., UMY, RF, KARS, KKVK, DCMSC, TRARS, NNSC, FST, BAPSSC, RKVK and KAPSSC at 690 bp. Slight amplification was observed at ~75 bp to 290 bp in UMY, RF, KARS, KKVK, DCMSC, TRARS, NNSC, FST of the population collected during 2014-15 and 2015-16. Based on these the samples were confirmed as *C. gonagra* (Fig 9 and 10).

3.9 Molecular Analysis of groundnut bruchid using 47 CMOsF / 47 CMOsR primer

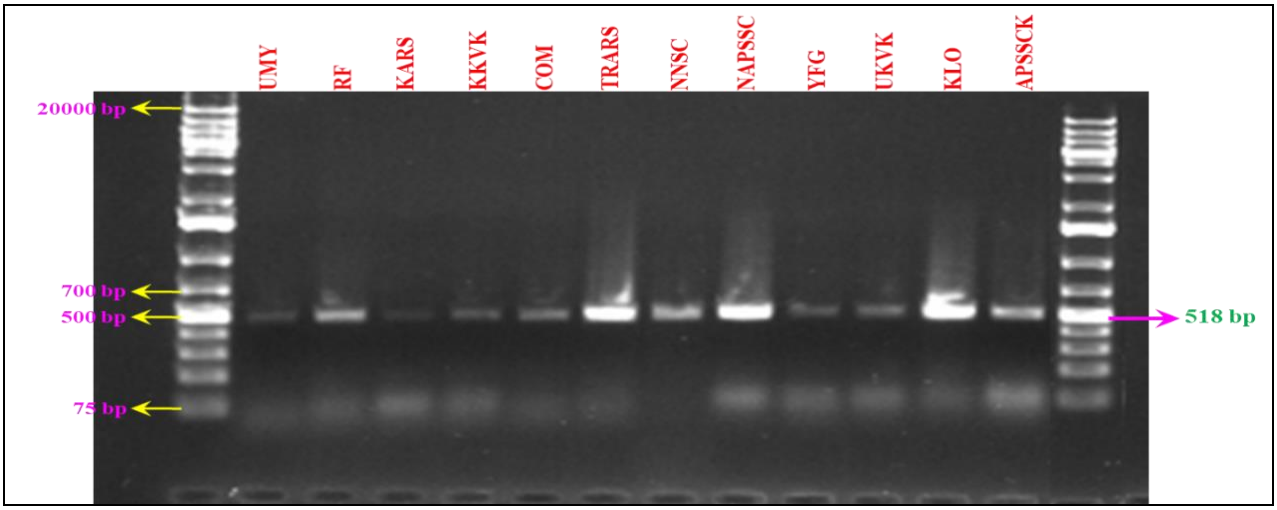
Out of 24 samples, 23 samples were amplified at 197bp with 47F/47R primer among the 23, a slight amplification was observed in KARS, APSSCK, CLK and absent in NNSC. Based on these the samples were confirmed as *C. gonagra* (Fig 11 and 12).

To confirm the phenotypic identification of *C. serratus* in the present study, the molecular characterization was done by using two specific primers *i.e.* CB1/CB2 and CIL/CIU (Sigma Company). The amplification of DNA was observed at 518 bp and 874 bp with CB1/CB2 and CIL/CIU primers respectively in the *C. serratus* population collected from different locations. In addition to this, with CIL/CIU primers, the amplification of DNA was observed at 190-200 bp, this observation led to the molecular characterization of *Caryedon* spp population for finding out any other species.

Four primers of *C. gonagra* (Sigma Company) *viz.*, A3CytBF/A3CytBR,CsF/CsR, A2CyF/A2CyR and 47CMOsF/47CMOsR were used in the present study. In majority of the samples, the DNA amplification was observed from 197 bp to 210 bp (Fig 5, 6, 7, 8, 11 and 12). Apart from

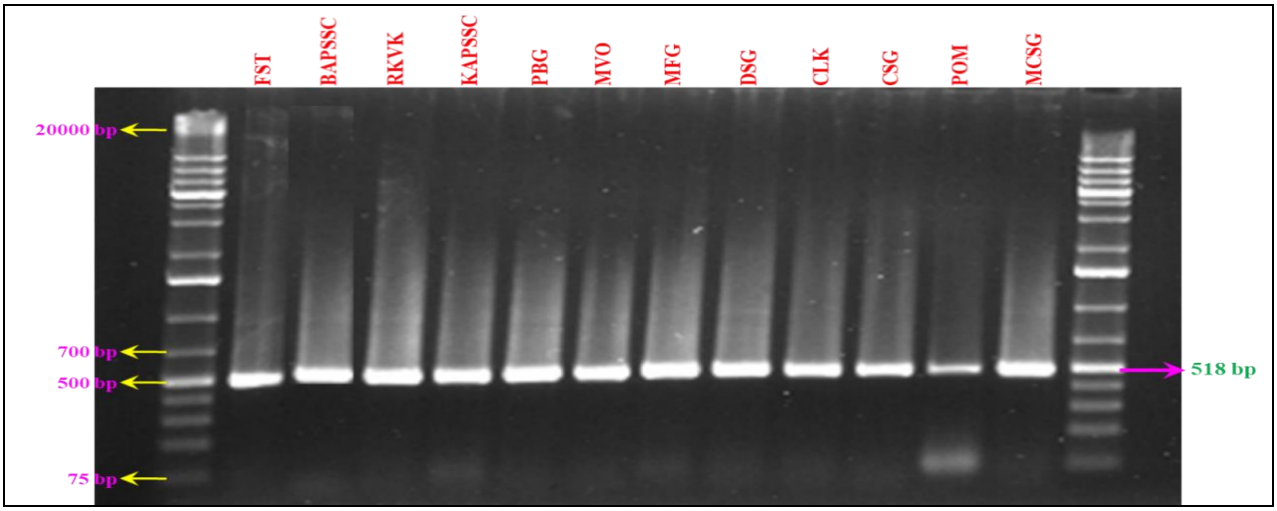
this, in case of CsF/CsR primers, DNA amplification was observed at 1500 bp also [2]. The above primers are specific for *C. gonagra*, and in the present studies, the amplification of DNA was observed in the above range and the population collected from UKVK, FST, RKVK, KAPSSC, PBG, MVO, DSG and CLK can be confirmed as *C. gonagra*. In the phenotypic characterization, and also by using specific primers in genotypic studies, the population of bruchid was confirmed as *C. serratus*. Hence, further studies are required for confirming the species with microsatellite primers and also by gene sequencing. *C. gonagra* and *C. serratus* appears as sister species which derived from same ancestor feeding on Mimosoideae and Caesalpinioideae, which might be due to their genetic relatedness [4].

The studies of phylogenetic and molecular studies of *C. serratus* feeding on *Cassia sieberiana* reported that, with the primers CB1 and CB2, the partial Cyt. B gene from 25 *C. serratus* populations amplified at 518 bp [13]. Further, an amplicon size of 874 bp with ITS1 region using CIL and CIU primers in "*serratus*" and 950 bp in *sieberian* noticed [13]. In detail studies of the morphological characters associated with the analysis of the partial Cyt.B end region using CB1 and CB2 primer revealed that the genetic distance between *C. serratus* and *C. gonagra* was successfully distinguished [10]. The studies on the effect of agro-ecological zones and contiguous basin crops of groundnut on the structuring and genetic diversity of *C. serratus* in the sub-region of West Africa and reported 37 haplotypes for the cytochrome b and 7 haplotypes for the 28S ribosomal gene. Genetic diversity as well as the phylogenetic relationships showed no significant differences in structuring according to agro-ecological zones where groundnut culturing areas compared to those areas where the cultivation of groundnuts is absent or low [6].



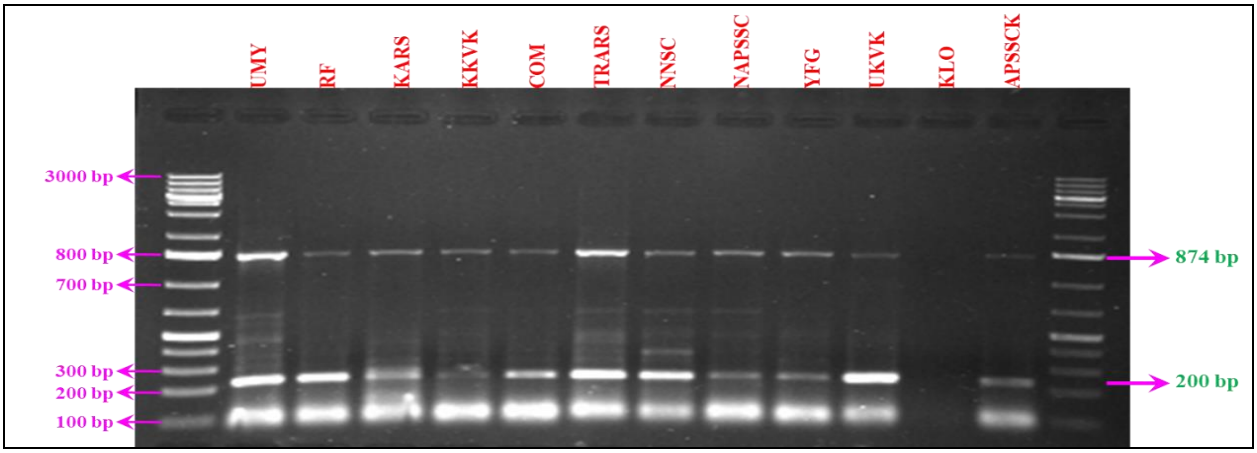
UMY: Market yard, Uravakonda; RF: Farmers Godown, Raketla; KARS: Seed godown, ARS, Kadiri; KKVK: Seed Godown, Kalikiri; DCMSC: DCMS, Seed godown, Chittoor; TRARS: Seed godown, RARS Tirupati; NNSC: Seed godown, NSC, Nandyal; NAPSSC: M/s Neelakanteswara farmers seed godown, Ayyalurmetta; YFG: Farmers godown, Yallurl; UKVK: Seed godown of Krishi Vigyan Kendra, Utukur; KLO: M/s Sri Lakshmi Narasimha Oil mill, Kadapa; APSSCK: Seed godown APSSC, Kadapa.

Fig 1: PCR amplification of DNA of *Caryedon* spp collected from different locations using Cytochrome B (Cyt. B) specific primers CB1/CB2 of *C. serratus* during 2014-15.



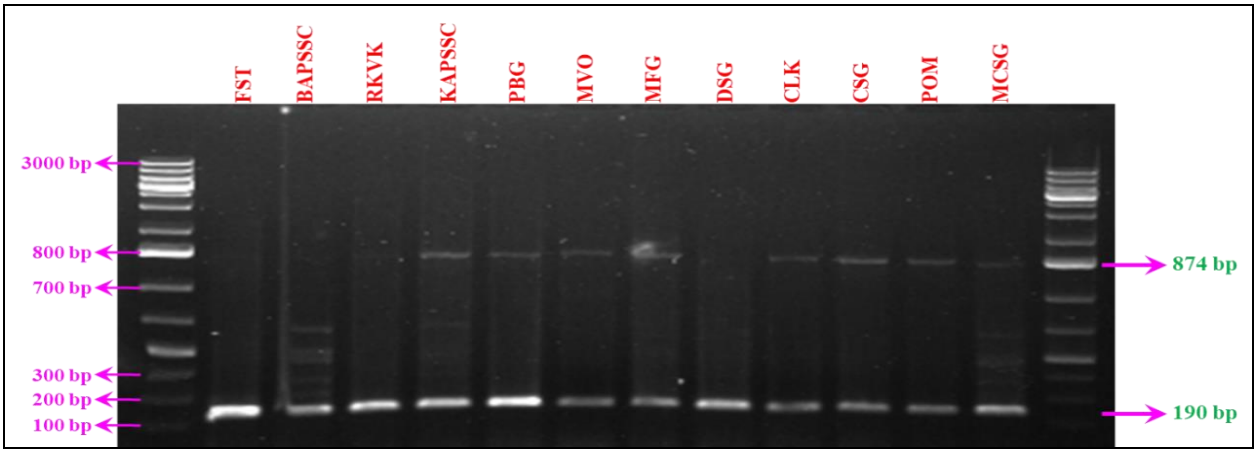
FST: Farmer seed Godown, Tharimela; BAPSSC: APSSDC, Prasannayapalli; RKVK: Seed godown KrishiVigyan Kendra, Reddipalli; KAPSSC: Seed godown, APSSDC, Kalahasthi; PBG: M/s Sri Balaji seed godown, Pileru; MVO: Farmers godown, Bangarupalem; MFG: Farmers seed godown, Madikerra; DSG: Seed Godown, Dhone; CLK: M/s Little Krishna seed processing plant, Chabolu; CSG: Seed godown, Chapadu; POM: Seed Godown, ARS, Utukur; MCSG: Mydukur cold storage seed godown, Mydukur

Fig 2: PCR amplification of DNA of *Caryedon* spp collected from different locations using Cytochrome B (Cyt. B) specific primers CB1/CB2 of *C. serratus* during 2015-16.



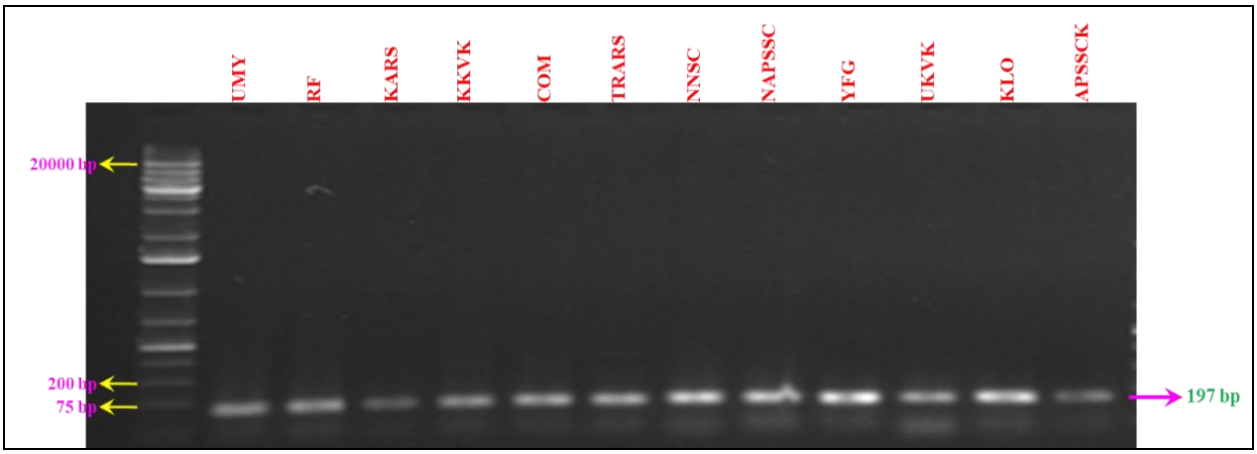
UMY: Market yard, Uravakonda; RF: Farmers Godown, Raketla; KARS: Seed godown, ARS, Kadiri; KKVK: Seed Godown, Kalikiri; DCMSC: DCMS, Seed godown, Chittoor; TRARS: Seed godown, RARS Tirupati; NNSC: Seed godown, NSC, Nandyal; NAPSSC: M/s Neelakanteswara farmers seed godown, Ayyalurmetta; YFG: Farmers godown, Yallurl; UKVK: Seed godown of Krishi Vigyan Kendra, Utukur; KLO: M/s Sri Lakshmi Narasimha Oil mill, Kadapa; APSSCK: Seed godown APSSC, Kadapa.

Fig 3: PCR amplification of DNA of *Caryedon* spp collected from different locations using ITS1 specific primer CIL/CIU of *C. serratus* during 2014-15.



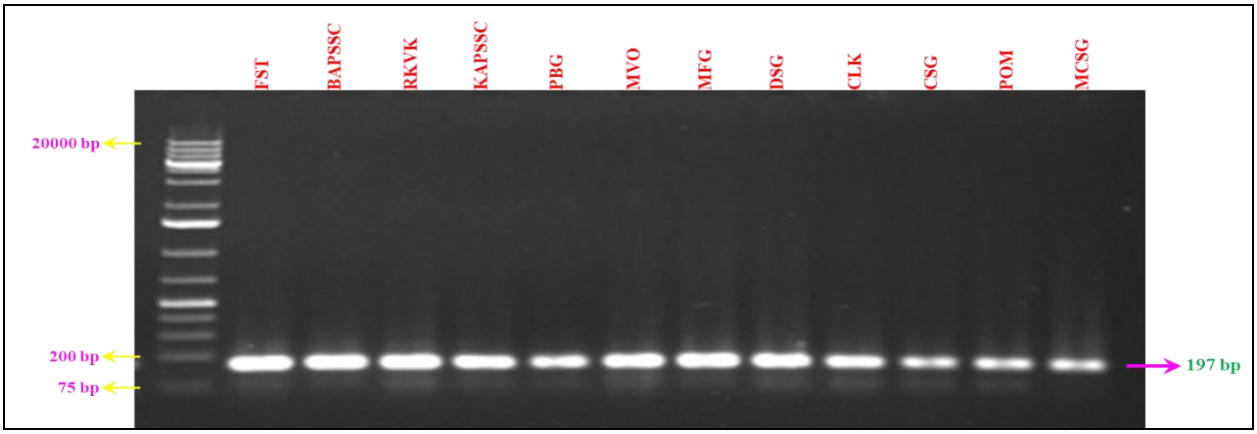
FST: Farmer seed Godown, Tharimela; BAPSSC: APSSDC, Prasannayapalli; RKVK: Seed godown KrishiVigyan Kendra, Reddipalli; KAPSSC: Seed godown, APSSDC, Kalahasthi; PBG: M/s Sri Balaji seed godown, Pileru; MVO: Farmers godown, Bangarupalem; MFG: Farmers seed godown, Madikerra; DSG: Seed Godown, Dhone; CLK: M/s Little Krishna seed processing plant, Chabolu; CSG: Seed godown, Chapadu; POM: Seed Godown, ARS, Utukur; MCSG: Mydukur cold storage seed godown, Mydukur

Fig 4: PCR amplification of DNA of *Caryedon* spp collected from different locations using ITS1 specific primer CIL/CIU of *C. serratus* during 2015-16



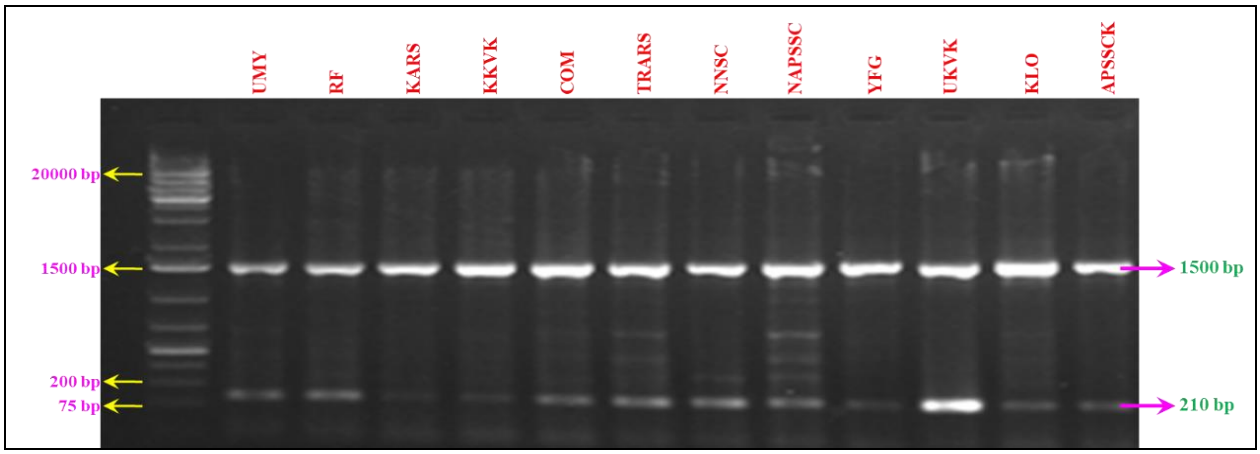
UMY: Market yard, Uravakonda; RF: Farmers Godown, Raketla; KARS: Seed godown, ARS, Kadiri; KKVK: Seed Godown, Kalikiri; DCMSC: DCMS, Seed godown, Chittoor; TRARS: Seed godown, RARS Tirupati; NNSC: Seed godown, NSC, Nandyal; NAPSSC: M/s Neelakanteswara farmers seed godown, Ayyalurmetta; YFG: Farmers godown, Yallurl; UKVK: Seed godown of Krishi Vigyan Kendra, Utukur; KLO: M/s Sri Lakshmi Narasimha Oil mill, Kadapa; APSSCK: Seed godown APSSC, Kadapa.

Fig 5: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers A3CytBF / A3CytBR of *C. gonagra* during 2014-15



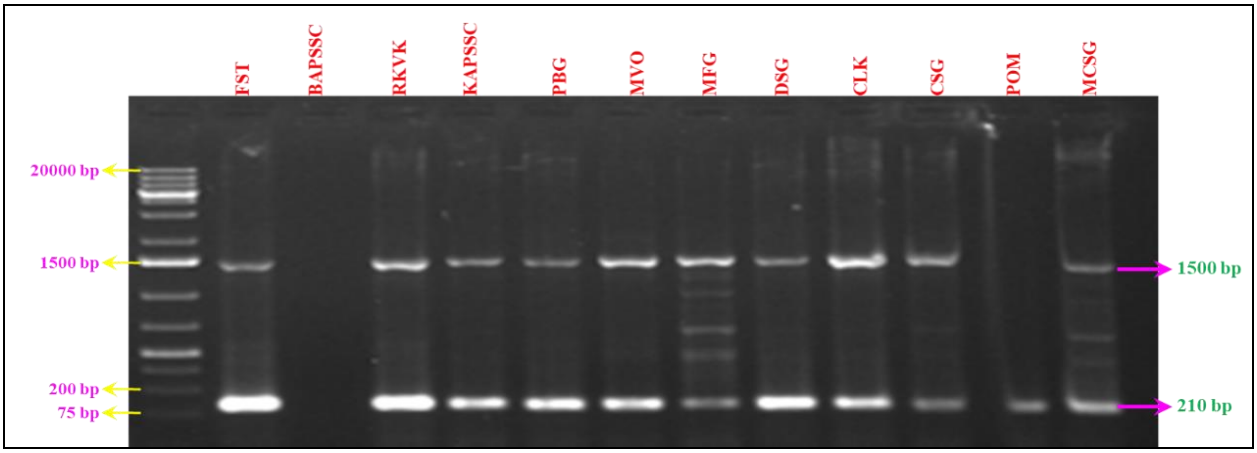
FST: Farmer seed Godown, Tharimela; BAPSSC: APSSDC, Prasannayapalli; RKVK: Seed godown Krishi Vigyan Kendra, Reddipalli; KAPSSC: Seed godown, APSSDC, Kalahasthi; PBG: M/s Sri Balaji seed godown, Pileru; MVO: Farmers godown, Bangarupalem; MFG: Farmers seed godown, Madikerra; DSG: Seed Godown, Dhone; CLK: M/s Little Krishna seed processing plant, Chabolu; CSG: Seed godown, Chapadu; POM: Seed Godown, ARS, Utukur; MCSG: Mydukur cold storage seed godown, Mydukur

Fig 6: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers A3CytBF / A3CytBR of *C. gonagra* during 2015-16



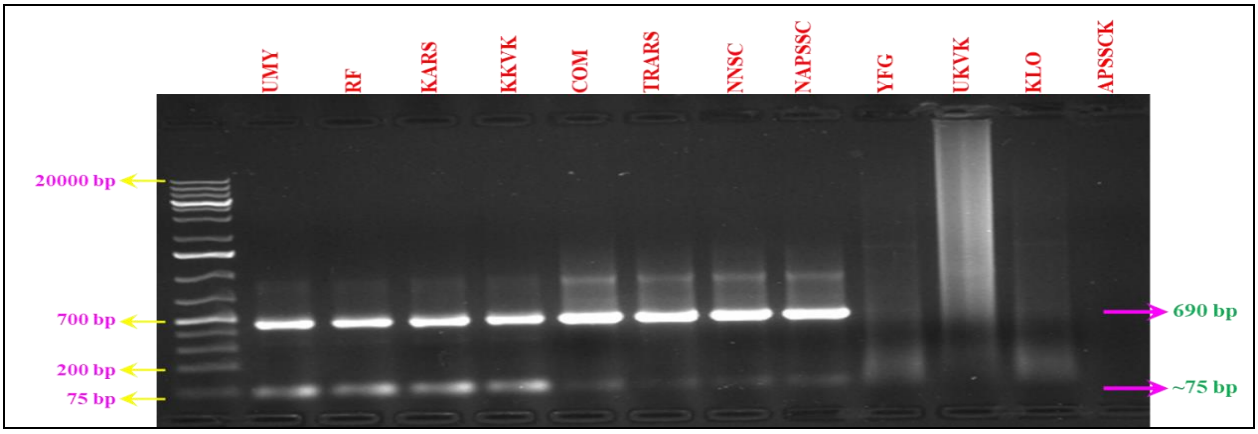
UMY: Market yard, Uravakonda; RF: Farmers Godown, Raketla; KARS: Seed godown, ARS, Kadiri; KKVK: Seed Godown, Kalikiri; DCMSC: DCMS, Seed godown, Chittoor; TRARS: Seed godown, RARS Tirupati; NNSC: Seed godown, NSC, Nandyal; NAPSSC: M/s Neelakanteswara farmers seed godown, Ayyalurmetta; YFG: Farmers godown, Yallurl; UKVK: Seed godown of Krishi Vigyan Kendra, Utukur; KLO: M/s Sri Lakshmi Narasimha Oil mill, Kadapa; APSSCK: Seed godown APSSC, Kadapa.

Fig 7: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers CsF / CsR of *C. gonagra* during 2014-15



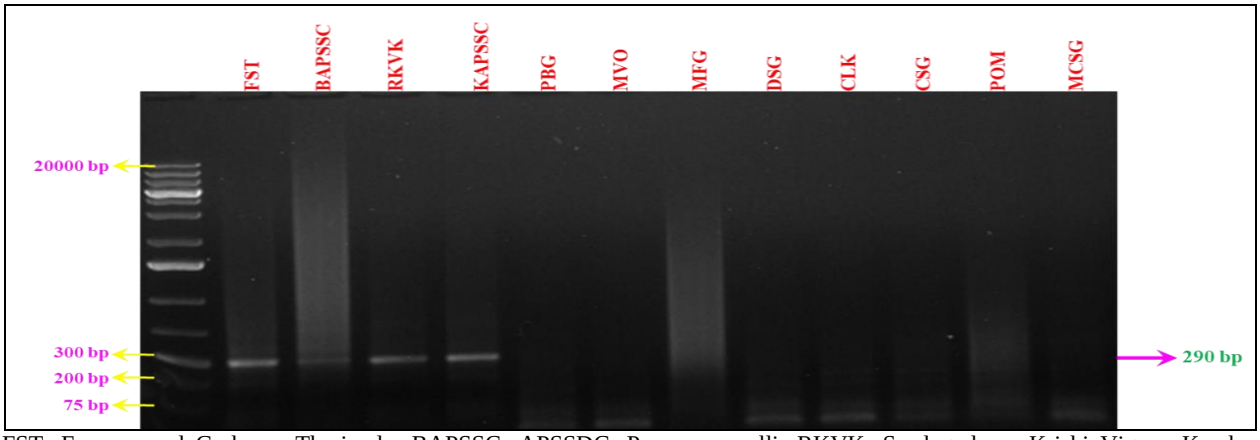
FST: Farmer seed Godown, Tharimela; BAPSSC: APSSDC, Prasannayapalli; RKVK: Seed godown Krishi Vigyan Kendra, Reddipalli; KAPSSC: Seed godown, APSSDC, Kalahasthi; PBG: M/s Sri Balaji seed godown, Pileru; MVO: Farmers godown, Bangarupalem; MFG: Farmers seed godown, Madikerra; DSG: Seed Godown, Dhone; CLK: M/s Little Krishna seed processing plant, Chabolu; CSG: Seed godown, Chapadu; POM: Seed Godown, ARS, Utukur; MCSG: Mydukur cold storage seed godown, Mydukur

Fig 8: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers Cs F / Cs R of *C. gonagra* during 2015-16



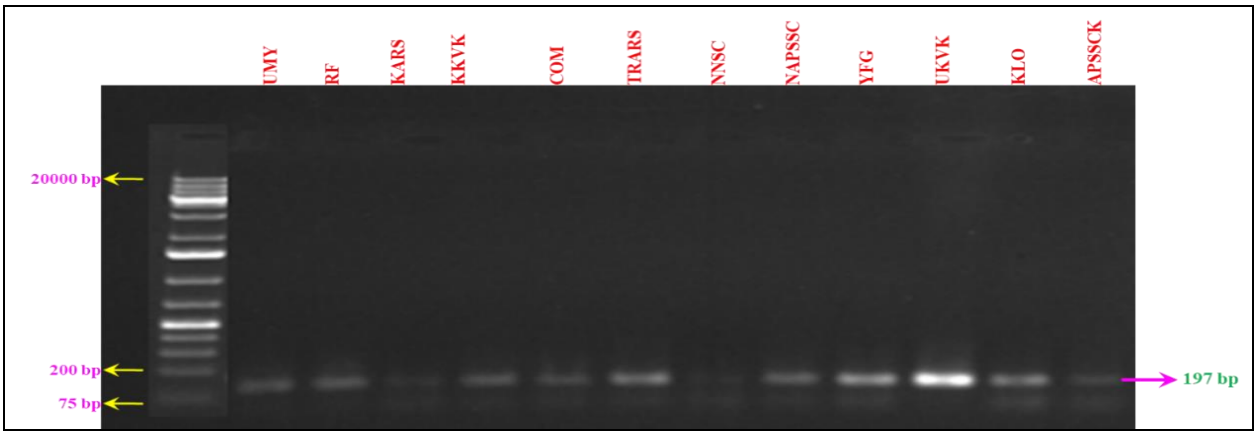
UMY: Market yard, Uravakonda; RF: Farmers Godown, Raketla; KARS: Seed godown, ARS, Kadiri; KKVK: Seed Godown, Kalikiri; DCMSC: DCMS, Seed godown, Chittoor; TRARS: Seed godown, RARS Tirupati; NNSC: Seed godown, NSC, Nandyal; NAPSSC: M/s Neelakanteswara farmers seed godown, Ayyalurmetta; YFG: Farmers godown, Yallurl; UKVK: Seed godown of Krishi Vigyan Kendra, Utukur; KLO: M/s Sri Lakshmi Narasimha Oil mill, Kadapa; APSSCK: Seed godown APSSC, Kadapa.

Fig 9: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers A2CytBF / A2CytBR of *C. gonagra* during 2014-15



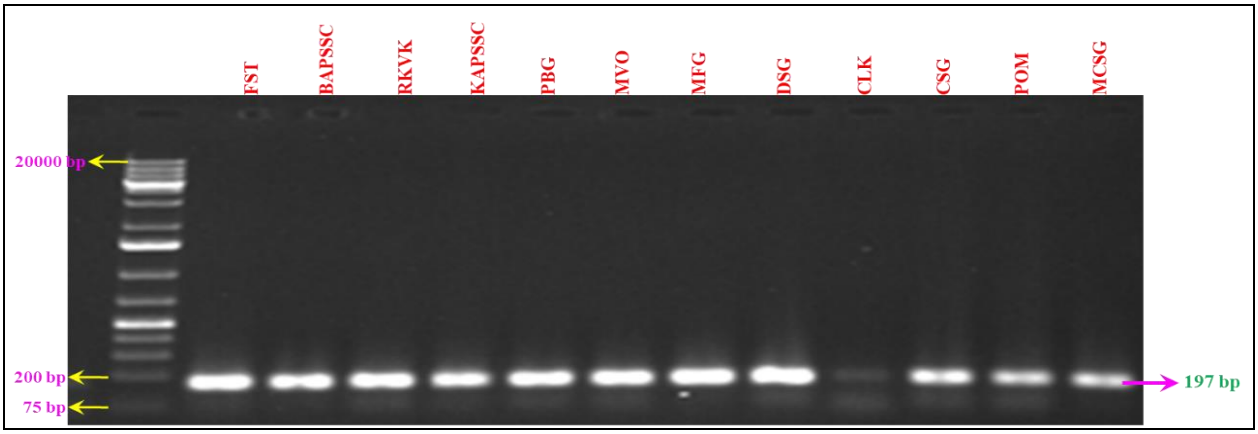
FST: Farmer seed Godown, Tharimela; BAPSSC: APSSDC, Prasannayapalli; RKVK: Seed godown Krishi Vigyan Kendra, Reddipalli; KAPSSC: Seed godown, APSSDC, Kalahasthi; PBG: M/s Sri Balaji seed godown, Pileru; MVO: Farmers godown, Bangarupalem; MFG: Farmers seed godown, Madikerra; DSG: Seed Godown, Dhone; CLK: M/s Little Krishna seed processing plant, Chabolu; CSG: Seed godown, Chapadu; POM: Seed Godown, ARS, Utukur; MCSG: Mydukur cold storage seed godown, Mydukur

Fig 10: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers A2CytBF / A2CytBR of *C. gonagra* during 2015-16



UMY: Market yard, Uravakonda; RF: Farmers Godown, Raketla; KARS: Seed godown, ARS, Kadiri; KKVK: Seed Godown, Kalikiri; DCMSC: DCMS, Seed godown, Chittoor; TRARS: Seed godown, RARS Tirupati; NNSC: Seed godown, NSC, Nandyal; NAPSSC: M/s Neelakanteswara farmers seed godown, Ayyalurmetta; YFG: Farmers godown, Yallurl; UKVK: Seed godown of Krishi Vigyan Kendra, Utukur; KLO: M/s Sri Lakshmi Narasimha Oil mill, Kadapa; APSSCK: Seed godown APSSC, Kadapa.

Fig 11: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers 47CMOsF/47CMOsR of *C. gonagra* during 2014-15



FST: Farmer seed Godown, Tharimela; BAPSSC: APSSDC, Prasannayapalli; RKVK: Seed godown Krishi Vigyan Kendra, Reddipalli; KAPSSC: Seed godown, APSSDC, Kalahasthi; PBG: M/s Sri Balaji seed godown, Pileru; MVO: Farmers godown, Bangarupalem; MFG: Farmers seed godown, Madikerra; DSG: Seed Godown, Dhone; CLK: M/s Little Krishna seed processing plant, Chabolu; CSG: Seed godown, Chapadu; POM: Seed Godown, ARS, Utukur; MCSG: Mydukur cold storage seed godown, Mydukur

Fig 12: PCR amplification of DNA of *Caryedon* spp collected from different locations using specific primers 47CMOsF/47CMOsR of *C. gonagra* during 2015-16

4. Conclusion

The present investigations revealed the genetic diversity in the groundnut bruchid *Careydon* reported from Rayalaseema region of Andhra Pradesh, India. The studies confirmed that, present *Careydon* collection in 24 locations of Andhra Pradesh identified with two different species *i.e., C. serratus* and *C. gongara*. Co-existence of *C. gongara* and *C. serratus* has been observed in the present investigations. The further studies can reveal the proportion of these two species and its damage potential at various regions of Andhra Pradesh.

5. Acknowledgement

I humbly thank the authorities of Acharya N.G. Ranga Agricultural University for the financial help in the form of deputation during my study period.

6. References

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