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Original Article

SCREENING AND CHARACTERIZATION OF DEVELOPING RESISTANT CULTIVAR AGAINST ODOIPORUS LONGICOLLIS (OLIVIER) (COLEOPTERA: CURCULIONIDAE) USING REFERENCE GENOTYPES IN INDIA

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ABSTRACT

Objective: The objective of the research was to develop a screening method for weevil resistance by using selected genotypes. The selected reference genotypes were screened against stem weevil to deals with the different parts in India.

Methods: The collected stem pieces of each cultivar were cut into 30 cm length and kept in an insect breeding chamber for two polypropylene cages is one into another. The bottom of the inner cage is made holes to drain the water from stem discs and the outer cage is without holes to collect it. Reproductively active and healthy weevils (4 females and 4 males) were selected and released into the breeding chamber for each commercial cultivar and screened against *Odoiporus longicollis* and observation was taken after 38 d. Based on the following method, different states of insect cultures were screened under *in vitro* conditions. This experiment was replicated four times for further statistical analysis.

Results: The resistant genotype had significantly more ($p<0.05$) mortality, and fewer than the susceptible genotypes. These results indicated that the genotypes can be used as reference genotypes in evaluating resistance or susceptibility against the banana stem weevil using Gas chromatography with antennogram detector. This implies that the infestation caused by the weevils varied in different genotypes and also this information will be further helpful in selecting the resistant cultivars for future in the field of Agriculture.

Conclusion: In commercial cultivars, no one can report the divergent and screening of stem weevil under *in vitro* conditions. In field conditions, that are not accuracy because, Climate change, Adaptation of host, migration and etc. For this instance, we performed the *in vitro* screening study and it finally revealed the susceptible and resistant genotypes based on the percent of weevil infestation are the same as well as no significant differences between different banana growing areas in India.

Keywords: Banana stem weevil (*Odoiporus longicollis*), Screening, Host-plant resistance, Resistant genotypes, Biodiversity, Gas chromatography with Electroantennogram detector (GC-EAD)

INTRODUCTION

Banana is the most agricultural food crop in all over the world, which is commonly grown in Southeast Asia. In the world, India is the largest producer of banana and 40 million tonnes of fruits produced in India annually [1-3]. Banana (*Musa* spp.) is one of the oldest fruits known to mankind as a good source of minerals and vitamins. Banana plantain which infests 470 species of insects and mites were reported [4]. In India, Banana corm weevil (BCW), Banana stem weevil (BSW), Leaf and fruit scarring beetle, Leaf-eating caterpillar are the major insect pests causing serious damage and more yield loss to this *Musa* cultivation [5].

The banana stem weevil, O*doiporus longicollis* (Olivier) (Coleoptera: Curculionidae), is a principal production constraint in a commercial cultivar of banana. The developmental period of Banana weevil from egg to adult has been estimated at 3 to 5 w under ambient conditions [6]. The biological control method as a feasible lifelong pest control strategy for stem weevil [7]. The biology, ecology and chemical control of *Odoiporus longicollis* have been studied in the past. In the last few years, efforts are on to evolve integrated pest management (IPM) strategies for efficient management of this pest. Literatures on the existence of host plant resistance of kairomones were completely lacking. In recent years, some progress has been achieved in the screening of banana germplasm against banana weevil and a number of cultivars have been identified as possible sources of resistance for breeding programs [8, 9]. In this connection, an attempt has been made to ascertain the presence of attractive weevil kairomones in commercial resistant cultivar so as to develop a semiochemical-based IPM strategy against the pest.

MATERIALS AND METHODS

Banana stem pieces of commercial cultivars (cv) such as Poovan, Pachaladan, Nendran, Red Banana, Monthan, Rasthali, Ney Poovan, Virupakshi, Karpuravalli, Grand Naine and Wild Balbisiana accession were collected from the different places of banana growing areas in Tamil Nadu and Kerala.

Collection of *Odoiporus longicollis*

Banana stem weevil collections were made from banana growing belts of Maharashtra (Bhusaval), Karnataka (Chamrajnagar), Tamil Nadu (Kolli hills), Kerala (Calicut) and AndhraPradesh (Kodur). The collected weevils were maintained and rearing in culture container at 23±2 °C for 16:8 h (L/D) in culture room (fig. 1).

Screening against *Odoiporus longicollis*

The collected stem pieces of each cultivar were cut into 30 cm length and kept in an insect breeding chamber for two polypropylene cages is one into another. The bottom of the inner cage is made holes drain the water from stem discs and the outer cage is without holes to collect it. Reproductively active and healthy weevils (4 females and 4 males) were selected and released into the breeding chamber for each commercial cultivars such as Poovan, Pachaladan, Nendran, Red Banana, Monthan, Rasthali, Ney Poovan, Hill Banana, Karpuravalli, Grand Naine and Wild Balbisiana accession were collected and screened against local insect culture of Tamil Nadu and observation was taken after 38 d. Based on the following method, different states of insect cultures were screened under *in vitro* conditions. This experiment was replicated four times for further statistical analysis.

Preparation of extracts

The Banana stem pieces were cut into small pieces and shade dried for 3-7 d. Then the dried pieces were powdered in a mechanical grinder. 100g of dried powder was mixed with 500 ml of Dichloromethane (DCM) solvent until the powder was fully immersed, incubated overnight at room temperature (37 °C) and filter through a Whatmann No.1 filter paper. The filtrate is then concentrated by rota vacuum evaporator. The concentrated sample was analyzed using GC-EAD.

Statistical analysis

Datas were presented as a mean value using SPSS software (Version 16.0). The differences between genotypes were performed by Analysis of Variance and bars with the same letters are not significantly different according to Least Standard Deviation (LSD) test at P<0.05.

RESULTS AND DISCUSSION

The collected stem weevils from Tamil Nadu culture were screened all the commercial cultivars of banana stem pieces (fig. 2). Among those eleven cultivars, we decided to Poovan and Red Banana as highly susceptible, Nendran and Pachaladan as moderately susceptible, Karpuravalli and Virupakshi as moderately resistant, Wild Balbisiana and Robusta as highly resistant for stem weevil based on the percent of infestation and mortality (table 1).

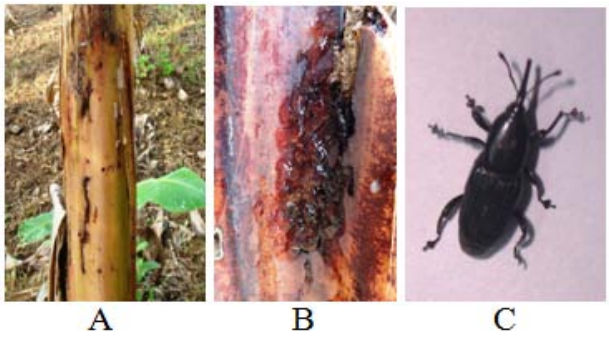

Fig. 1: A) Infected stem at field condition B) Gel exudation C) Banana stem weevil

Table 1: Commercial variety of banana stem were tested against *O. longicollis*

S. No.	Cultivars	Fecundity (Mean ± SE)					
		Egg	Larva	Cocoon	Adult	Mortality	Infestation (%)
1	Poovan	16.00±1.52^{ab}	13.00±2.08^{a}	9.00±2.30^{a}	21.00±2.08^{a}	0.00±0.00^{b}	54.06±2.29^{a}
2	Red Banana	18.00±3.78^{a}	9.33±1.45^{ab}	6.00±3.21^{ab}	17.33±1.85^{ab}	0.00±0.00^{b}	45.81±1.68^{b}
3	Monthan	11.66±2.18^{a-d}	10.00±2.08^{ab}	5.00±2.00^{a-c}	16.66±3.17^{ab}	0.00±0.00^{b}	31.49±0.53^{c}
4	Nendran	9.66±1.76^{a-d}	7.33±3.84^{a-c}	3.66±1.20^{a-c}	15.00±1.52^{b}	0.00±0.00^{b}	18.67±0.35^{d}
5	Pachaladan	13.00±2.88^{a-c}	7.00±3.21^{a-c}	3.00±2.08^{bc}	14.66±1.20^{b}	0.00±0.00^{b}	10.41±0.27^{e}
6	Rasthali	9.00±4.16^{a-e}	4.00±1.73^{b-d}	2.00±2.00^{bc}	11.66±2.40^{bc}	0.00±0.00^{b}	6.33±0.59^{f}
7	Virupakshi	6.00±3.21^{c-e}	0.00±0.00^{d}	0.33±0.33^{bc}	5.00±1.52^{de}	0.00±0.00^{b}	5.21±0.11^{f}
8	Ney poovan	7.00±3.21^{b-e}	0.00±0.00^{cd}	0.33±0.33^{bc}	5.00±1.52^{cd}	0.00±0.00^{b}	4.45±0.24^{f}
9	Karpuravalli	5.00±3.60g^{c-e}	2.00±0.57^{cd}	1.00±1.00^{bc}	4.66±0.88^{de}	0.00±0.00^{b}	3.83±0.32^{f}
10	Robusta	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}	3.00±0.57^{e}	1.00±0.57^{a}	0.39±0.09^{g}
11	Attikol	2.33±2.33^{de}	2.00±1.52^{cd}	0.00±0.00^{c}	3.00±0.00^{e}	1.00±0.00^{a}	0.28±0.02^{g}

*Means followed by same letters in the table indicate not significantly different (p>0.05) by LSD.

Feeding preference indicated that Poovan and Nendran were the most preferred and Robusta the least preferred clones [10]. The highest level of infestation was noticed on Nendran followed by Red banana [11].

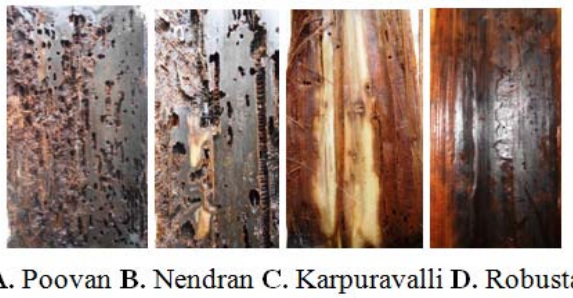

Fig. 2: Infested stem from Susceptible/resistant variety under *in vitro* conditions

Susceptible/Resistant cultivar against different states of banana stem weevil

The following susceptible and resistance genotypes of banana stem pieces were collected and kept in an insect breeding chamber. Reproductively active and healthy weevils (2 females and 2 males) of different states were selected and released in the selected genotypes of Highly susceptible, Moderately susceptible, Moderately resistant and Highly resistant *viz.* Poovan (AAB), Nendran (AAB), Karpuravalli (ABB) and Robusta (BB) stem pieces for screening to identify the diversity of stem weevils under *in vitro* conditions. After 38 d of weevil introduction, the feeding behaviour and mortality were observed and collected newly hatched pupae and adults from different locations.

Adults

Based on screening results, Poovan was recorded as the maximum fecundity of 9 at Maharashtra culture and minimum fecundity of 5 at Tamil Nadu culture (table 2). Robusta was recorded as the maximum mortality of Kerala, Karnataka and Tamil Nadu and minimum mortality of Maharashtra culture with no population development.

Pupae

At the pupal stage, Poovan was the maximum number of 10 at Karnataka culture and minimum of 6 at Tamil Nadu culture. The mortality was found to be more at Andhra Pradesh and Tamil Nadu culture in the cv Robusta.

Grubs

At grub stage, Poovan was recorded as the maximum number of 13 at Kerala and minimum of 9 at Tamil Nadu culture. Robusta was recorded as a maximum of 3 at Kerala and minimum of 1 at Maharashtra culture.

Eggs

The eggs were laid at the inner part of the leaf sheath and approximately once a female weevil lays on 5 to 8 eggs in the form of

a cluster. The maximum of 19 was collected in Karnataka and minimum of 13 at Maharashtra culture. In Robusta, maximum of 4 eggs was collected Maharashtra culture and minimum of 2 were collected in Tamil Nadu culture.

Population difference between *O. longicollis* was did not show any phylogeographic distribution and overall genetic differentiation among the populations were highly significant ($p<0.001$) [12]. Most successful attack of bananas involves Host–plant location, acceptance and suitability. Resistance modalities interfering with these processes include anti xerosis (non-preference) and antibiosis [13]. In this regard, there is no significant difference in the weevil populations of different banana growing areas in India.

Table 2: Susceptible/resistant variety of banana stem were tested against *O. longicollis*

	Cv.	Total fecundity (Mean ± SE)			
		Adults	**Pupae**	**Grubs**	**Eggs**
TN	P	5.75±1.60^{a}	6.25±1.88^{a}	9.00±2.61^{a}	13.50±3.30^{a}
	N	4.00±1.15^{ab}	4.25±1.31^{ab}	6.25±1.60^{ab}	9.50±2.02^{ab}
	K	2.25±0.85^{bc}	2.25±0.85^{ab}	3.75±1.49^{ab}	6.50±2.50^{ab}
	R	0.25±0.25^{c}	0.50±0.50^{b}	1.75±1.18^{b}	2.50±1.65^{b}
KL	P	8.00±1.00^{a}	9.50±1.19^{a}	13.75±1.79^{a}	16.50±2.25^{a}
	N	7.75±0.85^{a}	8.00±1.08^{a}	10.75±1.18^{a}	13.50±1.32^{a}
	K	3.75±0.25^{b}	4.00±0.40^{b}	6.50±0.64^{b}	8.00±0.40^{b}
	R	0.25±0.25^{c}	0.75±0.47^{c}	3.00±1.47^{b}	3.25±1.49^{c}
KN	P	7.25±0.62^{a}	10.75±1.18^{a}	11.75±1.10^{a}	19.75±2.56^{a}
	N	6.25±0.25^{a}	8.75±0.85^{a}	10.25±0.75^{a}	13.75±1.49^{b}
	K	3.50±0.28^{b}	3.50±0.64^{b}	6.75±0.94^{b}	9.00±0.81^{b}
	R	0.25±0.25^{c}	1.00±0.70^{b}	2.25±1.10^{c}	3.50±1.44^{c}
AP	P	6.00±2.27^{a}	7.25±2.32^{a}	10.75±3.11^{a}	13.25±3.70^{a}
	N	4.75±1.18^{ab}	6.25±1.79^{a}	9.50±1.84^{a}	11.75±2.25^{ab}
	K	2.50±1.04^{ab}	3.00±1.22^{ab}	5.25±1.93^{ab}	6.75±2.49^{ab}
	R	0.50±0.50^{b}	0.50±0.50^{b}	2.00±1.22^{b}	3.25±1.97^{b}
MH	P	9.00±1.35^{a}	10.5±1.84^{a}	12.00±0.91^{a}	18.50±1.32^{a}
	N	5.25±1.10^{b}	6.25±1.70^{ab}	10.25±1.31^{a}	15.00±1.58^{ab}
	K	4.50±1.19^{b}	5.25±1.03^{b}	9.00±1.22^{a}	10.75±1.54^{b}
	R	0.75±0.75^{c}	0.75±0.75^{c}	1.75±0.85^{b}	4.00±2.41^{c}

*Means followed by same letters in table indicate not significantly different ($p>0.05$) by LSD, P-Poovan N-Nendran K-Karpuravalli R-Robusta, TN-Tamil Nadu K-Kerala KN-Karnataka AP-Andhra Pradesh MH- Maharashtra

Characterization of weevil active volatile components of susceptible and resistant cultivars

Banana leaf sheath volatile compounds were collected from the susceptible cv of Poovan and resistant cv. of Robusta by solvent extraction method. The extract was subjected to the analysis of GC-EAD for identifying the stem weevil active components. Weevil attractive components were identified using GC-EAD profiles for the cv Poovan was recorded as 68% (19.8) and 18% (18.2) respectively (fig. 3).

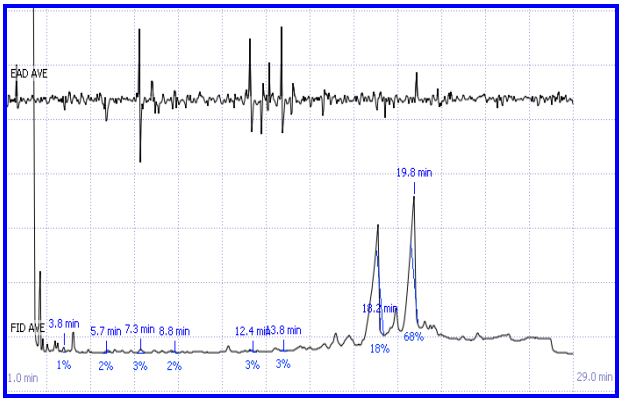

Fig. 3: GC-EAD profile of susceptible cv. Poovan

Whereas, Robusta does not show any response indicating that active weevil volatiles in the extract (fig. 4). For this reason, we approved cv. Robusta was resistant and cv. Poovan for susceptible to stem weevil.

Microwave oven assisted extract was prepared from banana pseudostem and tested its kairomonal activities on *O. longicollis* using Electroantennogram technique. It was found that the microwave oven assisted extract elicited the maximum EAG response in the female, whereas, Solvent extract was found higher antennal activity in the male. The Air-entrainment extract elicited poor responses in both male and female *O. longicollis* [14]. The 7 d decayed pseudo stem, and its extract indicated that the attractant of 75 % and 53.35 % respectively. The results suggest that decayed pseudostem based attractant can be used as the trapping agent for the management of *O. longicollis* [15].

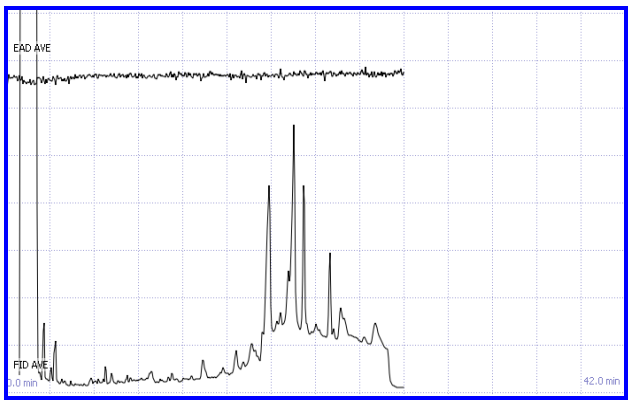

Fig.4: GC-EAD profile of resistant cv. robusta

The data indicated that Nendran and Poovan were better suited for the population development of the insect. Meanwhile, significant differences were limited in this screening data suggests that more fiber content in leaf sheath of resistant cultivar Robusta might reduced the larval hatching rates and eggs were decomposed compared to other cultivars. These results suggested that several

factors may contribute to antibiosis, antixenosis and tolerance based resistance against banana weevil and that resistance modalities might vary among cultivars and or genome groups.

CONCLUSION

Field observation data were reported that all the germplasm varieties of banana. Yet now, *in vitro* screening reports were lacking against stem weevil. In commercial cultivars, no one can report the divergent and screening of stem weevil under *in vitro* conditions. In field conditions, that are not accuracy because, Climate change, Adaptation of the host, Migration and etc. For this instance, we performed the *in vitro* screening study and it finally revealed the susceptible and resistant genotypes based on the percent of weevil infestation are the same as well as no significant differences between different banana growing areas in India.

Resistance variety of banana was proved by Electroantennogram detector profiles. This information can be further useful for breeding of resistant cultivars against *O. longicollis* to reduced yield loss and also try to perform the pheromone traps to control all varieties of Banana.

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CONFLICT OF INTERESTS

All authors have none to declare

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