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Field evaluation and *In vivo* screening of *Musa* germplasm against banana stem weevil, *Odoiporus longicollis*

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Abstract
Banana stem weevil (*Odoiporus longicollis*) has been a serious threat to banana production in Asian countries. *Musa* spp. has gained significance as it provides food security for millions of people. Screening of 316 *Musa* accessions for their susceptibility to banana stem weevil revealed that all the screened genotypes (316 *Musa* accessions), belongs to different genomic groups (AA: 26, AAA: 28, AB: 23, AAB: 101, ABB: 102, ABBB: 8, BB: 24, Rhodochiamys: 4) were susceptible. The susceptibility is categorized as follows: less, moderate, highly and most highly susceptible, and are discussed in detail. The present study indicated that 24 very less and 44 less susceptible *Musa* accessions shall be recommended and popularized among the banana growing farmers after characterization of other parameters like crop duration, yield and market value.

Keywords: *Musa* germplasm screening, banana stem weevil, *Odoiporus longicollis*, leaf sheath, Pseudostem, field, susceptible, resistant

Introduction
Banana is a horticultural fruit crop and cultivating worldwide, and India is one of the largest producer. Recently, banana was exported with 101314.37 Metric ton (MT) to different countries [1]. Banana production is limited by two weevil pests viz., the banana stem weevil (BSW), *Odoiporus longicollis* and Banana corm weevil (BCW), *Cosmopolites sordidus* [2,3]. Besides weevils, few other insect pests such as *Spodoptera litura, Olepa ricini, Pentalonia nigronervosa, Erionota torus, Basilepta subcostata*, bagworm, thrips etc are also infesting and affecting plant growth and yield from planting to fruit harvesting [4]. The banana stem weevil has wider distribution causing severe infestation and yield loss ranging from 10-90 % and it has been reported in Asian pacific countries [5-8]. Female *O. longicollis* lay eggs in the outer leaf sheath of pseudostem. The hatched grub feeds on the soft tissue inside the leaf sheath, then pseudostem and reaches upto bunch stalk by making tunnels. The grub damage leads to weakening of pseudostem making it more prone to light or heavy winds [9-11]. Approximately 3-10 adults, 4-7 grubs and 3-6 pupal cases has been recorded in the severely infested banana plants [12]. Swabbing or spray of chemical pesticides Chlorpyrifos on the pseudostem up to 50-100 cm from base at bimonthly interval or until bunch emergence adopted by farmers as a precautionary measure for management of banana weevils. Padmanaban *et al.* (2001) [12] reported that improper cultivation practices leads to increased weevil population in the field and it becomes difficult to manage them through chemical methods. Even though, chemical pesticides are effective, they also kill the natural enemies like entomopathogenic fungi, nematode, predators and parasite [13]. The longitudinally split banana pseudostem has been used as a trap which is practiced in South Asian and other banana growing countries for monitoring and management of weevils [8,14]. Moreover, the rice chaffy entomopathogenic fungi, *B. bassiana* and nematode smeared on longitudinal split banana stem trap have been successfully used for eco-friendly management of banana weevils but it requires replacement of stem trap once it gets dried [15, 16]. Recently, researchers have reported that the *O. longicollis* attraction towards "male aggregation pheromone" such as "2-methyl-4-heptanol" but it was less effective. They suggested that the use of 2-methyl-4-heptanol in combination with pseudostem extract which resulted in significant attraction of the weevils [17-19].

In the current context of environmental issues, it is essential to manage the banana associated insect pests without harming the beneficial organism and environment. Kiggundu *et al*. (2007) [20] screened "*Musa* germplasm" against *C. sordidus* to identify resistant sources and indicated that existence of weevil resistant factors in the germplasm. Similarly, Ajitha *et al*. (2017) [21] reported that secondary metabolites of *Musa* cultivars confer resistance against infestation by stem weevil, *Odoiporus longicollis*. Banana accessions were screened against banana stem weevil and results have been reported from various labs but their screening was done only for restricted accessions [12, 22-27]. Alagesan *et al*. (2016) [16] also studied the response of commercial cultivars to banana stem weevil under *in vitro* conditions and they reported that Nendran (AAB), Poovan (ABB) and Karpuravalli (ABB) are highly susceptible to banana stem weevil. Further, Padmanaban *et al*. (2001) [12] suggested that after initial screening under *in vitro* and field conditions, they need to be screened intensively under field cage method to identify the most promising resistant accessions. Hence, the current study was carry out to identify the resistant Musa accessions from total 316 No's which are available at gene bank field, Indian Council for Agricultural Research-NRC for Banana (ICAR-NRCB) for their reaction to the Banana stem weevil, *Odoiporus longicollis*.

Materials and Methods

Collection and laboratory rearing of *O. longicollis*

The adults *O. longicollis* (Coleoptera: Curculionidae) collected from the banana plantations at different places of Tamil Nadu, India were maintained in the laboratory for a week. Three pairs of banana stem weevils were introduced into the 30 L of perforated Plastic Container where 2 pieces of 30 cm length pseudostem supplied and closed with the lid (Fig.1).

Fig 1: Rearing setup of banana stem weevil. A. Full bucket setup. B. Inner bucket with sidewall perforated for aeration. C. Inner bucket perforated at bottom to collect the water drain out from pseudostem. D. Perforated cap for ventilation.

The pseudostem was replaced once in a twenty days interval. The cocoons were covered with 70 % wet cotton and kept in 100 mL plastic container for adult emergence. The freshly emerged weevils were separated based on their sex using "rostral characters" [26]. Twenty days old, newly emerged adults were selected for *Musa* screening purpose. In order to identify the resistance *musa* accessions against banana stem weevil, field evaluation in the endemic areas (where *O. longicollis* incidence is high) and *in vivo* screening using field cage method against *O. longicollis* were performed.

Field screening

Musa field gene bank available at ICAR-NRCB, Tiruchirappalli, Tamil Nadu and farmer's field at Theni were utilized for field screening against banana stem weevil during 2012-2018. There were totally 316 *Musa* accessions (The accession numbers provided based on "Musa Germplasm Information System (MGIS)"- http://nrcb.res.in/mgis.html) under different genomic groups are presented in percentage in Fig.2. The percent incidence was recorded and the data was analysed statistically.

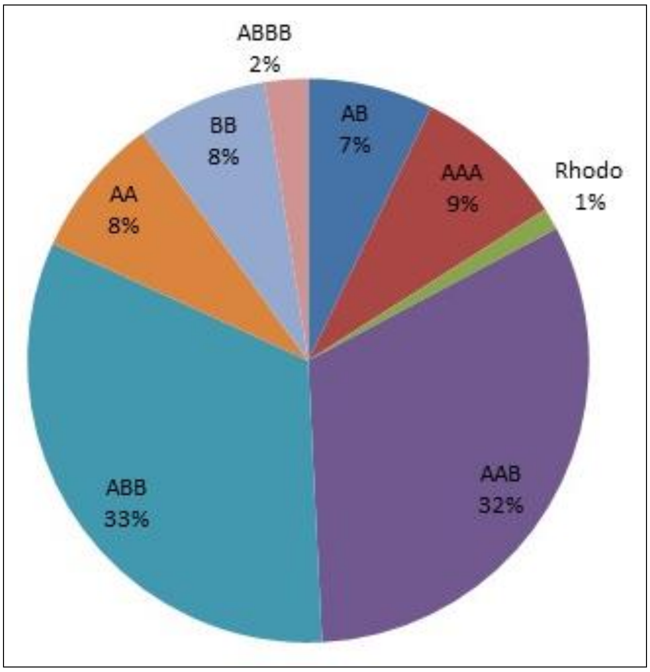

Fig 2: Distribution of *Musa* accessions under different genomic groups

In vitro screening (using *Musa* leaf sheath/ pseudostem)

The outer most leaf sheath/pseudostem of each *Musa* accession (10 month old) were collected from the farm. The leaf sheath was cut into 4 pieces of 15 cm height and 3 cm width and kept in the 5 L perforated plastic container along with three pairs of banana stem weevil. After 6 and 10 days, leaf sheath and stem respectively were opened and recorded egg, grub and feeding damage. The experiment was replicated thrice and the mean percentage was calculated.

Cage screening of less susceptible Musa accessions

Five months old Musa accessions were randomly subjected to cage screening at ICAR-NRC for banana field. Three pairs of weevils per plant were released into the cage. After 35 days, the damage symptoms, number of grub and percent of feeding damage were recorded. The cage screening method are shown in Fig. 3. Observations were recorded on three replicates and mean percentage was calculated. The statistical analysis was performed using PAST software.

Fig 3: Cage screening of less susceptible *Musa* accession against banana stem weevil

Results

The results of field screening revealed that 19.93, 14.55, 9.81 and 55.69 % of *Musa* accessions were found to be susceptible to banana stem weevil infestation ranging from less, moderate, highly and most highly susceptible respectively (Fig.4).

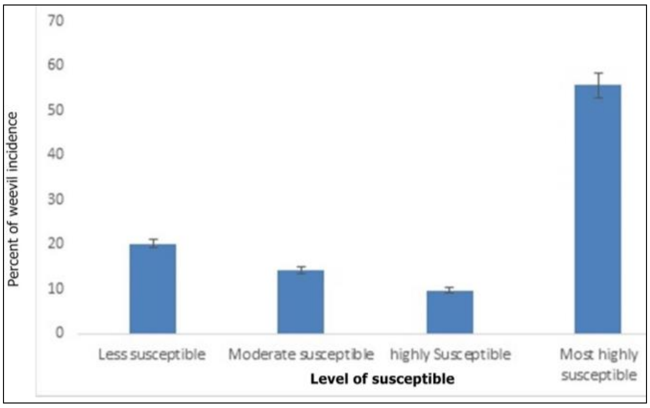

Fig 4: Field evaluation of *Musa* germplasm in response to *O. longicollis.* Values represent percentage ± SE.

However, the banana stem weevil can able to oviposit on the pseudostem but the egg hatching or larval feeding was not recorded in the less susceptible *Musa* accessions. Moreover, the field screening approach revealed that all the *Musa* germplasm accessions are not infested in a particular time since the maturation of varieties vary with the genomic and their sub groups. Interestingly, all genomic groups except Rhodo, AA and AB showed higher susceptibility (>50 %) to Banana stem weevil (Fig.5) The ornamental banana plants belongs to Rhodochiamys family should be studied in biochemical and molecular level for understanding the mechanism behind the weevil resistance.

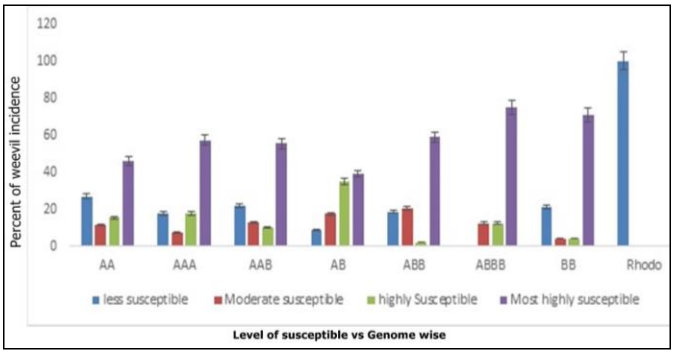

Fig 5: Comparative analysis of weevil response in the field to *Musa* germplasm between genomic groups. Values represent percentage ± SE.

The dead plants under field condition (named as most highly susceptible) due to high level of infestation and from highly to moderate susceptible accessions. The field infested *Musa* spp. is shown in Fig. 6. The highly and most highly susceptible accessions cannot be used for cross breeding to develop weevil resistance Musa accessions.

Fig 6: Infestation of banana plantain by Banana stem weevil, *Odoiporus longicollis* in the farmers field. Arrow indicates the larval damage symptoms produced by the grub of *Odoiporus longicollis*

The leaf sheath/stem based screening is a rapid method to identify the susceptibility of banana cultivars against banana stem weevil. This method requires about 6 and 10 days for leaf sheath and pseudostem respectively to record the oviposition and young grub feeding damage in an air-chamber of leaf sheath (Figs. 7 a and b).

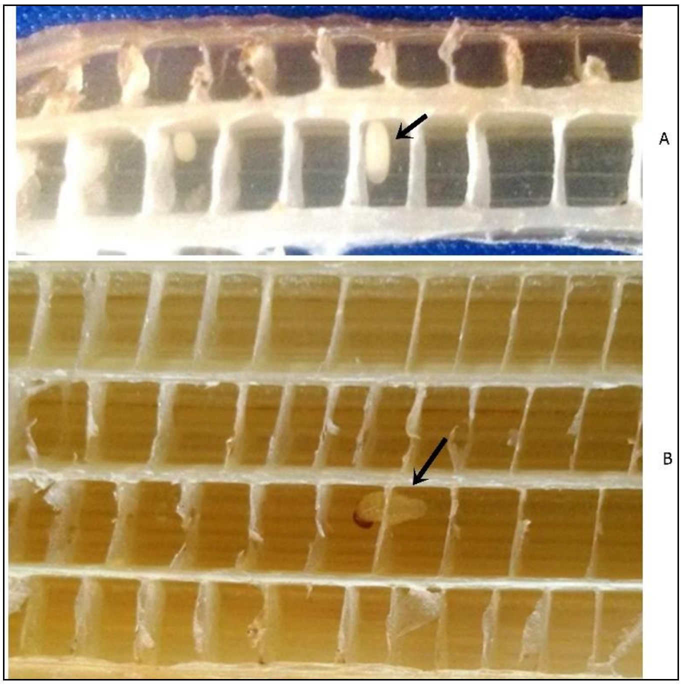

Fig 7: *O. longicollis* egg and grub. A. Arrows indicate the egg inside the air chamber. B. Arrow indicates the grub feeding inside the air chamber.

Further to confirm the field data, 54 accessions belongs to different genomic groups (AA: 5, AAA: 7, AAB: 11, AB: 13, ABB: 14, ABBB: 1 and BB: 3) were randomly selected for *in vivo* (using cage set up) screening approach. The cage screening experiment revealed that those 24 very less susceptible accessions (Table.1) recorded against weevil attack. Further, 44 less susceptible (Table.2) cultivars from field evaluation also recorded. The oviposition points with indication of jelly exudation were found in less susceptible accessions but due to plant defence mechanism, no egg and larval feeding were recorded (Fig.8).

Table 1: List of very less susceptible *musa* accessions identified from field and cage screening method.

S. No	Accession Number	Accession Name	Genome	Remarks
1.	1030	Chendwat	AA	No attack
2.	1181	Pagalphad wild	AA	No attack
3.	1836	Siguzani	AA	Oviposition found
4.	0378	Lacadan	AAA	No attack
5.	0633	GCTCV-215	AAA	No attack
6.	0249	Pacha	AAB	No attack
7.	0537	Padathi	AAB	No attack
8.	0397	Malaikali	AAB	Less jelly exudation
9.	0367	Soniyal	AAB	Oviposition and jelly exudation
10	0737	KNR mutant	AB	Oviposition found
11	0482	Padalimoongil	AB	No attack
12	0699	Poovilachudan	AB	Jelly exudation found
13	0430	Rigitchi	ABB	No attack
14	0493	Ashy Batheesa	ABB	Oviposition
15	0644	Bluggoe	ABB	No attack
16	0065	Desshikadali	ABB	Ovipositions found
17	0103	Ennabenian	ABB	Jelly exudation
18	0097	Nepali chinia	ABB	Oviposition and jelly exudation
19	0253	Klueteparod	ABBB	Oviposition found
20	2065	Jurmony	BB	Less oviposition and jelly exudation
21	0000	*Musa Rubra*	*Rhodo*	No attack
22	1260	*Musa ornata*	*Rhodo*	No attack
23	1376	*Musa laterita*	*Rhodo*	No attack
24	1718	*Musa Velutina*	*Rhodo*	No attack

Table 2: List of less susceptible *musa* accessions (1-30 %) under field condition

S. No	Accession No	Genome	Name of the accession
1.	0642	AA	M.ac. sspburmanocoides
2.	1631	AA	M.ac. sspburmanicca
3.	1712	AA	M. a type burmanioicols assam
4.	0009	AAA	Borjahaji

5.	0017	AAA	Manjahaji
6.	0670	AAA	2390-2
7.	0023	AAB	Krishnavazhai
8.	0160	AAB	Cherapadathi
9.	0164	AAB	CO-I
10.	0190	AAB	Pachaladan
11.	0191	AAB	Padathi
12.	0211	AAB	Kalibow
13.	0355	AAB	Sakkarchyna
14.	0387	AAB	Mysore Ethan
15.	0410	AAB	Thenkaali
16.	0489	AAB	Ennabenian
17.	0495	AAB	Nendrakali
18.	0497	AAB	Attrusingan
19.	0499	AAB	Mannan
20.	0519	AAB	Hoobale
21.	0637	AAB	Pisang Nangka
22.	0692	AAB	FiguePommeGeante
23.	0703	AAB	Kalie than
24.	1005	AAB	Sabri
25.	0059	ABB	Agnimalbhog
26.	0086	ABB	Birbutia
27.	0087	ABB	Chinia
28.	0096	ABB	Kothia
29.	0106	ABB	Madavazhai
30.	0121	ABB	Karimbontha
31.	0163	ABB	Chirapunji
32.	0171	ABB	Kallumonthan
33.	0227	ABB	Boddoda bukkisha
34.	0231	ABB	Jamulapelam collection
35.	0338	ABB	Peykunnan
36.	346	ABB	Gauria
37.	0347	ABB	China
38.	0403	ABB	Bainsa
39.	0427	ABB	Ginde
40.	0538	ABB	Peykunnan
41.	0067	BB	Bhimkol
42.	0508	BB	Musa balbisiana
43.	2028	BB	Attikol
44.	2064	BB	Srisailam collection

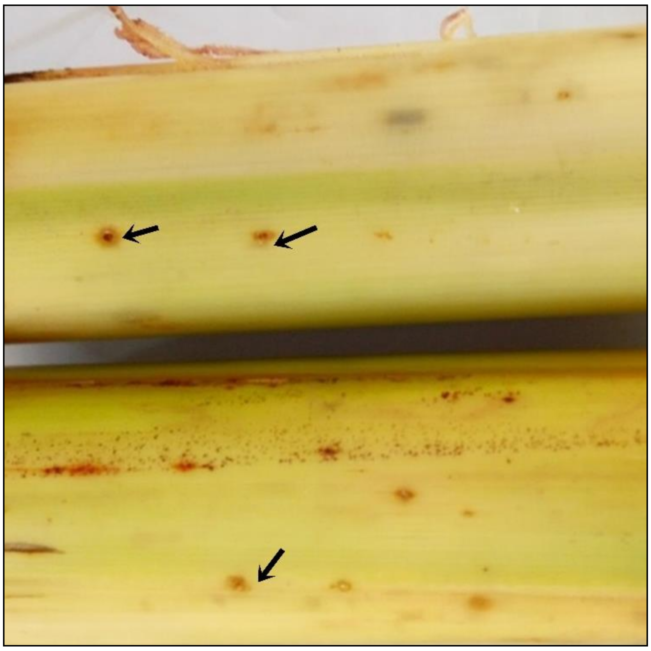

Fig 8: Ovipositional symptoms of Banana stem weevil on the banana stem. Arrows indicate the Ovipositional point on the outer leaf sheath of stem.

It may be due to anti-xenosis (reduced or no oviposition) and antibiosis (inhibit grub development) factors present in the less and moderately susceptible *Musa* accessions. The results of both field and cage screening did not showed significant difference (p>0.05) in screening part and we further recommend that cage screening is good choice to identify the weevil resistant *musa* accessions (Fig. 9).

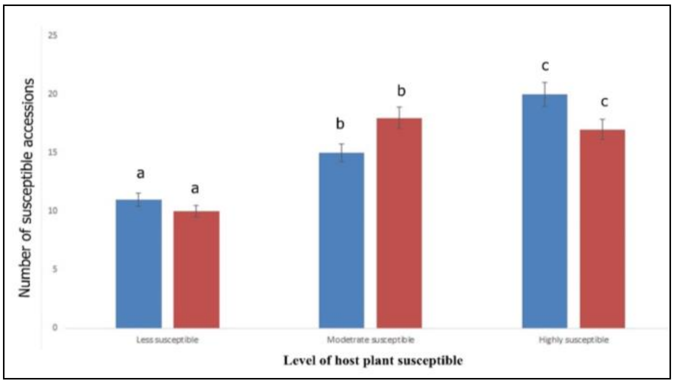

Fig 9: Comparative analysis of banana stem weevil response in the field and cage screening to *Musa* germplasm. Blue colour: field evaluation and Red colour: cage screening. Values represent percentage ± SE. The following same superscript over error bar indicates that p-values are not significantly different at p >0.05.

However, further work is needed to elucidate the biochemical aspect of sap synthesis in plant defence mechanism against weevil attack. The cage screening method holds good technique to separate the less susceptible varieties from the highly susceptible ones. Overall results revealed that less susceptible *Musa* accessions that were identified in the present study could be used for cross breeding to obtain weevil resistant *Musa* varieties.

Discussion

The screening of *musa* accessions against banana stem weevil revealed that few accessions identified as less susceptible. The less susceptible *musa* accessions may be have some plant defence mechanism like anti-biosis/xenosis against weevil as reported earlier [20,23]. Further, we suggest that the high yielding moderate and less susceptible varieties (from 5th month planting and up to harvesting banana bunch) may safe guard from weevil attack using contact pesticide or botanicals as an effective control measure for stem weevil management. The commercial cultivars grown in Andhra Pradesh like Sugundhalu (AAB), Rasthali (AAB: silk) and Karpuravalli (ABB: Pisang Awak) were susceptible to *O.longicollis* and it was reported by Reddy *et al* (2015) [27]. They suggested to use IPM tool for manage the pest by eco-friendly approaches including clean cultivation practice, monitoring weevils in the field periodically using longitudinally split pseudostem trap and kill them with *B. Bassiana/*nematode, and application of botanical pesticides. Earlier finding of Visalakshi (1989) [28], Kamala Jayanthi and Varghese (1999) [29] and Alagesan *et al*. (2016) [16] also indicated the use of eco-friendly approaches for banana stem weevil management. Since, the leaf sheath and pseudostem is not live plant and due to lack plant immune response, weevil made choice on oviposition and grub feeding damage on all the *musa* accessions. Alagesan *et al*. (2016) [16] reported that this method is more convenient and effective to screen the *Musa* accessions against banana stem weevil when compared to field screening which takes more time owing to long crop duration. However, the present study suggest that leaf sheath and stem based screening is less effective to identify the resistant *Musa* accessions.

Interestingly, Kavitha *et al*. (2015; 2016) [23, 25] reported that cv. Aattinkombu and Thenkaali as resistant to banana stem weevil as they have insect resistant mechanisms. They reported that the feeding of above cultivars to banana stem weevil leads to cytopathological and haemolymph changes in the grub, *O. longicollis.* Similarly, previous research indicate that the adult weevils are do not discriminate the resistant and susceptible cultivars for oviposition but the less susceptible cultivar do not affected due to occurrence of weevil resistant factors “antibiosis mechanism” or “effect of plant defence compounds” [30, 31]. Morevoer, Milburn *et al*. (1990) [32] also reported that sap (jelly exudate) of *Musa spp*. have rich iron content along with other essential ions such as $Mg^{2+,}$ K^+, Cl^- and NO^{3-}. These ions may be acted as desiccates or toxin to the egg. In conclusion and prospective view, *Musa* screening for resistance to banana stem weevil is the first and foremost critical step towards the development of resistant varieties. The accessions which were found to be very less and less susceptible (1-30 % infestation) could be used either directly in breeding for the development of stem weevil resistance in the existing cultivars or it can be used in gene pyramiding programmes towards the development of improved breeding lines. The very less susceptible accessions contain semio-chemicals and secondary metabolites have greater prospects in the banana stem weevil management.

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Disclosure

I on behalf of the entire author declare that we do not have conflict of interest.

Authors’ contributions

BP, SU and MK designed the study. BP, MK and KKA recorded the field observation. BP, MK and KKA conducted the *in vitro* screening experiments. BP, MK and KKA interpreted the data. MK, MMS and BP wrote the paper. All authors accepted to submit the manuscript for publication.

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