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Screening and Biochemical Analysis on Blackgram Genotypes for Resistance against Storage Pest Bruchine [*Callosobruchus maculatus* (F.)]

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ABSTRACT

Background: Blackgram [*Vigna mungo* (L.) Hepper] is a rich source of protein. It is one of the major crops essentially involved in daily human diets. However, storage pest bruchine [*Callosobruchus maculatus* (F.)] is a major production constraint for legumes. A research was formulated to assess the bruchine resistance in 20 blackgram genotypes along with the biochemical analysis to find out the active biochemical components responsible for the resistance activity.

Methods: The experiment was carried out during August- October, 2019 at Entomology Laboratory, National Pulses Research Center, Vamban, India. The experimental material comprised of 20 blackgram genotypes which were screened for bruchine resistance. Further, confirmatory trial was conducted with selected resistant entries and highly susceptible entries during October- December, 2019. Both experiments were carried out in completely randomized design and replicated three times. GC-MS analysis on the resistant and susceptible entries were performed to ascertain the active biochemical components conferring resistance.

Result: Among the genotypes, TU 68 had comparatively late developmental time (days), less number of adult emergence, higher mean developmental period (days), less susceptibility index, less seed damage (%) and less seed weight loss (%). Genotype TU 68 was found to be resistant in the confirmatory trial also. Less number of adult emergence and higher mean developmental period indicated the delayed developmental period which is a mechanism of bruchine resistance. GC-MS analysis on resistant (TU 68) and susceptible (MDU 1) genotypes indicated the presence of active biochemical compounds with insectifuge activity in TU 68. Hence, TU 68 could be utilized in the hybridization programmeas donor for bruchine resistance.

Key words: Blackgram, Bruchine beetles, *Callosobruchus maculatus*, GC-MS analysis, Screening, Resistance.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] is an important pulse cropsin South Asian continent. It is a major source of dietary protein. In India, this crop occupies an area of 4.50 million hectares with the production of 2.83 million tonnes (Anonymous, 2019). Storage pests especially the bruchine (*Callasobruchus sp*.) affects the postharvest produce during storage (Swella and Mushobozy, 2009). Bruchine beetles (*Callosobruchus maculatus*) (Chrysomelidae: Bruchinae) causes loss in both quantity and quality during storage in tropics and sub-tropical areas (Duraimurugan *et al*., 2011). The post-harvest damages varies based on the prevalent *Callasobruchus sp*. but *C. maculatus* causes more yield loss (Soundararajan *et al*., 2013). Bruchine beetles mainly infest the legumes *via* pods, seeds in field and storage conditions. It can multiply quickly and start disseminating into the uninfected storage lots (Dasbak *et al*., 2009). Adult of *C. maculatus* has a lifespan of 1-2 weeks for mating and Oviposition. It can stay without any requirement of food or water throughout its life span (Beck and Blumer, 2014). These beetles have the ability to withstand a high degree of inbreeding (Tran and Credland, 1995). The beetlesl acks the “snout” of a true weevil (Curculionidae) and are reddish-brown in colour, with black and gray elytral markings with two black spots at center. The abdomen extends out from the elytra and it also found to have two black spots in last segment of the abdomen. *C. maculatus* are sexually dimorphic in nature and males are easily distinguished from the females as females are larger in size than the males. Pesticides and fumigants are used to control storage pests in legumes but their application at higher doses leads to the

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accumulation of toxic residues in the treated products may mixed up in the daily diets too (Shaheen and Khaliq, 2005; Sharma and Thakur, 2014). However, identification of resistant genotypes by means of genetic improvement is an environment friendly approach to prevent bruchine infestation (Uddin and Adesiyun, 2012). The present study was made to assess the resistance nature of 20 blackgram genotypes against *C. maculatus.* Attempts were also made to identify the active biochemical components involved in the resistant genotype through GC-MS analysis.

MATERIALS AND METHODS

Experimental design

The experiment was conducted out during August- October, 2019 at the Entomology laboratory, National Pulses Research Center, Vamban, India. The experimental material consisted of 20 blackgram genotypes (Table 1). Tolerant genotype with lower rate of adult emergence on 50th days of infestation and four highly susceptible entries *viz*., MDU 1, VBN 6, VBN 8 and ADT 3 were further screened as a confirmatory trial during October-December, 2019. Seeds of each cultivar were stored at -20°C for 48 hours to avoid carry over infestation from field. Both experiments were carried out in completely randomized design and replicated three times.

Insect culture

Among the various species, *C. maculatus* covers the major proportion of nearly 90% in the seed lots at Vamban. Beetles were collected and multiplied on green gram seeds of VBN 4 [*Vigna radiata* (L.) Wilczek] variety. Good aeration was provided through small pin holes on the sides of the container. The *C. maculatus* male and female insects were identified morphologically with two key traits such as: a) presence of less dense setae on the ventral side of the 2, 3, 4th abdominal segments (sternites) of the female and b) presence of serrate type of antenna in both male and female may distinguish from other species. c) The females look larger than males. d) Females are darker in colour than males, while males are brown in colour. Freshly emerged 1-2 day old adults were collected from the stock culture and used for bioassay.

Bioassay for bruchid resistance

The assay procedure of Dongre *et al.* (1996) was followed with some modification. Modification such as instead of two pairs of adults, five pairs of adults were released on 50 number of seeds of each genotype placed in a 15 cm diameter plastic petriplates. The insects were allowed to remain in petriplates for five daysfor Oviposition. After five days the adults were removed from petriplates. Observations were recorded on:

a) 100-seed weight (g).
b) Seed lusture and seed surface.
c) Number of eggs per 50 seeds, Oviposition on 50 seeds of the blackgram genotypes were counted using Leica compound microscope with 10x magnification and the digital images were visualized using Leica application suite version 3.4.0,

Table 1: Descriptions of blackgram genotypes and its pedigree used in the study.

Genotypes	Pedigree	Seed lusture	Seed surface	Hundred seed weight (g) ± SE
ADT 3	Pureline selection from Tirunelveli local	Dull	Rough	3.1±0.0^{gh}
ADT 5	Pureline selection from Kanpur blackgram	Dull	Rough	3.1±0.1^{gh}
ADT 6	Vamban 1 × VBG 04-006	Dull	Rough	3.8±0.1^{bcdef}
APK 1	ADT × RU 1	Dull	Rough	3.8±0.0^{bcde}
CO 5	Pure line selection from Musiri type	Dull	Rough	4.1±0.1^{abcd}
CO 6	DU 2 × VB 20	Dull	Rough	3.2±0.0^{fgh}
KKM 1	COBG 653 × VBN 3	Dull	Rough	4.6±0.1^{a}
LBG 752	LBG 402 × LBG 20	Shiny	Smooth	4.5±0.1^{a}
LBG 787	LBG 685 × IPU 98-1	Shiny	Smooth	3.1±0.1^{h}
Mash 114	Mash 338 × RBI 1	Dull	Rough	3.6±0.1^{defgh}
Mash 1008	SML 32 × Mash 1	Dull	Rough	4.3±0.1^{ab}
MDU 1	ADB 2003 × VBG 66	Dull	Rough	4.2±0.1^{abc}
TMV 1	Mithiulunthu × KM 1	Dull	Rough	3.6±0.0^{defgh}
TU 68	TU 94-1 × *Vigna mungo* var. *silvestris*	Dull	Rough	3.5±0.1^{efgh}
VBN(Bg) 4	CO 4 × PDU 102	Dull	Rough	3.2±0.1^{fgh}
VBN 6	Vamban 1 × *Vigna mungo* var. *silvestris*	Dull	Rough	4.1±0.1^{abcd}
VBN(Bg) 7	Vamban 3 × *Vigna mungo* var. *silvestris*	Dull	Rough	3.4±0.0^{efgh}
VBN 8	Vamban 3 × VBG 04-008	Dull	Rough	4.3±0.0^{ab}
VBN 9	Mash 114 × VBN 3	Dull	Rough	3.3±0.3^{fgh}
VBN 11	PU 31 × CO 6	Dull	Rough	3.6± 0.0^{defg}

Values followed by the same letter along a column are not significantly different (P>0.05) from each other based on Tukey's honest significant difference (HSD) Test.

d) Mean number of eggs per seed (Sewsaran *et al.* 2019),
e) Developmental time (egg + larval + pupal stages) (days) *i.e.,* the time taken for the first adult emergence on the genotypes from the date of adult release.
f) Total number of adult emergence, after 25 days of adult release, the daily observation of adult emergence on the genotypes were performed up to 50 days after infestation (DAI). The emerged adults from the genotypes were counted and removed daily.
g) Mean developmental period (days):

$$MDP(days) = \frac{d_1a_1+d_2a_2+d_3a_3+...+d_na_n}{\text{Number of adults emerged}}$$

Where
d_1- Day at which the adults started emerging (1st day).
a_1- Number of adults emerged on d_1^{th} day.

h) Howe's Index of susceptibility:

$$\text{Howe's index of susceptibility} = \text{Log}_e \frac{F_1}{D} \times 100$$

Where
F_1- The total number of emerging adults.
D- MDP in days.

Using the Index of Susceptibility, genotypes were categorized based on Mensah (1986).

Index of susceptibility	Rating
0.0-2.5	Resistant (R)
2.6-5.0	Moderately resistant (MR)
5.1-7.5	Moderately susceptible (MS)
7.6-10.0	Susceptible (S)
>10.0	Highly susceptible (HS)

i) Seed damage (%), number of seeds damaged out of 50 seeds taken for study were counted and seed damage percentage was calculated at 50 DAI. Based on the seed damage (%), the genotypes were classified as Highly resistant (HR) (0-10%), resistant (R) (10.1-20%), moderately resistant (MR) (20.1-40%), susceptible (S) (40.1-80%) and highly susceptible (HS) (80.1-100%).
j) Seed weight loss (%), the final weight of the 50 seeds of each genotype after 50DAI was taken and the weight loss percentage was calculated. Seed damage and seed weight loss were estimated on 50 days after adult infestation (DAI). The adults emerged we re counted on daily basis and removed from the petriplates to avoid secondary infestation.

Gas chromatography-Mass Spectrometry (GC-MS) analysis

The seeds of identified tolerant genotype (TU 68) and highly susceptible genotype (MDU 1) for bruchine infestation were subjected to GCMS analysis (Equipment (Model: BrucherScion 436-GC with Detector TQ Quadrupole Mass Spectrometer). The analysis was carried as per the standard methods given by Huang *et al.* (2012). The individual components form the seeds were identified based on the retention time. It was compared with the components known from the NIST library database (U.S. Department of Commerce) version-2011.

Statistical analysis

All the observed data were transformed using square root transformation technique except the percentage data. Percentage data was subjected to arcsine data transformation. Analysis of variance for completely randomized design was carried out (Gomez and Gomez, 1984). Further Tukey's honest significant difference (HSD) post-hoc test was carried out with STAR statistical software (version 2.0.1) developed by IRRI, Philippines.

RESULTS AND DISCUSSION

In the present investigation 20 blackgram (*V. mungo*) genotypes were subjected to bruchine (*C. maculatus*) infestation to assess the level of resistance among the genotypes. The results of bruchid infestation among 20 blackgram genotypes are furnished in and confirmatory results in Table 2 and 3. The results revealed the significant differences among the genotypes for all traits. The hundred seed weight ranges from 3.1 g (ADT 3, ADT 5 and LBG 787) to 4.6 g (KKM 1) (Table 1). With regard to seed lusture, all the genotypes except LBG 752 and LBG 787 (shiny) are found to be dull in nature. Oviposition is one of the important behaviour of an insect for continuation of its race and for their population establishment (Sehgal and Sachdeva, 1985). Oviposition ranged from 55.7 eggs (TU 68) to110.3 eggs (VBN 11) on 50 seeds. All genotypes were confirmed for the presence of at least one number of egg per seed based on the mean number of eggs/seed before adult emergence. Hence, the seed size and seed lusture nature does not affect the level of oviposition by the bruchine. As all genotypes are invariably had eggs on seeds taken for study. Hence, it clearly indicates that the mechanism of resistance here is not the anti-xenosis. Similar results were reported by Tripathi *et al.* (2015) and Yadav and Pant (1974) in which they reported *Callosobruchus sp.* may oviposit on any seed even the seeds may not be suitable for its development. The developmental time of the bruchine beetles among the genotypes ranges from 21.7 days (CO 6) to 38.0 days (TU 68). TU 68 shows delayed emergence of bruchine beetles. Hence, it may not favour complete development of adults. Trypsin inhibitors in cowpea, alpha amylase in kidney bean and arcelin in wild bean, phyto-hemagglutinin in black beans had been found to affect the growth and development of the bruchid (Ishimoto and Kitamura, 1989) respectively. Likewise, chemical factors present in the seeds of resistant genotype prevent the hatching of eggs. The adult emergence starts from the seeds of all genotypes from 20 days after infestation (DAI) onwards. Maximum adult emergence was observed during the interval of 40-50 DAI (Swamy *et al.*, 2016). Adults emergence at 50 DAI ranged from 7.0 (TU 68) to 46.7 (MDU 1). The genotype TU 68 showed lesser emergence at 50 DAI and maintains its stable resistance even upto 90 DAI among the

Table 2: Initial screening of blackgram genotypes against bruchid infestation.

Genotypes	No. of eggs/50 seeds ± SE	Mean no. of eggs/ seed ± SE	Developmental time (egg + larval + pupal stages) ± SE	Total no. of Adult emergence ± SE	Mean developmental period (days) ± SE	Index of susceptibility (IS) ± SE	IS score	Seed damage (%) ± SE	SD score	Seed weight loss (%) ± SE
ADT3	70.0±2.5^{abc}	1.4±0.1^{ab}	24.0±1.0^{efg}	33.0±1.2^{bcde}	32.7±0.8^{bcd}	9.3±0.2^{cdefg}	S	66.0±2.3^{cdef}	S	36.8±1.1^{abc}
ADT5	74.3±7.4^{abc}	1.5±0.2^{ab}	29.0±0.6^{b}	44.0±2.7^{abc}	34.1±0.6^{bcd}	9.6±0.2^{bcdefg}	S	88.0±5.3^{abc}	HS	28.2±4.4^{bcde}
ADT6	108.0±7.2^{a}	2.2±0.1^{a}	28.0±0.0^{bc}	30.3±2.2^{cde}	34.2±0.9^{bcd}	8.7±0.4^{efg}	S	60.7±4.4^{def}	S	33.5±2.9^{abcd}
APK1	77.3±9.2^{abc}	1.6±0.2^{ab}	26.0±0.0^{cde}	48.3±0.9^{a}	31.9±0.6^{bcdef}	10.6±0.3^{abcde}	HS	95.3±1.3^{a}	HS	43.5±2.1^{a}
CO5	55.7±5.0^{c}	1.1±0.1^{b}	26.3±0.3^{cde}	33.0±1.0^{bcde}	30.3±1.5^{cdef}	10.1±0.4^{abcdef}	HS	66.0±2.0^{cdef}	S	38.8±1.9^{abc}
CO6	86.7±5.8^{abc}	1.7±0.1^{ab}	21.7±0.3^{h}	42.0±5.3^{abc}	27.4±0.3^{ef}	11.8±0.3^{a}	HS	82.0±5.1^{abcde}	HS	44.0±2.9^{a}
KKM1	78.7±3.8^{abc}	1.6±0.1^{ab}	23.3±0.7^{fgh}	42.7±1.2^{abc}	30.6±0.8^{cdef}	10.7±0.3^{abcd}	HS	85.3±2.4^{abcd}	HS	40.9±3.0^{abc}
LBG752	97.3±5.5^{ab}	2.0±0.1^{ab}	26.0±0.0^{cde}	46.3±0.9^{ab}	33.5±0.2^{bcd}	9.9±0.0^{abcdef}	S	92.7±1.8^{ab}	HS	38.1±0.7^{abc}
LBG787	69.3±2.6^{abc}	1.4±0.1^{ab}	26.0±0.0^{cde}	22.0±1.0^{e}	31.8±0.4^{bcdef}	8.5±0.1^{fg}	S	44.0±2.0^{f}	S	26.4±2.4^{cde}
MASH1008	77.3±5.4^{abc}	1.6±0.1^{ab}	26.0±0.0^{cde}	42.7±2.4^{abc}	32.2±0.3^{bcde}	10.1±0.1^{abcdef}	HS	85.3±4.8^{abcd}	HS	39.8±5.2^{abc}
MASH114	85.0±6.2^{abc}	1.7±0.1^{ab}	24.7±0.3^{defg}	27.7±3.4^{de}	32.1±1.0^{bcdef}	9.0±0.5^{defg}	S	55.3±6.8^{ef}	S	29.9±2.2^{abcde}
MDU1	94.0±11.7^{abc}	1.9±0.2^{ab}	24.0±0.6^{efg}	46.7±1.2^{ab}	29.9±0.5^{def}	11.2±0.2^{abc}	HS	93.3±2.4^{ab}	HS	41.7±1.7^{ab}
TMV1	66.3±2.6^{abc}	1.3±0.1^{ab}	22.7±0.3^{gh}	35.3±2.6^{abcd}	27.2±0.1^{f}	11.4±0.2^{ab}	HS	70.7±5.2^{bcdef}	S	30.8±2.6^{abcde}
TU68	55.7±1.0^{c}	1.2±0.0^{b}	38.0±0.6^{a}	7.0±0.6^{f}	43.5±0.4^{a}	3.9±0.2^{h}	MR	14.0±1.2^{g}	R	17.8±2.1^{e}
VBN(Bg)4	57.7±2.0^{bc}	1.2±0.0^{b}	24.3±0.3^{efg}	36.0±3.1^{abcd}	31.9±1.6^{bcdef}	9.8±0.7^{bcdef}	S	72.0±6.1^{bcdef}	S	38.4±2.0^{ab}
VBN6	64.7±11.3^{bc}	1.3±0.2^{b}	25.3±0.3^{def}	30.7±5.2^{cde}	31.9±0.8^{bcdef}	9.3±0.7^{cdefg}	S	61.3±6.6^{def}	S	33.5±0.5^{abcd}
VBN(Bg)7	94.0±16.2^{abc}	1.9±0.3^{ab}	27.0±1.0^{bcd}	46.0±1.5^{ab}	35.8±2.3^{b}	9.4±0.6^{cdefg}	S	90.7±2.9^{abc}	HS	42.2±1.5^{ab}
VBN8	67.7±1.8^{abc}	1.4±0.0^{ab}	27.0±0.0^{bcd}	27.3±3.0^{de}	36.8±0.9^{b}	7.8±0.1^{g}	S	54.7±5.9^{ef}	S	25.9±2.3^{cde}
VBN9	58.3±4.1^{bc}	1.2±0.1^{b}	28.0±0.0^{bc}	27.7±2.9^{de}	32.5±1.2^{bcd}	8.9±0.5^{defg}	S	55.3±5.7^{ef}	S	21.8±4.1^{de}
VBN11	110.3±21.1^{a}	2.2±0.4^{a}	26.3±0.3^{cde}	43.7±1.8^{abc}	35.2±1.0^{bc}	9.3±0.4^{cdefg}	S	87.3±3.5^{abcd}	HS	35.6±4.6^{abcd}

Values followed by the same letter along a column are not significantly different (P>0.05) from each other based on Tukey's honest significant difference (HSD) test.

genotypes taken for the study (Fig 1). The mean developmental period among the genotypes taken for the study was ranged from 27.2 days (TMV 1) to 43.5 days (TU 68). The mean developmental period (MDP) days at 50 DAI also indicated the prolonged developmental period of adult emergence in the genotype TU 68. This might be due to presence of certain chemicals in seeds which delay the growth of grub as observed by Somta *et al.* (2008). Similar findings were reported by Tripathi *et al.* (2015). The index of susceptibility (IS) has showed the moderate resistance nature of the TU 68 towards the bruchine infestation. The less seed damage percentage (14.0%) and less seed weight loss percentage (17.8%) also confirmed the resistant nature of the TU 68 genotype than the other genotypes towards the bruchine infestation. In confirmatory trial, the tolerant genotype TU68 and four susceptible entries *viz.,* ADT 3, MDU1, VBN 6 and VBN 8 were evaluated for their reaction against bruchine infestation. The results indicated that the genotype TU 68 was found promising against bruchine infestation for oviposition, developmental time, adult emergence, mean developmental period, index of susceptibility, seed damage and seed weight loss. Seed weight loss (%) was one of the important criterion to assess resistance against bruchid infestation due to the economic value of the seed. Among the genotypes TU 68 had less seed weight loss when compared to other genotypes both in initial trial and the confirmatory trials.

To identify the active biochemical compounds that are responsible for the resistance against the *C. maculatus,* tolerant genotype (TU 68) and the highly susceptible genotype (MDU 1) were subjected to GC-MS analysis. The results indicated the presence of 18 chemical compounds among the tolerant genotype and highly susceptible genotype of blackgram (Table 4). The GC-MS chromatogram plot of the resistant (TU 68) and susceptible (MDU 1) genotypes against the retention time has been presented as Fig 2 and Fig 3 respectively. Among the compounds identified, two chemical compounds are found to be distinguishing between the tolerant (TU 68) and the highly susceptible genotype (MDU 1). The compound 9, 12, 15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) with the retention time of 26.13 min is found to have higher peak area of 12.79% in the tolerant genotype TU 68 while highly susceptible genotype MDU 1 had 1.96%. Another compound hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl esterwith the retention time of 23.49 min is only found in the tolerant genotype TU 68 with a peak area of 8.63% but not in the highly susceptible genotype MDU 1. Gnanavel and Saral (2013) and Tayade *et al.* (2013) reported that these two compounds have the insectifuge property. Hence these chemical compounds may be responsible for the delayed emergence and prolonged developmental period in the tolerant genotype TU 68.

Based on the foregoing discussion, it may be concluded that TU 68 had comparatively less number of oviposition, delayed developmental time, less adult emergence,

Table 3: Confirmation screening of blackgram genotypes for bruchid resistance.

Genotypes	No. of eggs/50 seeds ± SE	Mean no. of eggs / seed ± SE	Developmental time(egg + larval + pupal stages) ± SE	Total no. of Adult emergence ± SE	Mean developmental period (days) ± SE	Index of susceptibility (IS) ± SE	IS score	Seed damage (%) ± SE	SD score	Seed weight loss (%) ± SE
TU68	100.7± 5.8^{c}	2.0± 0.1^{c}	40.7± 1.2^{a}	5.7± 0.7^{b}	46.0± 0.6^{a}	3.3± 0.2^{b}	MR	11.3±1.3^{c}	R	11.8±1.2^{c}
ADT3	101.3± 3.4^{c}	2.0± 0.1^{c}	31.3± 0.3^{bc}	31.0± 1.5^{a}	38.3± 0.6^{b}	7.9± 0.1^{a}	S	62.0±3.1^{b}	S	36.3±1.0^{a}
MDU1	125.0± 5.7^{abc}	2.5± 0.1^{abc}	28.7± 0.3^{c}	46.0± 1.2^{a}	37.5± 0.8^{b}	8.9± 0.2^{a}	S	92.0±2.3^{a}	HS	33.1±1.8^{a}
VBN6	128.0± 4.6^{ab}	2.6± 0.1^{ab}	35.3± 2.3^{ab}	36.7± 3.5^{a}	39.4± 0.9^{b}	8.1± 0.2^{a}	S	73.3±7.0^{ab}	S	25.6±3.4^{ab}
VBN8	106.7± 7.3^{bc}	2.1± 0.2^{bc}	30.3± 1.5^{bc}	30.7± 6.0^{a}	37.0± 1.4^{b}	8.5± 0.2^{a}	S	61.3±12.0^{b}	S	20.0±2.6^{bc}

Values followed by the same letter along a column are not significantly different (P>0.05) from each other based on Tukey's Honest significant difference (HSD) test.

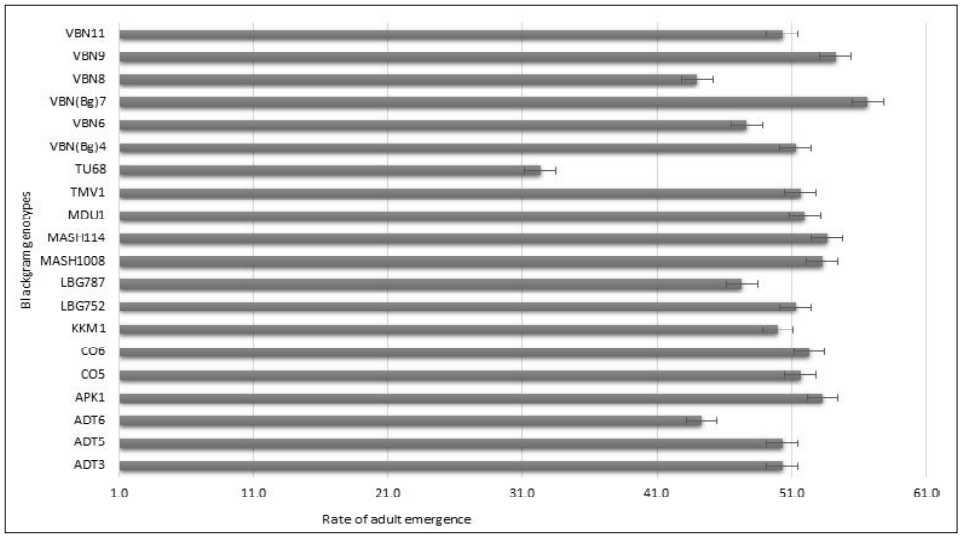

Fig 1: Adult emergence on 90 days after infestation (DAI) in blackgram genotypes.

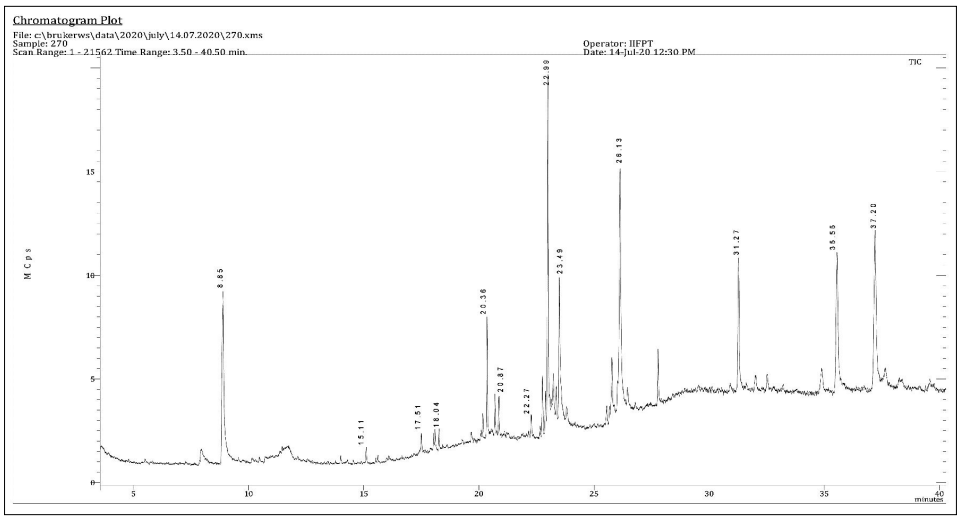

Fig 2: GC-MS chromatogram plot of blackgram genotype TU 68.

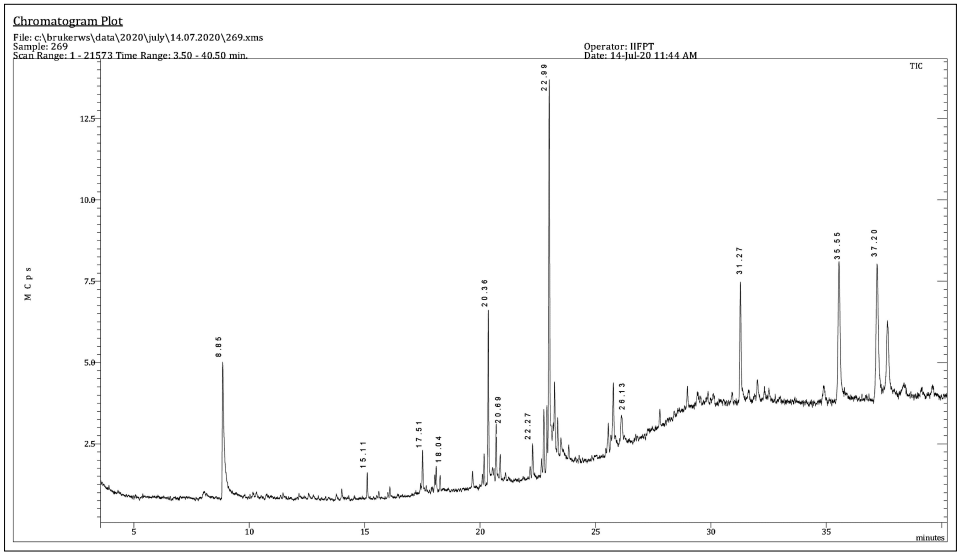

Fig 3: GC-MS chromatogram plot of blackgram genotype MDU 1.

Table 4: Compounds identified in the blackgram genotypes using GC-MS analysis.

RT (min)	Name of the compound	Molecular formula	Molecular weight (g/mol)	(MDU1) Peak area %	(TU68) Peak area %
8.85	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3) -β-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	11.49	14.39
15.11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.96	0.45
17.51	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	1.52	0.58
18.04	5,8,11-Heptadecatrien-1-ol	$C_{17}H_{30}O$	250	1.18	0.86
18.09	Octadecanedioic acid	$C_{18}H_{34}O_4$	314	0.78	0.46
18.27	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	0.71	0.63
20.36	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	7.43	4.40
20.69	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{40}O_4$	356	2.30	1.73
20.87	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	1.12	1.50
22.27	9,12-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	266	1.40	0.81
22.76	Cyclopentolate	$C_{17}H_{25}NO_3$	291	3.02	2.24
22.89	7,10,13-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320	3.25	1.90
22.99	cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302	21.73	15.27
26.13	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$	352	1.96	12.79
31.27	γ-Tocopherol	$C_{28}H_{48}O_2$	416	8.17	7.38
35.55	Glycine, N-[(3α,5β,12α)-3,12-dihydroxy-24-oxocholan-24-yl]-	$C_{26}H_{43}NO_5$	449	16.61	11.84
37.20	γ-Sitosterol	$C_{29}H_{50}O$	414	16.3	14.16
23.49	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	-	8.63

RT- Retention time.

prolonged mean developmental period, less susceptibility index, less seed damage (%) and less seed weight loss (%) in both the experiments. The compounds 9, 12, 15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl esterare may be responsible for the bruchine resistance in the seeds of TU 68. Kumar *et al*. (2009) reported that bruchine resistant varieties were not suitable for feeding and quick development of the life stages of the bruchine. There is a concern about the consumption of resistant varieties by human beings like hard seed coat, unfavorable chemical factors and non-preference nature (War *et al*., 2017). Hence complete resistance will not be suitable for the human consumption. TU 68 was found to have moderate resistance nature and hence it may not have adverse effect like wild species. However, further analysis of quality assessment will be helpful to confirm the usefulness of this genotype. TU 68 is a derivative of TU 94-2 × *Vigna mungo* var. *silvestris*. Presence of antibiosis nature in *Vigna mungo* var. *silvestris* was reported by Soundararajan *et al.* (2013). The antibiosis factors responsible for the reduced oviposition, reduced seed damage and prolonged developmental period might be transferred from *Vigna mungo* var. *silvestris*. Hence, TU 68 could be utilized in bruchine resistance breeding programme.

CONCLUSION

Genetic resistance is a better method than chemical methods to reduce bruchine damage in storage (Somta *et al*., 2006). Hence, the present investigation was carried out to identify the resistant sources, confirmation of their resistance and the active biochemical compounds present in resistant source. The genotype TU 68 was found to be resistant in initial test as well as the confirmatory trials. It was found to have the active biochemical compounds with insectifuge activity. Hence, TU 68 could be utilized in the hybridization programmeas donor to evolve cultivars resistant to bruchine beetles.

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