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Host Plant Resistance and Analysis of Chemical Compounds Responsible for Bruchid Resistance in Greengram *Vigna radiata* (L.) Wilczek

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ABSTRACT

Background: Greengram *Vigna radiata* (L.) Wilczek, is an important legume crop that serves as a low-cost source of protein. The bruchid (*Callosobruchus spp.*) is a serious storage pest affecting greengram and other pulse crops. Thus, a study was designed to investigate bruchid resistance (*Callosobruchus chinensis*) in inter sub-specific derived lines of greengram and to identify chemical compounds responsible for resistance.

Methods: The experimental material comprised of 200 inter sub-specific derived lines of F_9 generation of VBN (Gg) 2 (susceptible to bruchid) × *Vigna radiata* var. *sublobata/2* (resistant to bruchid) and a susceptible check variety. The bruchid screening experiment was carried out in completely randomized design and replicated twice with 50 seeds in each replication by adopting no choice test. Out of 200 lines evaluated for bruchid screening, seed damage due to bruchid was less than 20 per cent in 11 lines, identified as resistant. However, three resistant lines *viz*., GGISC 124, GGISC 140 and GGISC 150 were taken for further confirmation for bruchid resistance and GC-MS analysis to discover the chemical compounds conferring resistance (Clarus SQ 8C, Perkin Elmer).

Result: In confirmation screening, seed damage due to bruchid (*Callosobruchus chinensis*) on 30th day was less than 20 per cent in three inter sub-specific lines *viz*., GGISC 124, GGISC 150 (17.00%) and GGISC 140 (18.00%), whereas the susceptible check [VBN (Gg) 2] reached 100 per cent adult emergence. The three inter sub-specific lines recorded susceptibility index of 0.046 (GGISC 124), 0.047 (GGISC 140) and 0.048 (GGISC 150) and classified as resistant. The susceptible check [VBN (Gg) 2] recorded the susceptibility index of 0.085. GC-MS study was carried out in resistant lines GGISC 124, GGISC 140, GGISC 150 and susceptible check VBN (Gg) 2. The results revealed that the existence of three compounds *viz*., 9, 12-octadecadienoic acid, methyl ester; Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester and Hexadecanoic acid, 2-oxiranyl methyl ester in resistant lines conferred resistance against *C. chinensis* in greengram.

Key words: Bruchid resistance, *Callosobruchus chinensis*, GC-MS analysis, Greengram, Inter sub-specific lines.

INTRODUCTION

Greengram *Vigna radiata* (L.) Wilczek is one of the major legume crops in India and a major component of many cropping systems. It is a low-cost source of nutritional protein (24-25%) and carbohydrate (56%). Greengram is a short duration, self-pollinated diploid grain legume crop with a genome size of 494 to 579 Mb (Liu *et al*., 2016). In India, the area under cultivation of greengram is 51.3 lakh hectares with the production of 30.9 lakh tonnes and the productivity of 601 kg/ha (Indiastat, 2022).

Bruchid is one of the major storage pests of pulses causing severe damage when it is unnoticed under storage. The bruchid, *Callosobruchus spp.* (Chrysomelidae: Coleoptera) is the dominant insect pest of pulse crops that causes 55 to 60 per cent loss in seed weight and 45.50 to 66.30 per cent loss in protein content and upto 30 per cent loss during storage. The bruchid infestation begins in the field, when adult beetles lay eggs on green pods and larva bore through the pod and feed on the growing seed, causing 1-2 per cent of the damage at field level. When the seeds are stored, the insects continue to feed, mature into adults, generate a secondary infestation and resulting in the complete destruction of the seeds in 3-4 months (Reddy *et al.,* 2021). Storage at low temperature, solar irradiation of the grains, hermetic storage, the use of biocontrol agents, the use of botanical extracts, chemical treatment with methyl

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bromide, carbon disulphide and aluminium phosphide have been employed to control the bruchid infestation during storage condition. Chemical control is effective, but it increases storage costs, harmful to humans and other animals owing to residues in food, raises the risk of insect resistance and also environmentally hazardous (Gbaye *et al*., 2011). In order to overcome these issues, host plant resistance emerged as a useful strategy for developing resistant varieties.

Gas chromatography-mass spectroscopy methods are used to identify the phytochemicals found in seeds, seedlings and also leaf extracts. It entails identifying and isolating secondary metabolites produced by plants. The use of gas chromatography and mass spectrometry to screen secondary metabolites allows for sensitive detection of biologically active chemicals. Many chemical compounds have been reported for bruchid (*Callosobruchus chinensis*) resistance in the greengram seeds of TC 1966 *viz*., Vignatic acid A (cyclopeptide alkaloid) (Kaga and Ishimoto, 1998) and cysteine-rich protein (*VrCRP* or *VrD1*) (Chen *et al.,* 2002). The compounds *viz.,* vicilins, para-amino-phenylalanine, lignins, quinines, alkaloids, saponins, polysaccharides, lectins, phytohemagglutinins (PHA), chitinase, beta-1,3-glucanase, peroxidase, provicilin, α-amylase inhibitors, trypsin inhibitors, canavalin, cyanogenic glycosides and phytic acid were also identified for bruchid resistance in greengram seeds (Khan *et al.,* 2003; Somta *et al*., 2007). The current study was aimed to identify the active chemical compounds involved in the bruchid resistant lines by GC-MS analysis.

MATERIALS AND METHODS

Two hundred inter sub-specific derived lines of F_9 generation of VBN (Gg) 2 (susceptible to bruchid) × *Vigna radiata* var. *sublobata/2* (resistant to bruchid) and the parents were subjected to bruchid screening. A total of 11 resistant lines were identified (data not shown). Out of this, three resistant lines were selected on the basis of low damage per cent. Independent bruchid screening was done along with susceptible check to confirm the resistant lines identified in the preliminary screening. The bruchid species used for screening was *Callosobruchus chinensis* and the mass culture of the bruchids were maintained on the greengram seeds. The bruchid screening experiment was carried out at Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, India during 2021-22 by using 'No choice test' in completely randomized design and replicated twice with 50 seeds in each replication (Dongre *et al*., 1993). Fifty seeds of each line were transferred into the petriplates and five pairs of bruchids were released and maintained under controlled laboratory conditions. Standard screening parameters *viz*., initial seed weight (g), final seed weight (g), days to first adult emergence, mean developmental period (days), weight of 10 pairs of adult bruchids (mg) and number of adults emerged on 30th day were recorded. Damage assessment was carried out on 30th day of inoculation when the susceptible check reached 100 per cent damage. The damage parameters *viz*., weight loss per cent of seed (Khattak *et al.,* 1987), susceptibility index (Howe, 1971) and seed damage per cent (Mariammal *et al.,* 2019) were assessed on 30th day of inoculation.

GC-MS analysis on three resistant lines and one susceptible check was performed to discover the active chemical compounds conferring resistance. The samples were prepared from powdered greengram by using standard methods given by Rivera *et al*. (2012) and the samples were suspended in HPLC grade methanol. The three resistant lines and a susceptible check were subjected to GC-MS analysis (Clarus SQ 8C with detector Perkin Elmer Mass Spectrometer) in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The mass spectrum of the GC-MS was compared with the components known from the NIST library database version-14. The spectrum data from four greengram samples were compared to known spectrum from the NIST, PubChem and Human Metabolome Databases.

RESULTS AND DISCUSSION

In the present investigation, out of 200 inter sub-specific lines screened, eleven lines were recorded as resistant (< 20% seed damage) (data not shown). However, three resistant lines with less seed damage 17.00% (GGISC 124, GGISC 150) and 18.00% (GGISC 140) were further taken for bruchid confirmation screening and also for GC-MS analysis along with a susceptible check [VBN (Gg) 2].

During confirmation screening, the bruchid screening parameters were recorded in three resistant lines and a susceptible check and are presented in Table 1. All the parameters recorded in resistant lines showed significant difference when compared to susceptible check. Among the lines, initial seed weight for 50 seeds was 1.21 g (GGISC 124), 1.26 g (GGISC 140), 1.54 g (GGISC 150) and 1.86 g [VBN (Gg) 2]. Three resistant lines showed less weight loss and recorded final seed weight (after 30th day) of 1.07 g (GGISC 124), 1.10 g (GGISC 140) and 1.37 g (GGISC 150). VBN (Gg) 2, a susceptible line showed the highest reduction in seed weight and recorded the final weight of 0.76 g.

The days to first adult emergence was prolonged in resistant lines when compared to VBN (Gg) 2. The days to first adult emergence recorded was 24 days (GGISC 124, GGISC 140, GGISC 150) and was only 20 days in susceptible check. The mean developmental period was longer in resistant lines when compared to VBN (Gg) 2. The mean developmental period recorded was 27 days (GGISC 140), 26 days (GGISC 124, GGISC 150) and 23 days in the susceptible check VBN (Gg) 2. Soumia *et al.* (2017) also reported longer developmental period in resistant lines when compared to susceptible lines in greengram. The weight of 10 pairs of bruchids were 45.00 mg in VBN (Gg) 2 and however sufficient number of bruchids was not emerged in the specified time (30 days) inorder to record weight of the bruchids. The bruchids emerged from resistant lines were observed as malformed and small in size than in the susceptible lines. Samyuktha *et al.* (2020) reported that the

Table 1: Screening of inter sub-specific lines for bruchid resistance.

	Initial seed weight (g)	Final seed weight (g)	Days to first adult emergence	Mean developmental period (days)	Weight of 10 pairs of adult bruchids (mg)	Number of adults emerged on 30th day	Weight loss of seed on 30th day (%)	Susceptibility index on 30th day	Seed damage on 30th day (%)
GGISC 124	1.21	1.07	24	26	-	9	11.59	0.046	17.00
GGISC 140	1.26	1.10	24	27	-	9	12.41	0.047	18.00
GGISC 150	1.54	1.37	24	26	-	9	11.37	0.048	17.00
VBN (Gg) 2 (Susceptible check)	1.86	0.76	20	23	45.00	50	59.15	0.085	100.00
Mean	1.47	1.07	23	26	-	19	23.63	0.057	38.00
Range	1.21-1.86	0.76-1.37	20-24	23-27	-	9-50	11.37-59.15	0.046-0.085	17.00-100.00
SEd	0.15	0.12	1.00	0.87	-	10.25	11.84	0.01	20.67
CD(P=0.05%)	0.48	0.40	3.18	2.76	-	32.62	37.69	0.03	65.77

Table 2: Analysis of chemical compounds in the resistant lines and the susceptible check by GC-MS.

Retention time (Min.)	Chemical compounds	Molecular formula	Molecular weight (g/mol)	Peak area (%)			
				GGISC 124	GGISC 140	GGISC 150	VBN (Gg) 2
21.77	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.369	3.389	3.487	0.170
22.45	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.249	9.399	9.361	19.653
24.94	9,12-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	1.032	2.960	3.063	-
25.63	9,12-octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	9.213	4.687	4.029	13.501
26.19	Octadecanoic acid	$C_{18}H_{36}O_2$	284	10.284	8.102	7.753	7.061
26.29	Hexadecanoic acid, 1-(hydroxy methyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	0.254	1.838	0.487	-
28.45	Hexadecanoic acid, 2-oxiranyl methyl ester	$C_{19}H_{36}O_3$	312	5.804	3.390	2.862	-

resistant genotypes expressed the antibiosis mechanism against bruchid infestation and caused the malformation and death of grub in greengram. The number of adults emerged on 30th day in resistant lines were nine, whereas in susceptible check 50 adults were emerged (Table 1). Sarkar and Bhattacharyya (2015) also reported high bruchid infestation in susceptible lines of greengram.

In the present study, damage assessment parameters *viz.,* weight loss per cent of the seed, susceptibility index and seed damage were worked and are presented in Table 1. The three resistant lines recorded less weight loss per cent of seed *viz*., 11.59 per cent (GGISC 124), 12.41 per cent (GGISC 140) and 11.37 per cent (GGISC 150), whereas susceptible check [VBN (Gg) 2] recorded the highest seed weight loss of 59.15 per cent (Table 1). Seram *et al.* (2016) and Harshitha *et al.* (2022) reported less weight loss per cent of seed in bruchid resistant lines of greengram.

The three resistant lines recorded susceptibility index less than 0.050 (resistant category) *viz*., 0.046 (GGISC 124), 0.047 (GGISC 140) and 0.048 (GGISC 150), whereas the susceptible check [VBN (Gg) 2] recorded the susceptibility index of 0.085 (Table 1). Neupane *et al.* (2016); Ghosh *et al.* (2022) and Harshitha *et al.* (2022) have also used the susceptibility index and weight loss per cent for determining the level of bruchid resistance in greengram and were found to be high in susceptible lines.

Seed damage per cent on 30th day was less than twenty per cent in three resistant lines *viz*., 17.00 per cent (GGISC 124, GGISC 150) and 18.00 per cent (GGISC 140), whereas the susceptible check (VBN (Gg) 2) reached 100 per cent adult emergence on 30th day of inoculation (Table 1). Harshitha *et al.* (2022) also reported the 100 per cent adult emergence on 30th day of inoculation in VBN (Gg) 2. Sarkar and Bhattacharyya (2015) and Soumia *et al.* (2017) reported that in susceptible varieties of greengram, the susceptibility index was more than 0.050 and the seed damage was more than 40 per cent. Soumia *et al.* (2017) reported that the reduction in adult emergence is an indication of the presence of antibiosis factors in seed that results in the prolongation of developmental period of bruchid in greengram.

The resistant lines *viz*., GGISC 124, GGISC 140, GGISC 150 and susceptible check VBN (Gg) 2 were subjected to GCMS analysis for identifying the chemical compounds responsible for resistance to *C. chinensis*. The data generated by gas chromatography showed that the composition of the various chemical compounds were present in three resistant lines and a susceptible check. The GC-MS chromatogram plot of the resistant lines GGISC 124, GGISC 140, GGISC 150 and susceptible check VBN (Gg) 2 obtained are shown in Fig 1 to 4, respectively. In the present study, a total of forty bioactive compounds were observed in GC-MS analysis. Among them, seven compounds showed difference in peak area (%), molecular formula, molecular weight and retention time (min.) (Table 2).

Among the seven compounds identified four compounds *viz*., Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; 9, 12-octadecadienoic acid (Z, Z) and Octadecaenoic acid were observed in all the four lines including susceptible check with some difference in per cent peak area. The compounds *viz*., Hexadecanoic acid, methyl ester; n-Hexadecanoic acid and 9, 12-octadecadienoic acid (Z,Z) were reported while studying bruchid resistant and susceptible lines in chickpea (Reddy *et al.,* 2021) and in blackgram (Ragul *et al.,* 2022) through GC-MS analysis. Bharathithasan *et al.* (2021) also reported insecticidal property of Octadecaenoic acid against insect pest of Areca nut.

Among the seven compounds identified, three compounds were found to distinguish the resistant (GGISC 124, GGISC 140, GGISC 150) and susceptible check [VBN (Gg) 2]. The first compound 9, 12-Octadecadienoic acid, methyl ester was observed with the peak area of 1.032 per cent, 2.960 per cent and 3.063 per cent in the resistant lines GGISC 124, GGISC 140 and GGISC 150, respectively with retention time of 24.94 minutes (Table 2). The second compound Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester was reported with the peak area of 0.254 per cent, 1.838 per cent and 0.487 per cent in the resistant lines GGISC 124, GGISC 140 and GGISC 150, respectively with the retention time of 26.29 minutes (Table 2). The third compound Hexadecanoic acid, 2-oxiranyl methyl ester was identified in the peak area of 5.804 per cent, 3.390 per cent

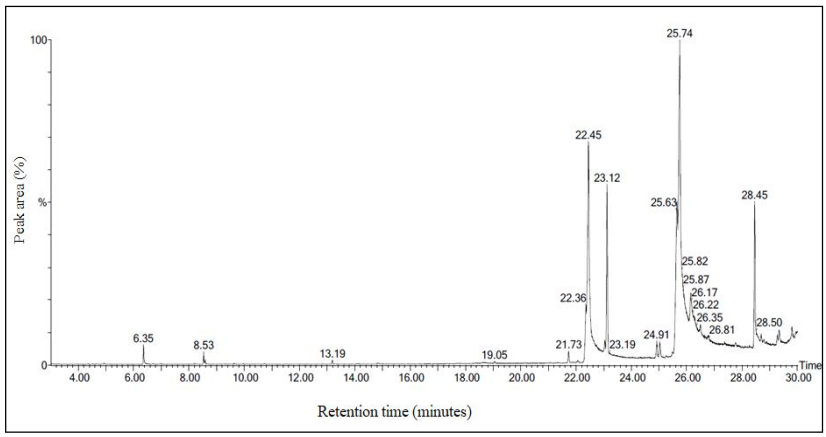

Fig 1: GC-MS chromatogram plot of GGISC 124.

and 2.862 per cent in the resistant lines GGISC 124, GGISC 140 and GGISC 150, respectively with the retention time of 28.45 minutes (Table 2). The compound 9, 12- Octadecadienoic acid, methyl ester was reported against bruchid infestation in chickpea (Reddy *et al.,* 2021). Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester and Hexadecanoic acid, 2-oxiranyl methyl ester were known to have insecticidal properties against insect pest of Areca nut (Bharathithasan *et al.,* 2021). Therefore, in the present study, 9, 12-Octadecadienoic acid, methyl ester; Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester and Hexadecanoic acid, 2-oxiranyl methyl ester were identified as the compounds responsible for bruchid (*Callosobruchus chinensis*) resistance in greengram.

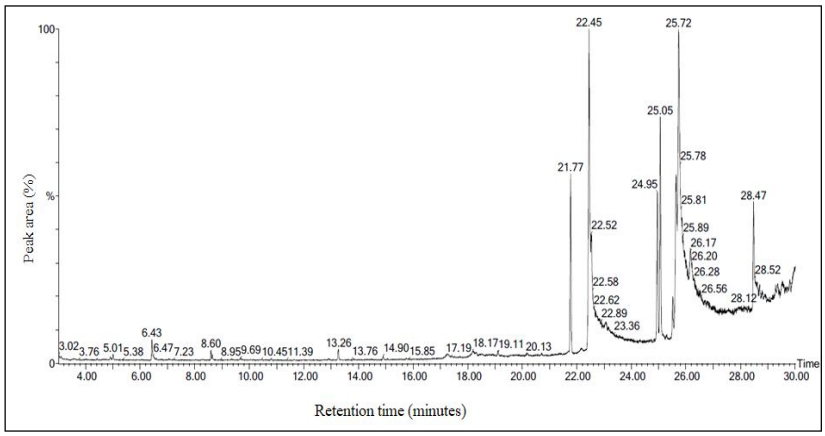

Fig 2: GC-MS chromatogram plot of GGISC 140.

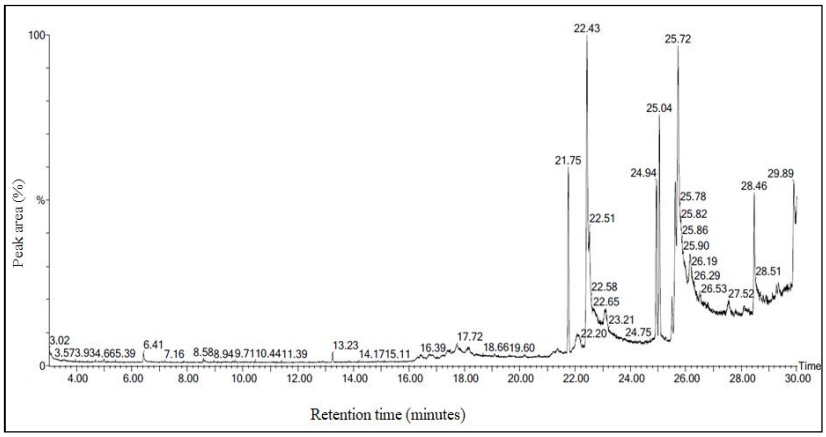

Fig 3: GC-MS chromatogram plot of GGISC 150.

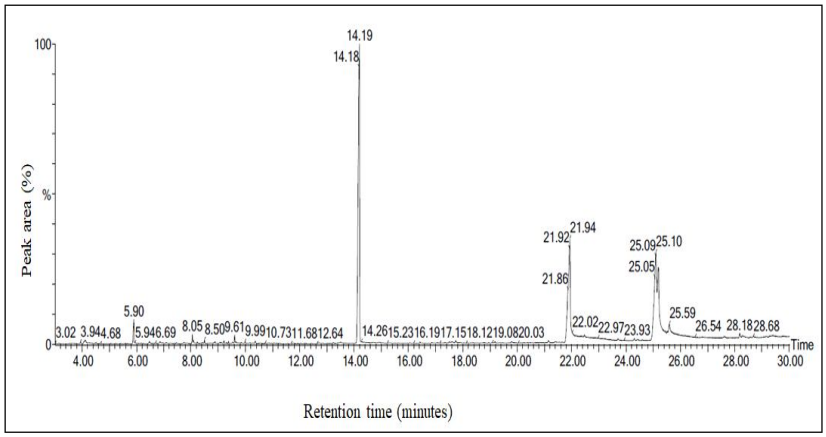

Fig 4: GC-MS chromatogram plot of VBN (Gg) 2.

CONCLUSION

Host plant resistance is an environmentally safe, cost effective and sustainable way to mitigate bruchid damage during storage. The inter sub-specific lines *viz.,* GGISC 124, GGISC 140 and GGISC 150 were found to be resistant against bruchids. The three chemical compounds *viz*., 9, 12-octadecadienoic acid, methyl ester; Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester and Hexadecanoic acid, 2-oxiranyl methyl ester were identified in GC-MS analysis and were responsible for bruchid (*Callosobruchus chinensis)* resistance in the above three lines of greengram. Thus, the three resistant lines can be utilized as a pre-breeding material and used as parents in the crossing programme to develop bruchid resistant varieties in greengram.

Conflict of interest

All authors declared that there is no conflict of interest.

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