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Deciphering Biochemical Traits in Selected RILs of Mungbean [*Vigna radiata* (L.) Wilczek] against Bruchids (*Callosobruchus maculatus* F.)

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ABSTRACT

Background: Mungbean [*Vigna radiata* (L.) Wilczek] is a vital pulse crop having a colossal nutritionary and gastronomic significance. The bruchid, *Callosobruchus maculatus* is recognised as the most heinous storage pest of leguminous crops that causes drastic economic loss. Crop breeders may use the nutritional and antinutritional traits of seeds to successfully combat storage pests.

Methods: The present investigation has been framed to compare the degree of seed damage and weight loss with the biochemical parameters, *viz*., starch, sugar, protein, phenol, tannin and alpha-amylase inhibitor, of mungbean seeds from parental and selected lines of F5 populations for bruchid resistance.

Result: The resistant parent, V2802BG and seven F_5 families (BSR-GG-1-49-2, BSR-GG-1-49-5, BSR-GG-1-56-5, BSR-GG-1-170-5, BSR-GG-1-198-2, BSR-GG-1-198-3, BSR-GG-1-160-1) were completely resistant to bruchid infestation with no seed damage. One of the probable reasons for the stunted development of *Callosobruchus maculatus* might be the high level of phenol. Seed weight loss and damage by bruchids were shown to be positively correlated with high levels of protein, starch and sugar; however, alpha-amylase inhibitors and tannins did not reach statistical significance at the 5% level.

Key words: Biochemical analysis, *Callosobruchus maculatus*, Damage scoring, Mungbean, Resistance screening, RILs.

INTRODUCTION

Mungbean or greengram [*Vigna radiata* (L.) R. Wilczek var. *radiata*], the third foremost pulse crop in the country, is superior to other pulse crops as they are more palatable, highly nutritive and easily digestible, with excellent source of vitamins (3%), protein (24-26%), carbohydrates (51%) and minerals (4%). It plays an essential role in ensuring food security to feed future generations owing to its wider adaptability, climate-smart nature and lesser water requirement.

The most destructive storage pests of this crop are the bruchids which mitigate the market and nutritional value of the seed by rendering them unfit for consumption and sowing. In the fields, during post-harvest storage and on matured pods and grains. *Callosobruchus maculatus*, a bruchid with a widespread distribution, is the most problematic species (Hajam and Kumar, 2022). Biocides, parasitoids, plant resistance mechanisms and physical methods have been developed as affordable and more environmentally sustainable alternatives to synthetic insecticides. However, host plant resistance is the most reliable and effective modality for bruchid control in mung bean. Exploring and exploiting host plant resistance to bruchids could be a viable option, due to the inherited insecticidal activity in various legumes *viz*., secondary metabolites, enzyme inhibitors, antinutritional seed proteins *etc*. have insecticidal activity in various legumes. Resistance-related defence mechanisms are highly exploited in crop breeding against insect pests. Even though the wild sources of bruchid resistance in mung bean have been reported and used to develop resistant lines, their appropriate use as commercial cultivars has been hampered by undesirable genetic linkages, which are reflected in the non-availability of resistant cultivars. TC 1966, a wild form of mung bean and a few pure line selections V2802BG and V2709 of Philipine and Indian origin, respectively, however, are immune to both *C. chinensis* and *C. maculatus* (Tripathi *et al*., 2020).

Physical, chemical and molecular characteristics that affect insects and alleviate damage and further yield loss were referred to as resistance features. Legumes have

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antinutritional components that impede digestion and render the seeds unpleasant when consumed uncooked. Yao *et al*. (2015) reported that bruchid resistant mung bean is as benign as traditional mungbean after conducting the sub-chronic oral toxicity test on Sprague-Dawley (SD) rats with bruchid resistance cultivars. Discerning and evaluating the compounds that make the seeds resistant and susceptible to bruchid are uncomplicated with the biochemical estimation of legumes. Since resistance conferred by several protein antimetabolites, such as protease inhibitors and lectins are controlled by a single gene or genes from the same family, they could be utilised in transgenic development. However, other secondary plant compounds, such as tannins and polyphenols, which are crucial for resistance to legumes, could not be developed due to their multigenic nature. In this context, research on the biochemical components of the developed resistant lines is imperative. The quantitative estimation of nutritional and antinutritional features in the parents and their selected lines was looked into to determine the potential biochemical underpinnings of bruchid resistance.

MATERIALS AND METHODS

Experimental material

The experiment was conducted between February-April 2021 in the laboratory of the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, India. Seeds of 25 families comprising 150 progeny lines in the F_5 generation maintained in the Agriculture Research Station, Bhavanisagar were obtained for the study. From the preliminary screening, the lines with different damaging score were selected for the biochemical studies.

Screening of selected mungbean lines

C. maculatus procured from Agricultural Research Station, Bhavanisagar, Tamil Nadu, were maintained for generations in the susceptible cultivar CO6 during the entire research period for a continuous supply of bruchids. Evaluation of bruchid resistance was carried out in breeding lines with different levels of bruchid infestation under the "no-choice" test (Tomooka *et al*., 2000). For this test, five pairs of freshly emerged adults were released on 50 number of seeds of each genotype placed in a 15 cm diameter plastic petriplates for five days and removed. All the plates were kept inside the Biological Oxygen Demand (B.O.D.) incubator at 27±2°C temperature and 65±5% relative humidity (RH) and the following observations, were recorded and calculated:

i. **Number of eggs laid on seeds:** Recorded with the help of an egg counting device.

ii. **Number of adults emerged from individual seeds:** Recorded daily to determine developmental period (days) and continued till cessation of emergence.

iii. Seed damage per cent (%) =

$$\frac{\text{Number of seeds damaged}}{\text{Number of seeds taken}} \times 100$$

Scoring was done based on seed damage per cent suggested by (Weigand and Tahhan 1990) (Table 1).

iv. Per cent survival rate (%) =

$$\frac{\text{No. of adults emerged}}{\text{Total no. of eggs laid}} \times 100$$

v. **Mean developmental period (MDP) in days:** MDP is the time taken for 50 per cent of the adults to emerge.

Mean developmental period (in days) =

$$\frac{d_1a_1 + d_2a_2 + d_3a_3 + \ldots..+d_na_n}{\text{Total number of adults emerged}}$$

Where,

d_1 = Day at which the adults started emerging (1st day).

a_1 = Number of adults emerged on d_1^{th} day.

vi. Index of suitability (Howe's index or growth index) =

$$\frac{\text{Log (per cent survival)}}{\text{Mean developmental period}}$$

Biochemical analysis of seeds

Estimating biochemical parameters in the seeds of selected mung bean lines were performed at the Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore, under laboratory conditions. The following methodology was used for the estimation:

I. Total starch and total soluble sugar by anthrone reagent method (McCready *et al*.,1950 and Somogy, 1952, respectively).

II. Protein using bovine serum (Lowry, 1951).

Table 1: Scoring for bruchid infestation (Weigand and Tahhan,1990).

Seed infestation (%)	Category	Scale
0%	Completely resistant or immune	1
1-9%	Resistant	3
10-69%	Moderately susceptible	5
70-99%	Highly susceptible	7
100%	Completely susceptible	9

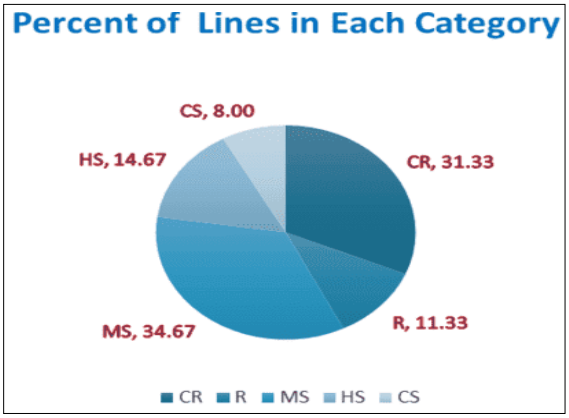


Fig 1: Different categories of F_5 breeding lines in percentage. *CR: Completely resistant lines; R: Resistant lines, MS: Moderately susceptible lines; HS: Highly susceptible lines; CS: Completely susceptible lines.

III. Total phenols using the Folin-Ciocalteau reagent method (Waterhouse, 2002).
IV. alpha-amylase activity using the method of (Bernfeld, 1955).
V. Tannins by Follin-Dennis spectrophotometric method (Pearson, 1976).

Statistical analysis

All experiments were performed in triplicates. The data on the biology of *C. maculatus* in parents and selected lines of mungbean were analyzed by adopting a completely randomized design. Analysis of variance (ANOVA) was performed using the SPSS package and R programming.

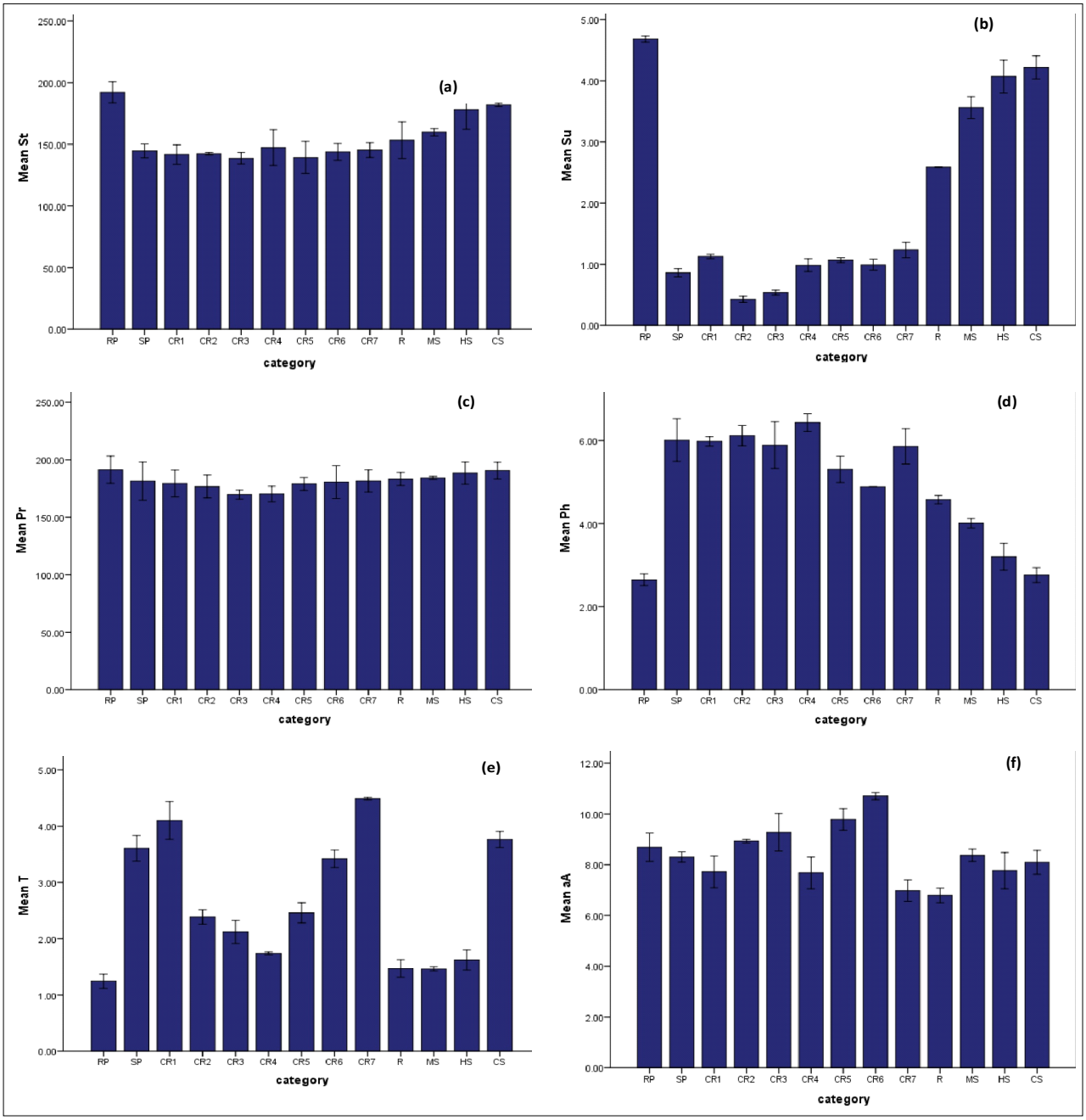


Fig 2: The biochemical traits of selected lines and parents a) starch (mg/g) b) sugar (mg/g), c) protein (mg/g), d) phenol (mg/g), e) Tannin(mg/g) f) alpha-amylase (%). Values are the mean (+/- SE) of three replicates.

The mean value of each character was compared with the critical difference (CD) values at p = 0.05. The one-tailed Pearson's correlation coefficient (r) was calculated by R programming using Scatterplot Matrix and Corrplot.

RESULTS AND DISCUSSION

Biological parameters of insects

The study revealed substantial variability in the expression of resistance to *C. maculatus* among 25 families, including 150 lines of parents CO6 and V2802BG, screened in a no-choice laboratory setting (Table 2). The 150 lines were scored and marked based on the damaging score (Fig 1). Among them, seven families were completely resistant (Table 2). Further biochemical research was conducted using the parents and selected lines listed in Table 3. Preference to bruchid damage during storage was assessed based on the number of eggs (NEL), adult emergence (AE), seed damage per cent (SDP) and weight loss per cent (WP). The findings revealed no significant difference in the total number of eggs deposited by the insect between the lines. However, AE, SDP and WP varied considerably between the lines and the variation in seed weight loss may be due to variation in adult emergence and per cent seed damage levels. The absence of adult emergence from the seeds of

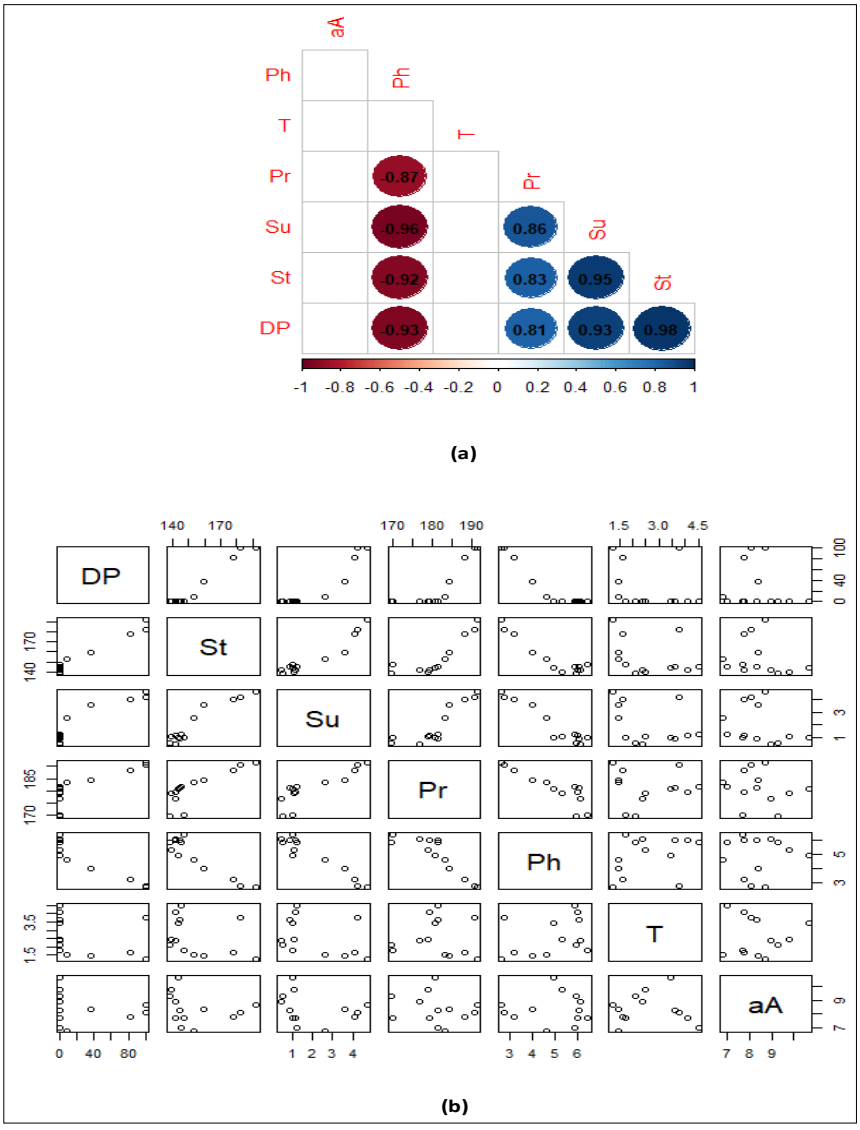


Fig 3: a) Scatterplot matrix for seed damage per cent and biochemical parameters to visualise a rough linear correlation between the parameters.

b) Corrplot between seed damage per cent and other biochemical parameters (sig level = 0.05, confidence interval =0.95). Here, the blank box - no correlation, red circle-negative correlation and blue circle- positive correlation.

*In the figure: aA- alpha-amylase inhibitor, Ph-Phenol, T-Tannin, Pr-Protein, Su-sugar, St- Starch, DP-Seed damage per cent.

V2802BG and progeny lines (BSR-GG-1-49-2-2, BSR-GG-1-49-5-2, BSR-GG-1-56-5-2, BSR-GG-1-170-5-2, BSR-GG-1-198-2-2, BSR-GG-1-198-3-2 and BSR-GG-1-160-1-2) confirmed their resistance to the bruchid infestation.

On the other hand, CO6 and BSR-GG-1-198-4-2 had 100% infestation (Table 4). The MDP also showed a negative trend with the lines that exhibited least damage. The results also agree with Tripathi *et al*. (2020), that the development period was significantly shorter in susceptible genotypes and significantly longer in resistant genotypes of cowpea. Antibiosis resistance may be to blame for extended development period of *C. macuatus* in cowpea genotypes, which results in a decrease in adult emergence.Several researchers used these parameters to identify resistant lines in legumes to bruchids (Majhi and Mogali, 2020).

Biochemical parameters of seeds

The principal biochemical traits, *viz*., starch, sugars, protein, phenol, tannin and alpha-amylase were analysed between the parents and the selected lines to *C. maculatus*.

Nutritional analysis (Starch, sugar and protein)

There is a strong negative linear correlation between starch, sugar and resistance that is significantly different between susceptible and resistant varieties. A higher quantity of starch (159 to 192 mg g^{-1}) and sugar (3.6 to 4.7 mg g^{-1}) were recorded in the susceptible parent and lines compared to

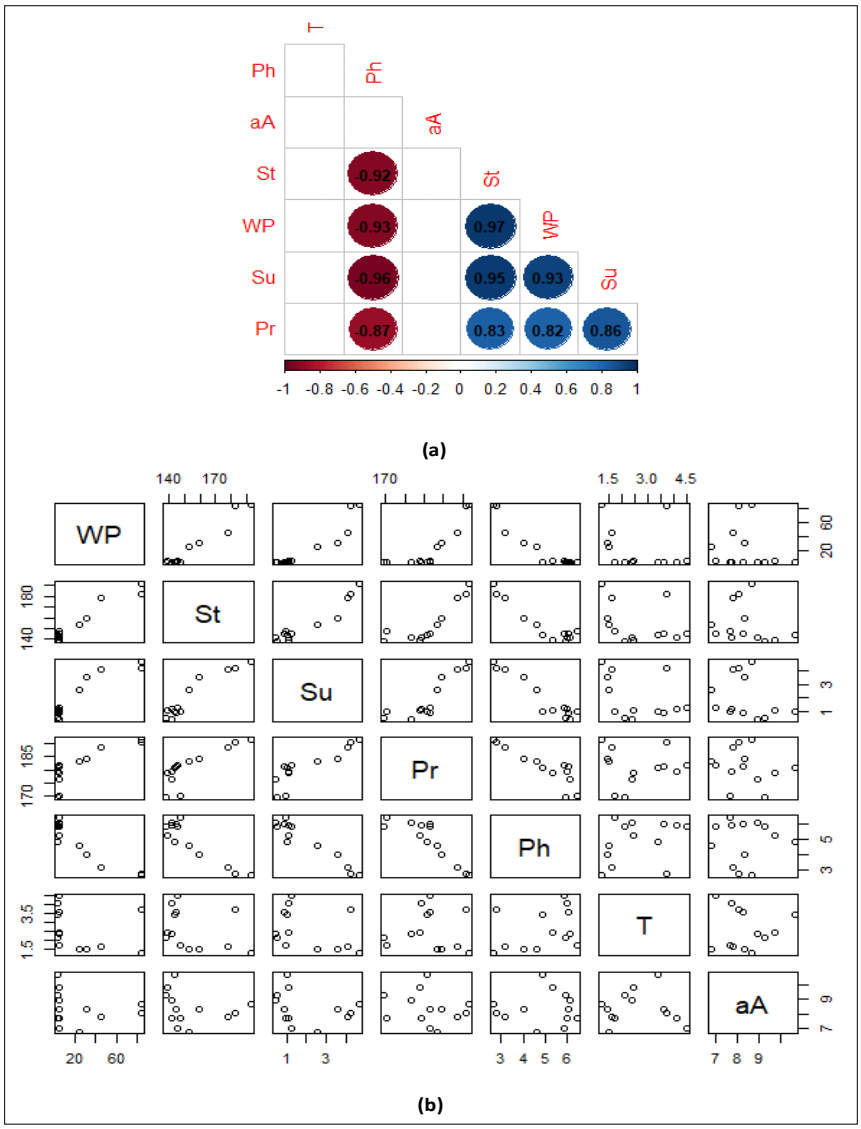


Fig 4: a) Scatterplot matrix for weight loss per cent and biochemical parameters to visualise a rough linear correlation between the parameters.

b) Corrplot between weight loss per cent and biochemical parameters (sig level = 0.05, confidence interval =0.95. Here, the blank box - no correlation, red circle – negative correlation and blue circle- positive correlation).

*aA- -alpha-amylase inhibitor, Ph-Phenol, T-Tannin, Pr-Protein, Su-sugar, St-Starch, WP-Weight loss per cent.

Table 2: Bruchid bioassay with no choice test to select the lines for biochemical analysis.

S. no.	Progeny lines	Seed damage per cent	Category	Score	S. no.	Progeny lines	Seed damage per cent	Category	Score	S. no.	Progeny lines	Seed damage per cent	Category	Score
1	BSR-GG-1-49-1-1	5	R	5	51	BSR-GG-1-56-4-3	10	MS	5	101	BSR-GG-1-198-2-5	0	CR	1
2	BSR-GG-1-49-1-2	5	R	5	52	BSR-GG-1-56-4-4	25	MS	5	102	BSR-GG-1-198-2-6	0	CR	1
3	BSR-GG-1-49-1-3	5	R	5	53	BSR-GG-1-56-4-5	20	MS	5	103	BSR-GG-1-198-3-1	0	CR	1
4	BSR-GG-1-49-1-4	5	R	5	54	BSR-GG-1-56-4-6	10	MS	5	104	BSR-GG-1-198-3-2	0	CR	1
5	BSR-GG-1-49-1-5	5	R	5	55	BSR-GG-1-56-5-1	0	CR	1	105	BSR-GG-1-198-3-3	0	CR	1
6	BSR-GG-1-49-1-6	5	R	5	56	BSR-GG-1-56-5-2	0	CR	1	106	BSR-GG-1-198-3-4	0	CR	1
7	BSR-GG-1-49-2-1	0	CR	1	57	BSR-GG-1-56-5-3	0	CR	1	107	BSR-GG-1-198-3-5	0	CR	1
8	BSR-GG-1-49-2-2	0	CR	1	58	BSR-GG-1-56-5-4	0	CR	1	108	BSR-GG-1-198-3-6	0	CR	1
9	BSR-GG-1-49-2-3	0	CR	1	59	BSR-GG-1-56-5-5	0	CR	1	109	BSR-GG-1-198-4-1	100	CS	7
10	BSR-GG-1-49-2-4	0	CR	1	60	BSR-GG-1-56-5-6	0	CR	1	110	BSR-GG-1-198-4-2	100	CS	7
11	BSR-GG-1-49-2-5	0	CR	1	61	BSR-GG-1-170-1-1	70	HS	7	111	BSR-GG-1-198-4-3	100	CS	7
12	BSR-GG-1-49-2-6	0	CR	1	62	BSR-GG-1-170-1-2	80	HS	7	112	BSR-GG-1-198-4-4	100	CS	7
13	BSR-GG-1-49-3-1	25	MS	5	63	BSR-GG-1-170-1-3	85	HS	7	113	BSR-GG-1-198-4-5	100	CS	7
14	BSR-GG-1-49-3-2	10	MS	5	64	BSR-GG-1-170-1-4	60	HS	7	114	BSR-GG-1-198-4-6	100	CS	7
15	BSR-GG-1-49-3-3	20	MS	5	65	BSR-GG-1-170-1-5	70	HS	7	115	BSR-GG-1-198-5-1	85	HS	7
16	BSR-GG-1-49-3-4	5	R	3	66	BSR-GG-1-170-1-6	1	R	5	116	BSR-GG-1-198-5-2	80	HS	7
17	BSR-GG-1-49-3-5	5	R	3	67	BSR-GG-1-170-2-1	50	HS	7	117	BSR-GG-1-198-5-3	90	HS	7
18	BSR-GG-1-49-3-6	20	MS	5	68	BSR-GG-1-170-2-2	40	MS	5	118	BSR-GG-1-198-5-4	100	CS	9
19	BSR-GG-1-49-4-1	10	MS	5	69	BSR-GG-1-170-2-3	10	MS	5	119	BSR-GG-1-198-5-5	90	HS	7
20	BSR-GG-1-49-4-2	10	MS	5	70	BSR-GG-1-170-2-4	25	MS	5	120	BSR-GG-1-198-5-6	100	CS	9
21	BSR-GG-1-49-4-3	30	MS	5	71	BSR-GG-1-170-2-5	40	MS	5	121	BSR-GG-1-160-1-1	0	CR	1
22	BSR-GG-1-49-4-4	20	MS	5	72	BSR-GG-1-170-2-6	15	MS	5	122	BSR-GG-1-160-1-2	0	CR	1
23	BSR-GG-1-49-4-5	5	R	3	73	BSR-GG-1-170-3-1	20	MS	5	123	BSR-GG-1-160-1-3	0	CR	1
24	BSR-GG-1-49-4-6	5	R	3	74	BSR-GG-1-170-3-2	5	R	3	124	BSR-GG-1-160-1-4	0	CR	1
25	BSR-GG-1-49-5-1	0	CR	1	75	BSR-GG-1-170-3-3	30	MS	5	125	BSR-GG-1-160-1-5	0	CR	1
26	BSR-GG-1-49-5-2	0	CR	1	76	BSR-GG-1-170-3-4	40	MS	5	126	BSR-GG-1-160-1-6	0	CR	1
27	BSR-GG-1-49-5-3	0	CR	1	77	BSR-GG-1-170-3-5	25	MS	5	127	BSR-GG-1-160-2-1	30	MS	5
28	BSR-GG-1-49-5-4	0	CR	1	78	BSR-GG-1-170-3-6	20	MS	5	128	BSR-GG-1-160-2-2	25	MS	5
29	BSR-GG-1-49-5-5	0	CR	1	79	BSR-GG-1-170-4-1	40	MS	5	129	BSR-GG-1-160-2-3	40	MS	5
30	BSR-GG-1-49-5-6	0	CR	1	80	BSR-GG-1-170-4-2	25	MS	5	130	BSR-GG-1-160-2-4	40	MS	5
31	BSR-GG-1-56-1-1	60	HS	7	81	BSR-GG-1-170-4-3	0	CR	1	131	BSR-GG-1-160-2-5	25	MS	5
32	BSR-GG-1-56-1-2	70	HS	7	82	BSR-GG-1-170-4-4	30	MS	5	132	BSR-GG-1-160-2-6	25	MS	5
33	BSR-GG-1-56-1-3	60	HS	7	83	BSR-GG-1-170-4-5	20	MS	5	133	BSR-GG-1-160-3-1	100	CS	9
34	BSR-GG-1-56-1-4	50	HS	7	84	BSR-GG-1-170-4-6	30	MS	5	134	BSR-GG-1-160-3-2	85	HS	7
35	BSR-GG-1-56-1-5	70	HS	7	85	BSR-GG-1-170-5-1	0	CR	1	135	BSR-GG-1-160-3-3	70	HS	7
36	BSR-GG-1-56-1-6	50	HS	7	86	BSR-GG-1-170-5-2	0	CR	1	136	BSR-GG-1-160-3-4	50	HS	7
37	BSR-GG-1-56-2-1	25	MS	5	87	BSR-GG-1-170-5-3	0	CR	1	137	BSR-GG-1-160-3-5	45	MS	5
38	BSR-GG-1-56-2-2	5	R	3	88	BSR-GG-1-170-5-4	0	CR	1	138	BSR-GG-1-160-3-6	50	HS	7
39	BSR-GG-1-56-2-3	0	CR	1	89	BSR-GG-1-170-5-5	0	CR	1	139	BSR-GG-1-160-4-1	10	MS	5
40	BSR-GG-1-56-2-4	20	MS	5	90	BSR-GG-1-170-5-6	0	CR	1	140	BSR-GG-1-160-4-2	20	MS	5
41	BSR-GG-1-56-2-5	5	R	3	91	BSR-GG-1-198-1-1	100	CS	1	141	BSR-GG-1-160-4-3	5	R	3
42	BSR-GG-1-56-2-6	5	R	3	92	BSR-GG-1-198-1-2	100	CS	1	142	BSR-GG-1-160-4-4	5	R	3
43	BSR-GG-1-56-3-1	10	MS	5	93	BSR-GG-1-198-1-3	90	HS	7	143	BSR-GG-1-160-4-5	10	MS	5
44	BSR-GG-1-56-3-2	20	MS	5	94	BSR-GG-1-198-1-4	20	MS	7	144	BSR-GG-1-160-4-6	10	MS	5
45	BSR-GG-1-56-3-3	15	MS	5	95	BSR-GG-1-198-1-5	85	HS	7	145	BSR-GG-1-160-5-1	0	CR	1
46	BSR-GG-1-56-3-4	20	MS	5	96	BSR-GG-1-198-1-6	100	CS	7	146	BSR-GG-1-160-5-2	0	CR	1
47	BSR-GG-1-56-3-5	10	MS	5	97	BSR-GG-1-198-2-1	0	CR	1	147	BSR-GG-1-160-5-3	20	MS	5
48	BSR-GG-1-56-3-6	15	MS	5	98	BSR-GG-1-198-2-2	0	CR	1	148	BSR-GG-1-160-5-4	0	CR	1
49	BSR-GG-1-56-4-1	30	MS	5	99	BSR-GG-1-198-2-3	0	CR	1	149	BSR-GG-1-160-5-5	10	MS	5
50	BSR-GG-1-56-4-2	20	MS	5	100	BSR-GG-1-198-2-4	0	CR	1	150	BSR-GG-1-160-5-6	10	MS	5

the resistant parent and lines (145 to 153 and 0.81 to 2.59 mg g^{-1}, respectively) (Fig 2a and b). Seed damage and weight loss per cent showed a strong positive linear correlation with starch and sugar. Saruchi and Thakur (2014) also reported higher carbohydrate levels in susceptible cowpea and chickpea, while Lazar (2014) found that the lower amount of total soluble sugar in the grain was responsible for mediating resistance to *C. maculatus*. The mean protein content ranged from 190.65 to 169.74 mg g^{-1} in the selected lines. BSR-GG-1-56-5-2 possessed the lowest protein content (169.74 mg g^{-1}), which is slightly higher in resistance parent (181.44 mg g^{-1}) and the susceptible line (BSR-GG-1-198-4-2) had significantly higher protein content than the resistant parent. However, it is lesser than the susceptible parent (191.42 mg g^{-1}) (Fig 2c). BSR-GG-1-56-5-2 also showed the most negligible weight loss (3.62%) (Table 4). Protein content in the selected materials positively correlated with quantitative losses, such as per cent seed damage and weight loss. The correlation coefficient was calculated for seed damage, weight loss and biochemical parameters of the parents and selected lines. The results were statistically significant, except for amylase inhibitors and tannins. Protein, total soluble sugar (TSS) (r = 0.92** and r = 0.93**) and starch contents (r = 0.98** and r = 0.97) were positively correlated with the percentage of seed damage and weight loss (r = 0.81** and r = 0.82**), in the selected lines and parents (Fig 3 and 4). The genotypes with the highest protein per cent are susceptible to bruchid attack compared to the genotypes with the lowest protein content, suggesting that increased protein content of the mungbean genotypes will undoubtedly increase the seed damage. The present result is consistent with that of Chandel and Bhadauria (2015). Positive correlations were found between soluble sugar content and quantitative losses, such as percent seed damage, in mungbean genotypes. The lines that contain high level of sugar is particularly vulnerable to bruchid attack, whilst the lines that contain the least amount of soluble sugar are less vulnerable. Highly significant positive correlation between total sugar, protein content and resistance to *C. chinensis* was also reported by Sekar and Nalini (2017). Although there was a slight deviation in the present study, it is consistent with the results of many previous findings. The results concluded that all nutritional traits analysed have a positive correlation with seed damage per cent and weight loss per cent.

Table 3: List of lines selected for screening and biochemical analysis.

Parents/ derived lines	Damage score	Category
CO6	9	Susceptible parent
V2802BG	1	Resistant parent
BSR-GG-1-49-2-2	1	Completely Resistant line 1
BSR-GG-1-49-5-2	1	Completely Resistant line 2
BSR-GG-1-56-5-2	1	Completely Resistant line 3
BSR-GG-1-170-5-2	1	Completely Resistant line 4
BSR-GG-1-198-2-2	1	Completely Resistant line 5
BSR-GG-1-198-3-2	1	Completely Resistant line 6
BSR-GG-1-160-1-2	1	Completely Resistant line 7
BSR-GG-1-49-1-2	3	Resistant line
BSR-GG-1-170-2-2	5	Moderately susceptible line
BSR-GG-1-198-5-1	7	Highly susceptible line
BSR-GG-1-198-4-2	9	Completely susceptible line

Antinutritional analysis (Phenol, Tannin and alpha-amylase)

Phenols are essential plant secondary metabolites involved in resistance against insect pests and resistance or susceptibility to pulse beetle is attributed to the biochemical content of seeds, such as phenol that are directly toxic to insects and/or act as feeding deterrents. In the present study, phenolic content showed a negative linear correlation (r = - 0.93** and r = -0.93**) (Fig 3 and 4) with seed damage per cent and weight loss per cent respectively in the resistant lines, which were significantly different (6.01 to 5.48 mg g^{-1}) from susceptible lines (2.65 to 4.01 mg g^{-1}) (Fig 2 d). In general, phenolic content significantly protects plants from insect herbivory. Kpoviessi (2021) in his result found a positive correlation between primary metabolite content and the infestation rate and non-protein antimetabolites are essential in conferring resistance to the seeds. The phenolic content of lines BSR-GG-1-49-2-2 and BSR-GG-1-56-5-2 in the present study was significantly higher than that of V2802BG (6.01 mg g^{-1}) and CO6 (2.65 mg g^{-1}). Phenols in legume seeds reduce the penetration of neonate larvae of bruchids. A halt in the larval period was observed in the present study, confirming the fact that a biochemical factor resists further development. In addition, phenol content in seeds prolong the larval development period and reduce the percentage of adult emergence, thereby increasing resistance to storage pests. Also the biochemical constituents such as tannins and amylase inhibitors play an important role in conferring resistance to bruchids in many cases. Kpoviessi *et al*. (2021) reported that the resistant cowpea genotypes exhibited higher levels of tannins. The high concentration of tannins in undamaged cowpea seeds conferred a biochemical defence that deterred, poisoned, or starved bruchid larvae. The alpha-amylase inhibitor is an essential biochemical substance in common beans. It plays a vital role in insect resistance, which can disrupt the digestive enzymes of bruchids and act as a biocontrol agent against bruchids. In contrast to these studies, the study revealed that tannins and alpha-amylase inhibitors (Fig 2e and f) were not significantly different between the susceptible and resistant lines and were not correlated with seed damage per cent and weight loss per cent (Fig 3 and Fig 4). However, Desroches *et al*. *(*1997) observed no significant effect of tannins on bruchid larvae penetration in *Vicia faba* seed, which supports the present study and the biochemical investigation revealed that the only compound significantly and negatively related with seed damage and

Table 4: Effect of *Callosobruchus maculatus* infestation on different mungbean lines.

Parents/ derived lines	NEL	AE	AEP	SDP	MDP	SI	WP
CO6	4.59 (20.00)	4.58 (20)	90 (100)	90 (100)	5.16 (25.33)	1.038 (0.079)	66.67 (84.26)
V2802BG	4.546 (19.67)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	12.38 (4.60)
CR1	4.508 (19.33)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	11.88 (4.24)
CR2	4.469 (19.00)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	12.311 (4.55)
CR3	4.471 (19.00)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	10.96 (3.62)
CR4	4.469 (19.00)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	12.55 (4.73)
CR5	4.509 (19.33)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	13.84 (5.73)
CR6	4.508 (19.33)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	10.97 (3.62)
CR7	4.546 (19.67)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	13.92 (5.79)
R	4.434 (18.67)	1.626 (1.67)	17.22 (8.97)	16.59(8.33)	5.608 (30.45)	1.015 (0.031)	30.19 (25.30)
MS	4.397 (18.33)	2.768 (7.33)	37.10 (39.97)	35.24 (36.67)	5.322 (27.33)	1.028 (0.059)	33.82 (31.01)
S	4.509 (19.33)	4.04 (16.33)	63.30 (84.48)	61.20 (81.67)	5.26 (26.67)	1.035 (0.072)	42.24 (45.21)
CS	4.583 (20.00)	4.583 (20)	90 (100)	90 (100)	5.164 (25.66)	1.038 (0.078)	66.34 (83.82)
SE(m)	N/A	0.041	1.135	0.85	0.021	0.001	0.606
CD	0.062	0.12	3.317	2.475	0.063	0.002	1.771

Original data: In parenthesis; transformed data by $\sqrt{x} + 1$.

*SP: Susceptible parent; RP: Resistant parent; CR1 to CR7: Completely resistant line 1 to 7; R: Resistant line, MS: Moderately susceptible line; HS: Highly susceptible line; CS: Completely susceptible line; *NEL- No. of eggs laid; AE- Adult emergence; AEP- Adult emergence percentage; SDP- Seed damage percentage; MDP- Mean development period; SI- Susceptibility index; WP- Weight loss percentage.

weight loss percent was the antinutrient phenol, which may possibly be involved in imparting resistance to the bruchids combined with the nutritional chemicals.

CONCLUSION

To prevent bruchid damage during storage, genetic resistance is preferable over chemical methods. Therefore, the present study was carried out to identify the resistant sources, confirm their resistance and determine active biochemical compounds in the resistant source. Based on the screening, seven F_5 families (BSR-GG-1-49-2, BSR-GG-1-49-5, BSR-GG-1-56-5, BSR-GG-1-170-5, BSR-GG-1-198-2, BSR-GG-1-198-3, BSR-GG-1-160-1) comprising 42 lines were identified to be adopted in the next generation sowing. These seven lines derived from the cross between CO6 and V2802BG can be deployed as a resistant donor in developing resistant cultivars to bruchids, with further confirmations. The increased phenol content of the resistant parents and lines compared to the susceptible lines may have contributed to the resistance in the seed. Additionally, the resistant lines had lower levels of nutritional characteristics like starch, sugar and protein than the susceptible lines. Breeding lines for insect resistance require thorough screening and investigation to identify factors involved in resistance that could be utilized in the development of lines with specific traits. A further selection of resistant lines could be made by assessing the appropriate traits in the host. Identification of resistant lines in mungbean would be highly economical for the farmers and also minimizes environmental and health hazards by eliminating the need for harsh chemical inputs.

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