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RESEARCH ARTICLE

Screening of Black Gram (*Vigna mungo*) and its Crop Wild Relatives against *Callosobruchus maculatus* (Fab.) and Correlation of Resistance with Seed Physical Parameters

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Black gram [*Vigna mungo* (L.) Hepper] a potential grain legume is greatly affected by bruchid infestation in production and post-harvest storage. Moreover, there is dearth of natural and reliable sources of bruchid resistance in cultivated black gram. Therefore, a set of 69 germplasm accessions, representing landraces of black gram and its crop wild relatives (CWR) were assessed for resistance against *Callosobruchus maculatus* (Fab.). Accession IC259504 (*V. vexillata*) showed complete resistance whereas IC424616 (*V. mungo*) was highly resistant to bruchid infestation based on the three key growth traits viz., adult emergence (AE), per cent seed weight loss (PSWL) and growth index (GI). Correlation heat matrix indicated AE was positively correlated with GI (r= 0.80) and PSWL (r= 0.72). Seed hardness showed a significant negative correlation with AE (r= -0.38). The resistant accessions could be utilized in various breeding programs for the development of bruchid resistant cultivars in black gram and its other related *Vigna* species.

Key Words: Black gram, Bruchid, Crop wild relatives, Landraces, Resistance, Wild *Vigna*

Introduction

Pulses occupy a significant position in Indian agriculture as they form the cheapest source of plant-based proteins (25-40%). They have been rightly called the "poor man's meat" providing an ideal blend of minerals and essential amino acids (Jayasena and Abbas, 2016; Vishwajith *et al*., 2020). Black gram [*Vigna mungo* (L.) Hepper], an Indian origin potential grain legume, is grown and consumed primarily in South East Asian region (Indhu *et al*., 2018). India tops in black gram production being cultivated over an area of 3.81 million ha, thus accounting for more than 70% of global production. In 2018-19, the national production of black gram accounted for 3.36 million tonnes, with Madhya Pradesh being the leading producer state (Anonymous, 2019). But production, storage and nutritional quality of black gram is significantly constrained by post-harvest damage from bruchids especially *Callosobruchus maculatus* (Fab.). Bruchid infestation during storage has been observed to cause around 50% decrease in seed weight and protein in black gram (Gujar and Yadav, 1978). Indiscriminate use of insecticides and other chemical control measures for its management present high risk of persistent toxic residues, resistant strains and environmental pollution (Soumia *et al*., 2017). Identifying natural and sustainable sources of bruchid resistance therefore appears to be a cost-effective and environmentally beneficial approach for reducing storage damage (Tripathi *et al*., 2020). However, bruchid resistance sources are scarce in cultivated black gram (Duraimurugan *et al*., 2014; Tripathy, 2016). Hence, the present experiment was undertaken to evaluate black gram germplasm and its crop wild relatives (CWRs) for resistance against *C. maculatus* under artificial infestation set up following no-choice protocol.

Materials and Methods

Experiments were carried out in the laboratory of Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi. Insect cultures were reared on a local black gram variety and maintained in B.O.D incubator at 28º and 65% RH. A total of 55 landrace accessions of black gram and 10 wild *Vigna* accessions representing its CWRs (Table 1) were screened during 2020-21 against the test insect, *C. maculatus* following "no-choice" artificial infestation

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Table 1. Collection sites of germplasm accessions

S.No.	Accession	State	S.No.	Accession	State
1	IC426766	Andhra Pradesh	34	IC569080	Odisha
2	IC436702	Andhra Pradesh	35	IC557279	Sikkim
3	IC436770	Andhra Pradesh	36	IC557292	Sikkim
4	IC541882	Andhra Pradesh	37	IC557300	Sikkim
5	IC598464	Andhra Pradesh	38	IC401376	Tamil Nadu
6	IC628759	Andhra Pradesh	39	IC436519	Telangana
7	IC628781	Andhra Pradesh	40	IC436644	Telangana
8	IC394168	Assam	41	IC626440	Tripura
9	IC394232	Assam	42	IC626441	Tripura
10	IC394276	Assam	43	IC626446	Tripura
11	IC394301	Assam	44	IC371765	Uttar Pradesh
12	IC394436	Assam	45	IC393526	Uttar Pradesh
13	IC394448	Assam	46	IC393543	Uttar Pradesh
14	IC394479	Assam	47	IC393545	Uttar Pradesh
15	IC394551	Assam	48	IC393550	Uttar Pradesh
16	IC394940	Assam	49	IC393551	Uttar Pradesh
17	IC395030	Assam	50	IC541046	Uttar Pradesh
18	IC545207	Assam	51	IC140825	Uttarakhand
19	IC398744	Bihar	52	IC436951	Uttarakhand
20	IC417875	Bihar	53	IC556551	Uttarakhand
21	IC628811	Jammu & Kashmir	54	IC449258	West Bengal
22	IC392275	Jharkhand	55	IC449265	West Bengal
23	IC424616	Jharkhand	56	IC524639	Andhra Pradesh
24	IC447791	Jharkhand	57	IC553505	Andhra Pradesh
25	IC393540	Karnataka	58	IC553510	Andhra Pradesh
26	IC471981	Kerala	59	IC553520	Andhra Pradesh
27	IC472021	Kerala	60	IC553532	Andhra Pradesh
28	IC472034	Kerala	61	IC553516	Andhra Pradesh
29	IC472054	Kerala	62	IC331454	Chattisgarh
30	IC396791	Madhya Pradesh	63	IC331457	Odisha
31	IC568908	Odisha	64	IC553517	Andhra Pradesh
32	IC568947	Odisha	65	IC259504	Goa
33	IC569057	Odisha			

*Germplasm were obtained from the National Genebank, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India

Table 2. Responses of various accessions to *C. maculatus* infestation in terms of growth parameters

S.No.	Trait	Minimum	Maximum
1	OP	9.67 (IC331454)	56.0 (IC436519)
2	AE (%)	0.00 (IC259504)	89.44 (IC553517)
3	Exit holes (No.)	0.00 (IC259504)	19.33 (IC371765.IC394479)
4	PSWL (%)	0.84 (IC259504)	63.48 (IC541046)
5	GI	0 (IC259504)	22.46 (IC524639)
6	MDP (days)	0 (IC259504)	13.0 (IC140825)

*OP- oviposition (per 20 seeds), AE- adult emergence, PSWL- % seed weight loss, GI- growth index, MDP- mean development period

protocol as described by Giga (1995). Experimental set up also contained four checks viz., KU-6, MASH-114, PU-11-14 and IPU-2-43. Identified resistant and susceptible accessions were validated to confirm their responses to bruchid.

Insect growth parameters: Newly emerged adults were released for oviposition (72 hrs) @ 2 pairs per 20 seeds each accession replicating 5 times. Observations on adult emergence (24 hr interval) were continued till no fresh adults appeared. Various insect growth parameters were calculated as follows:

$$\text{Adult emergence (AE) \%} = \frac{\text{(no. of adults emerged/}}{\text{No. of eggs laid)}} \times 100$$

(Howe, 1971).

$$\text{Mean development period (MDP)} = \frac{D_1A_1 + D_2A_2...+D_nA_n}{}$$

Table 3. Reaction of studied accessions to *C. maculatus* based on GI

Groups	Range	Number of accessions	List of accessions
I	0	1	IC259504
R	0.1-2.0	4	IC424616, IC436519, IC598464, IC626440
MR	2.0-4.0	9	IC140825, IC436644, IC436702, IC541882, IC557300, IC569057, IC569080, KU-6, MASH-114
MS	4.0-6.0	16	IC371765, IC393540, IC394168, IC393545, IC394232, IC394448, IC394940, IC396791, IC449265, IC472054, IC556511, IC557279, IC568908, IC568947, IC626441, IC394551
S	6.0-8.0	12	IC392275, IC393526, IC394276, IC394301, IC395030, IC398744, IC401376, IC426766, IC471981, IC472034, IC557292, IC628781
HS	>8.0	27	IC393543, IC393550, IC393551, IC394436, IC394479, IC417875, IC436770, IC436951, IC447791 IC449258, IC472021, IC541046, IC545207, IC626446, IC628759, IC628811, IC331454, IC524639, IC553505, IC553510, IC553516, IC553517, IC553520, IC553532, PU-11-14, IPU-2-43, IC331457

*I- immune; R- resistant; MR-moderately resistant; MS- moderately susceptible; S- susceptible; HS- highly susceptible

Total no. of adults emerged

where D_1 is the day on which adult emergence commenced (first day), A_1 is the number of adults emerging on D_1th day (Howe, 1971)

$$\text{Growth index (GI)} = \frac{\text{AE}}{\text{MDP}} \text{ (Jackai and Singh, 1988)}$$

$$\text{Percent seed weight loss (PSWL)} = \frac{(N_1 - N_2)}{N_1} \times 100$$

where N_1 is weight of fresh seeds (g) and N_2 is weight of damaged seeds (g) (Eker *et al*., 2018)

Seed parameters: Quantitative and qualitative seed traits were also studied to comprehend the physical basis of resistance in the accessions. Observations on qualitative parameters of seed viz., seed coat colour, texture, lustre, seed shape and hilum concavity were recorded as per descriptor list of IBPGR (1985). The quantitative physical traits included 100-seed weight, seed length and width, length-width ratio and seed hardness. Hundred seed weight (in grams) was determined by weighing 100 seeds of uniform size in an analytical balance. Seed length and width were measured in millimetre using Vernier calliper. Texture analyzer was employed to measure the seed hardness and expressed in Newton.

Statistical Analysis: The strength of the relationship and extent of correlation between physical seed parameters, and specific growth parameters of bruchid was analysed by simple linear correlation estimation with the help of PAST-3 (Paleontological Statistics) software (Hammer *et al*., 2001).

Results and Discussion

Insect growth parameters: Studies showed that accessions had significant differences in their response to *C. maculatus* in terms of growth traits viz., total oviposition, number of exit holes, AE%, GI, PSWL and MDP (Table 2). Accession IC259504 (*V. vexillata*) exhibited complete resistance and IC424616 (landrace from Jharkhand) showed high resistance to bruchid infestation in terms of three vital life parameters viz., AE, PSWL and GI. Based on GI, the accessions were categorized as immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible (Table 3). Two checks (KU-6 and MASH-114) were observed to be moderately resistant whereas, other two checks (PU-11-14 and IPU-2-43) showed high degree of susceptibility to *C. maculatus*.

Physical seed parameters: Considerable variability was observed for quantitative seed traits (Table 4). The studied accessions exhibited wide variation in their qualitative seed traits (Figure 1). Majority of the accessions were being smooth textured (88.23%), black coloured (55.88%) coupled with dull appearance (86.76%), ovoid shaped (52.94%) with non-concave hilum (52.94%).

Table 4. Variable response of studied accessions to *C. maculatus* infestation in terms of quantitative seed parameters

S.No.	Trait	Minimum	Maximum
1	SH (N)	11.64 (IC553510)	77.37 (IC472021)
2	HSW (g)	0.76 (IC331454)	5.91 (IC628781)
3	SL (mm)	2.33 (IC331454)	5.98 (IC436770)
4	SW (mm)	1.63 (IC553532)	4.47 (IC628759)
5	LWR	1.13 (IC436519)	1.74 (IC436770)

* SH- seed hardness, HSW-100-seed weight, SL- seed length, SW- seed width, LWR- length-width ratio

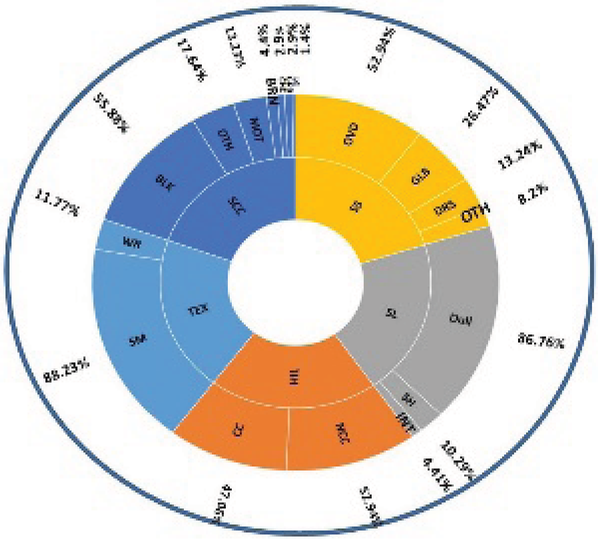

Fig. 1. Frequency distribution of qualitative seed traits
*SS seed shape (OVD ovoid, GLB globose, DRS drum shaped, OTH others); SL seed lustre (Dull dull, SH smooth, INT intermediate); HIL hilum (NC non concave, CC concave); TEX seed texture (SM smooth, WR wrinkled); SCC seed coat colour (BLK black, MOT mottled, BRN brown, CHC chocolate, GRB greenish brown, LG light green, OTH others)

Correlation studies: Correlation heat matrix (Figure 2) of growth and quantitative seed traits of various accessions revealed a significant positive correlation of AE with GI (r= 0.80) and significant negative correlation with MDP (r= -0.54) and seed hardness (r = -0.38). This was in conformity with earlier reports (Soumia *et al*., 2017; Amusa *et al*., 2018) where chemical deterrent inside the seed and physical barriers on seed coat obstructed adult emergence and prolonged the developmental period. Moreover, PSWL was significantly positively correlated with AE (r=0.83) and number of exit holes (r=0.71) which was congruent with earlier findings of Dasbak *et al*. (2009) and Tripathi *et al*. (2020). This could be attributed to the fact that larva of the bruchid is the sole damaging stage on account of its feeding on starchy seed contents. Significant positive correlation of total oviposition with 100-seed weight (r=0.51) indicated bruchid's preference to lay eggs on seeds with more storage reserves. Further, in the present study number of eggs laid by bruchid did not exhibit strong and uniform correlation with any of the studied qualitative seed parameters of the accessions. Similar findings were also reported by Pankaj *et al*. (2011).

Conclusion

In the present study, it was found that the black gram and its CWR accessions exhibited considerable variability in their physical seed attributes. Besides, the growth parameters of the bruchid, *C. maculatus* varied significantly among the accessions. Resistant accessions identified in the study namely, IC259504 and IC424616 displayed immunity and high degree of resistance respectively in terms of three vital growth parameters (AE, PSWL and GI). Hence, these validated accessions could be further utilized in breeding programmers to introgress bruchid-resistant genes into agronomically improved black gram cultivars which could increase the production manifold and prolong shelf life.

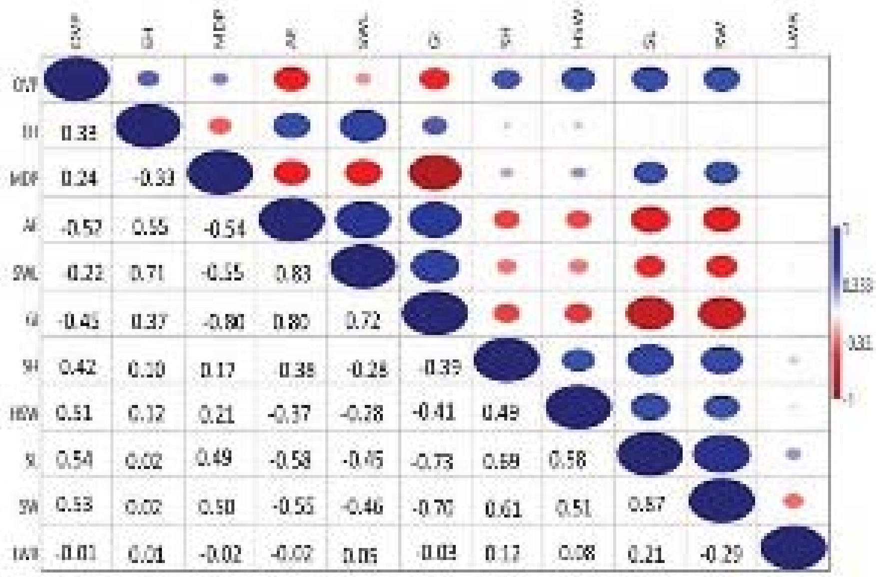

Fig. 2. Correlation heat map of insect parameters and physical traits of seeds
*Wtl % seed weight loss; Ade % adult emergence; GI growth index; EMH emergence holes; MDP mean development period; SH seed hardness; HS 100-seed weight; LWR length-width ratio. Significant correlations are coloured either in blue (positive) or red (negative).

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Conflict of Interest

The authors declared no conflict of interest in the content of the manuscript and study undertaken.

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