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Identification of tolerant genotypes against pulse beetle as a source to reduce post harvest losses in pigeonpea [*Cajanus cajan* (L.) Millisp.]

Satheesh Naik S.J., Amrit Lamichaney, Abhishek Bohra,
R.K. Mishra, Farindra Singh, Dibendu Datta, I.P. Singh, N.P. Singh

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ABSTRACT

The present study intends to screen 52 pigeonpea genotypes for bruchid infestation under controlled conditions using no-choice assay. The results revealed significant differences among the genotypes considering parameters like growth index (GI), egg numbers and adult emergence. The genotypes *viz.,* ICP 89049, IPA 37 and Dholi dwarf DB had low average values for GI (0.45, 0.48 and 0.48 respectively), number of eggs after 20 days (14.5, 21.5 and 28), and adults emerged after 30 days of oviposition (9.0, 7.0 and 4.5, respectively) when compared to the genotypes Asha (higher GI: 1.10) and IPA 7-6 (having 98.0 eggs after 20 days of incubation). Concerning associations among different traits, the GI had significant positive correlations with number of eggs laid (0.484), and number of adults emerged at 15-30 days (0.638). The GI showed a negative relationship with proportion of seed coat (-0.162) and seed hardness (-0.197). The traits that are less preferred by the bruchids include hard seed with less seed diameter and high proportion of seed coat. The tolerant genotypes *viz*. ICP 89049, IPA 37 and Dholi dwarf DB identified here could be deployed in breeding programs for reducing post harvest losses in pigeonpea.

Key words: Bruchids, Growth index, Oviposition, Pigeonpea.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp] is an important multi-purpose grain legume crop of rainfed agriculture. Globally, it is cultivated in about 82 countries in an area of 5.40 m ha with a production of 4.49 mt and an average productivity of 829 kg ha^{-1} (FAOSTAT, 2016). In India, pigeonpea is cultivated on 4.65 m ha area with an average productivity of 800 kg ha^{-1} (http://agricoop.gov.in/sites/default/files/3rd_advance_eng_2018). A range of biological and physical factors influence seed quality in pigeonpea. A survey on postharvest loss in pigeonpea revealed that the storage alone contributes 10-15% of total loss (Grolleaud, 2002). The losses can be broadly categorized as losses in mass, quality and nutritive value and hygienic deterioration. Bruchids are the most important storage pests among the various biological agents that cause storage loss in pigeonpea. These include three major species *viz., Collosobruclus chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.) of which *C. maculatus* remains the important in pigeonpea at Indian conditions (Prabhakar, 1979).

Bruchids begin their infestation when the pods are in the ripening stage at the field and are subsequently carried with the grain to the stores after harvesting, considerably deteriorating both grain quality and quantity (Howe and Currie, 1964). However, Dasbak *et al.* (2009b) demonstrated that postharvest storage losses due to bruchids in pigeonpea should not be attributed to field-to-store infestation rather to re–infestations or cross-infestations in the store itself. The post harvest losses due to bruchids in various pulses have been reported to vary between 30-40% within a period of six months (Akinkurolere *et al.* 2006; Soumia *et al.* 2017).

ICAR-Indian Indian Institute of Pulses Research, Kanpur-208 024, Uttar Pradesh, India.

Corresponding Author: Satheesh Naik S.J., ICAR-Indian Indian Institute of Pulses Research, Kanpur-208 024, Uttar Pradesh, India. Email: satheeshnaikagri@gmail.com

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Proper storage of agricultural harvest is key to minimize post harvest losses and sustainable development goals (DAC, National Agriculture Policy Report 2000). Although, chemical control remains the effective means of controlling bruchids, the use of resistant sources is a reliable, eco-friendly and sustainable approach (Sarwar *et al.* 2006). Developing pigeonpea varieties that could combine both grain and pod resistance helps achieving enhanced and durable level of resistance to bruchid attack.

Efforts have been made to identify traits that contribute to bruchid resistance in pigeonpea, such as reduction in insect oviposition and adult insect emergence due to the physical and chemical characteristics of the plant host (Fitzner *et al.* 1985; Bamaiyi *et al.* 2000), however information on seed traits conferring bruchid resistance in pigeonpea remains limited. Majority of studies have concentrated more

on field than storage. The present study aimed to identify important measurable seed trait (s) that could allow selection and breeding of pigeonpea genotypes with enhanced tolerance to bruchids. Exploitation of such traits in breeding programs would help in minimizing post harvest losses.

MATERIALS AND METHODS

Screening of pigeonpea genotypes against *C. maculatus*

Fifty two pigeonpea genotypes were assayed at ICAR-Indian Institute of Pulses Research, Kanpur during 2016-2017. The seeds were free from any other pest infestation.

Insect culture

Pure culture of *C. maculatus* was maintained by sub-culturing at regular interval so as to ensure continuous supply of insects for the experiment. The adults were sexed using key characters (Arora, 1977) and 20 pairs of freshly emerged adults were allowed to oviposition on healthy seeds for 24 hours to get uniform age insects for further studies. The culture was maintained at 27 ± 1°C and 65 ± 5% relative humidity in the laboratory cupboard.

Bioassay for bruchid resistance

No Choice test

Fifty two pigeonpea genotypes were screened by no choice test for their comparative resistance to *C. maculatus* under laboratory conditions in a complete randomized design with two replications. Twenty seeds of each entry were kept in a culture bottles separately with 1 cm diameter holes on lid. Five pairs of freshly emerged *C. maculatus* adults were released into a culture bottles and allowed for oviposition (Duraimurugan *et al.* 2014). Three days later the released adult were removed and the seeds with eggs were kept under laboratory conditions (Gibson and Raina, 1972). The number of eggs per 20 seeds, number of adult bruchids emerged after 30 days, relative egg laying preference and relative adult emergence were recorded (Howe, 1971).

Quantitative seed parameters

Seed hardness was determined by compression test using a texture analyser (TA + Di, Stable Micro Systems, UK). Pressure was exerted on the individual grain until it cracked and the cracking point was recorded and expressed in Newton (Mohsenin, 1980). The basic dimensions of the seed (length, width and thickness) were measured using a digital vernier caliper with an accuracy of 0.001mm. Diameter of seeds was measured by following the equation of Shkelqim *et al.* (2010).

$$Dg= (L\times W\times T)^{1/3}, \text{ Diameter } (mm^2) = \pi Dg^2$$

Where,

Dg is geometric mean diameter (mm), L is length, (mm), W is Width (mm) and T is thickness (mm).

Hundred seed weight was obtained by weighing 100 uniformly sized seeds and expressed in grams. Proportion of seed coat to cotyledon was estimated by separating seed coat and cotyledons form 10 seeds, dried in oven at 80°C for 24 hours and expressed in per cent.

Qualitative seed parameters

Seed coat colour and coat colour patterns were recorded in four categories *viz*., brown, grey, cream and dark brown for colour and uniform, mottled, mottled–red spot at hilum and uniform-red spot at hilum for colour patterns. Likewise, seed shape was recorded in three categories *viz.,* oval, elongate and globular after 50 days of harvest using DUS guideline descriptors (DUS Guidelines for Pigeonpea, 2007).

Statistical analysis

Data on quantitative traits were statistically analyzed using SAS Version 9.2 and PAST Version 3.18. One way analysis of variance (ANOVA) was carried out to assess difference in susceptibility in which means were compared based on the least significant difference (LSD) at P=0.05. A one-tailed Pearson's correlation coefficient analysis was performed to indicate the relationship between GI and other seed and insect growth parameters. Multivariate linear regression was performed by considering GI as dependent variable and other seed and insect traits as independent variables to assess the outcome of a response variable and model the relationship between the explanatory and response variables. The correlation coefficient matrix was subjected to principal component analysis. The two components were plotted in biplot mode in various combinations. Only the biplots of the first two most informative components were presented.

RESULTS AND DISCUSSION

Pigeonpea grains in storage suffer losses due to bruchid infestation. Therefore, farmers typically sell it as soon as possible after harvest. Storing the grain and selling it at a time when the prices have risen due to scarcity of dal in the market could provide an economic incentive for the farmers to store; however, their need for cash after harvest time, the lack of bruchid tolerant varieties, paucity of effective storage systems deter famers to sell pigeonpea at lesser prices during glut in the market. Because of the wide range of maturities, pigeonpea seed needs to be stored for variable periods of time (up to nine months). Bruchids, major storage pests of pigeonpea, causes substantial losses (Grolleaud, 2002). Keeping this in view the present study was conducted to identify possible tolerant donors for use in pigeonpea breeding programme.

Screening of pigeonpea genotypes against *C. maculatus*

None of the genotypes were immune to *C. maculatus*. However, laboratory screening in terms of GI revealed significant variations in their reaction to *C. Maculatus*. GI ranging 1.10 to 0.45 with an average of 0.85 (Table 1). On the basis of GI, genotypes were grouped into four categories *viz.* (1) resistant (≤ 0.50), (2) moderately resistant (0.51-0.70), (3) moderately susceptible (0.71-0.90) and (4) susceptible (≥ 0.91) (Table 2).

Table 1: Statistical summary of quantitative characteristics for pigeonpea (N= 52) and bruchids.

Traits	Range	Mean± SE	σ^2	SD	CV (%)	Mean for reaction categories			
						R	MR	MS	S
GI	1.1- 0.45	0.85±0.02	0.02	0.13	15.76	-	-	-	-
NE	98-14.5	62.43±2.79	404.21	20.10	32.20	-	-	-	-
NA@14D	9.5-0	4.77±0.38	7.57	2.75	57.70	-	-	-	-
NA@15-30D	21-4.5	12.19±0.49	12.45	3.53	28.94	-	-	-	-
SSA	19.27-12.93	15.61±0.19	1.87	1.37	8.75	14.98	15.58	15.62	16.03
SH	279.66-153.37	206.71±4.29	959.21	30.97	14.98	221.01	225.02	205.17	193.52
PSC	16.67-10.31	13.45±0.22	2.62	1.62	12.03	14.56	13.32	13.45	12.69
100SW	24.72-8.51	12.10±0.43	9.77	3.13	25.84	10.88	11.02	12.28	12.22

GI= Growth Index, NE= No. of eggs, NA@14D= No. of adults at 14 days, NA@15-30D= No. of adults at 15-30 days, SSA= Seed diameter (mm), SH= Seed hardness (N), PSC= Proportion of seed coat (%), 100SW= 100 Seed weight (g).
N= Sample size, σ^2= Variance, SE= Standard error, SD=Standard deviation, CV= Coefficient of variation, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible.

Table 2: Reaction of pigeonpea genotypes to *C. maculatus*.

Category	Growth index scale	Number of genotypes	Names of the genotypes
Resistant	≤ 0.50	3	ICP 89049, IPA 37, Dholi dwarf DB.
Moderately resistant	0.51-0.70	4	MA 3B mutant, Banda phalera, IPA 242, MAL 13 bold.
Moderately susceptible	0.71-0.90	41	IPA 7-4, Dholi dwarf, IPA 6-1, PI 397430, KPBR 80-2-1, PUSA 9, IPA 7-8, BDN 2, Maruti, MA 3A, BDN 1, PUSA 992, BSMR 736, IPA 7-7, Bahar, Type 7, MA 3C Mutant, ICPL 67B, UPAS 120, IPA 204, IPAV 16-1C, IPA 7-5, IPAPB-7-2-1-7, MA 3, DA 11, IPA 409, Ranchi local bold, MA 6, IPAPB 7-2-1, MAL 13, KPL 44, NDA 1, Amar, KPL 43, IPA 403, IPA 92, IPAV 16-1, Azad, IPA 61, IPA 406, IPA 234.
Susceptible	≥ 0.91	4	IPA 7-6, NDA 2, IPA 308, Asha.

Table 3: Pearson's linear correlation coefficient.

Traits	GI	NE	NA@14D	NA@15-30D	SSA	SH	PSC	100SW
GI	-	0.484*	0.159	0.638**	0.112	- 0.197	-0.162	0.128
NE		-	0.007	0.361*	0.292*	0.143	-0.355*	0.277*
NA@14D			-	-0.659**	-0.042	- 0.090	0.050	0.048
NA@15– 30D				-	0.117	- 0.080	-0.163	0.059
SSA					-	0.397*	-0.497*	0.854**
SH						-	-0.316*	0.356*
PSC							-	- 0.426*
100SW								-

*Correlation significant at p=0.05; **significant at p=0.01.
GI= Growth Index, NE= No. of eggs, NA@14D= No. of adults at 14 days, NA@15-30D= No. of adults at 15-30 days, SSA= Seed Diameter (mm), SH= Seed hardness (N), PSC= Proportion of seed coat (%), 100SW= 100 Seed weight (g).

Table 4: Variation explained by Principal Components Analysis.

PC axis	Eigen value	Total variation explained		Important characters with maximum weightage
		% variance	Cumulative	
1	2.8076	35.0950	35.0950	SSA, 100SW, PSC
2	1.9073	23.8410	58.9360	NA@15– 30D, NA@14D, GI, SH, NE
3	1.3485	16.8570	75.7930	NA@14D, GI, NE, SH

GI= Growth Index, NE= No. of eggs, NA@14D= No. of adults at 14 days, NA@15-30D= No. of adults at 15-30 days, SSA= Seed Diameter (mm), SH= Seed hardness (N), PSC= Proportion of seed coat (%), 100SW= 100 Seed weight (g).

Out of 52 genotypes, three genotypes *viz.,* ICP 89049, IPA 37 and Dholi dwarf DB were found to be resistant, with GI less than 0.50. Four genotypes with GI between 0.51 and 0.70 were classified as moderately resistant, while 41 were found to be moderately susceptible. Remaining four genotypes having GI above 0.91 were susceptible (Table 2). Earlier, Chandel and Bhadauria (2015) reported substantial variation in terms of degree of damage by *C. maculatus* in 12 pigeonpea varieties. Dasbak *et al.* (2009a) reported varied influence of pigeonpea genotypes on bruchids infestation and identified ICPL 87 and ICPL 161 to be less susceptible to bruchids.

Physical seed parameters of pigeonpea genotypes

Seed hardness, seed diameter, proportion of seed coat, 100-seed weight and seed colour revealed significant variability among 52 genotypes (Table 1). Seed hardness among 52 genotypes ranged from 279.66 (IPA 7-5) to 153.37 (BDN 1) Newton with an average of 206.71 Newton. Average hardness of resistant and moderately resistant genotypes to *C. maculatus vis-a-vis* moderately susceptible and susceptible genotypes (categorized on the basis of GI) was observed to 221.01, 225.02, 205.16 and 193.52 Newton, respectively. Seed diameter (mm) of different genotypes ranged from 19.27 (Ranchi local) to 12.93 (Maruti) with an average of 15.61 mm. Average seed diameters of resistant, moderately resistant, moderately susceptible and susceptible genotypes were 14.98, 15.58, 15.62 and 16.03 mm, respectively (Table 1). The proportion of seed coat ranged from 16.67 (ICP 89049) to 10.31% (Bahar) with mean of 13.45%. Average seed coat proportion of resistant, moderately resistant, moderately susceptible and susceptible genotypes was 14.56, 13.32, 13.45 and 12.69%, respectively (Table 1). Hundred-seed weight ranged from 24.72 (IPAV 16-1) to 8.51 g (ICPL 67B) with an average of 12.10 g. Average values for 100-seed weight of resistant, moderately resistant, moderately susceptible and susceptible genotypes were observed to be 10.88, 11.02, 12.28 and 12.22 g, respectively (Table 1). Anamika and Jayalaxmi (2016) reported that the rate of increase in insect population is adversely affected by seed traits of resistant variety by hampering oviposition. Hard seed coat and more proportion of seed coat deter access of bruchids into the grains and thereby make it unsuitable for oviposition.

Resistance of C. maculatus vis-a-vis seed parameters

Seed hardness (-0.197) and proportion of seed coat (-0.162) had negative relation with GI of *C. Maculatus* (Table 3). Seed coat acts as a physical defensive trait of seed that protects the embryo from external calamities like diseases, pests and insects. Dasbak *et al.* (2009a) proposed testa thickness as a direct measure for estimation of proportion of seed coat content, and a comparison of it with susceptibility index or number of eggs laid suggested that an increase in testa thickness results into increased tolerance to bruchid. This inverse relationship could be attributed to the difficulty in larval penetration owing to the presence of thick grain coat barriers. The 100-seed weight had a significant positive relation with number of eggs laid (0.277) and GI (0.128) of *C. maculatus*, respectively (Table 3), that suggested preference of bruchids on large-sized seeds for feeding and development. Lamichaney *et al.* (2017) reported that large seeds have low proportion of seed coat. Therefore, preference of large sized seeds by bruchid may be because of low content of seed coat as they showed significant

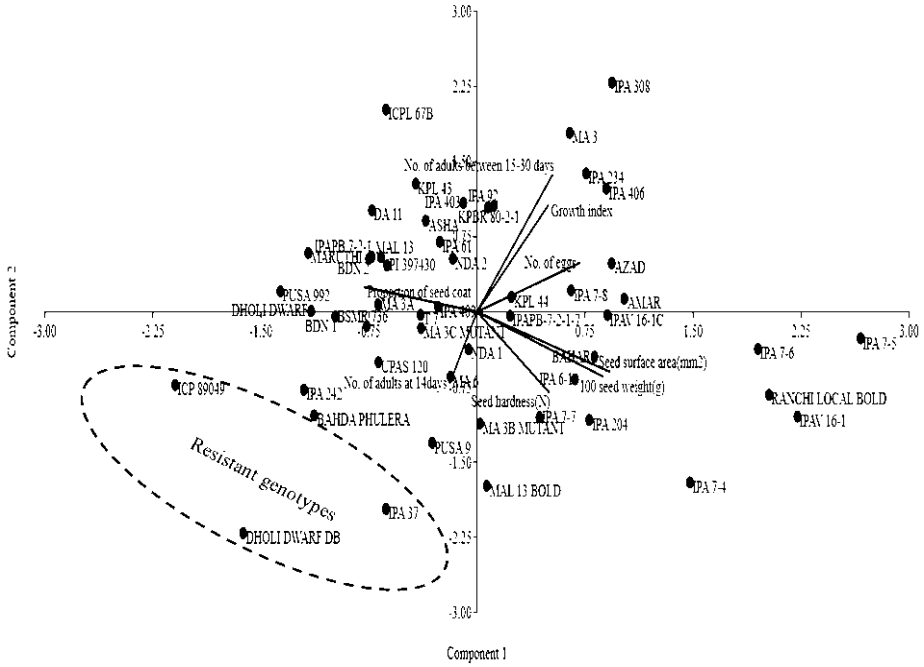

Fig 1: Scatter plot displaying the distribution of genotypes based on its reaction to bruchid infestation. ICP 89049, IPA 37 and Dholi dwarf DB marked in circle are tolerant types.

negative relationship (-0.426). In other crops like rice, grain size was positively correlated with mean GI of *S. oryzae* (Tripathi *et al.* 2017). Soumia *et al.* (2017) also established a positive correlation in greengram between susceptibility and grain size. Seed coat colour varied from brown to grey to dark brown regardless of their (resistant or susceptible) response to bruchids. Similarly, pattern of seed coat colour (uniform and mottled), and seed shape (elongate and globular) did not show any clear-cut relationship with GI (data not presented). It has been evident that grain hardness serves as a significant barrier for penetration by storage pests such as *Sitophilus zeamais* in maize (Zakka *et al.* 2013) and *Sitophilus oryzae* (L.) in rice (Tripathi *et al.* 2017).

Principal component analysis (PCA)

The PCA conducted on 52 pigeonpea genotypes using seed and bruchid quantitative characters revealed that the first three components (PCs) accounted for about 35, 24 and 17% of variances, with 75.79% as cumulative variance (Table 4). The scatter plot of ordinance using two most informative principals is illustrated in Fig 1. The genotypes ICP 89049, IPA 37 and Dholi dwarf DB formed a distinct cluster of resistant types.

CONCLUSION

Considerable variations for resistance/susceptibility to *C. maculatus* were observed through this study. No accession could show immune reaction to infestation, however, three genotypes (IPA 37, ICP 89049 and Dholi dwarf DB) were found to be the less preferred by *C. maculatus* and same can be utilized in pigeonpea breeding program. A smaller seeds with higher seed coat content seems to be the ideal traits for reducing the damage of bruchids during storage.

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