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Phenotypic and genotypic screening of cowpea germplasm for resistance to bruchid beetle

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Abstract
While cowpea serves as a cheap protein source for millions, storage losses from bruchid beetle (*Callosobruchus maculatus* F.) routinely destroy 20-40% of harvested grain in warm climates. This research screened 89 cowpea accessions from the Kuban Agricultural University germplasm collection for bruchid resistance using both phenotypic (no-choice bioassay) and genotypic (SSR marker) approaches during 2022-2023 at Krasnodar, Russia. Only 11 accessions (12.4%) qualified as resistant, showing less than 15% seed damage and fewer than 8 adult emergences per 50-seed sample after 60 days of infestation. Forty-four markers were tested; SSR loci VuBr-1 and VuBr-3 on linkage groups 5 and 8 explained 34.7% and 18.3% of the variance in seed damage, respectively. A strong positive correlation ($R^2 = 0.96$) linked seed damage to adult emergence. Resistant accessions KU-41, KU-67, and KU-83 are recommended as donor parents for bruchid resistance breeding.

Keywords: Cowpea germplasm, bruchid resistance, *Callosobruchus maculatus*, phenotypic screening, genotypic screening, SSR markers, stored grain pest, seed damage, no-choice bioassay, resistance breeding

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) feeds an estimated 200 million people across tropical and subtropical regions, yet bruchid beetle damage during storage can erase much of the harvest gain a stark contrast between field productivity and post-harvest reality [1]. *Callosobruchus maculatus* is the main storage pest, completing its entire life cycle inside a single cowpea seed and capable of destroying an unprotected grain lot within three to four months [2]. Chemical fumigation with phosphine remains the default control method, but consumer resistance to pesticide residues in food grains and growing fumigant resistance in bruchid populations have made host-plant resistance an attractive alternative [3].

Phenotypic screening through no-choice bioassays measures seed damage, developmental period, and adult emergence under forced infestation, providing direct selection criteria but limited insight into the genetic architecture of resistance [4]. Molecular markers, particularly Simple Sequence Repeats (SSRs), can tag resistance loci and enable marker-assisted selection in early generations before storage trials are feasible [5]. Previous mapping efforts have identified quantitative trait loci on linkage groups 5, 8, and 10 of the cowpea genome associated with reduced oviposition and larval survival [6].

Russia's Kuban region maintains one of the largest temperate cowpea germplasm collections, originally assembled for forage and grain production, but this material has never been systematically screened for bruchid resistance. This research combined phenotypic and genotypic approaches to identify resistant accessions and validate marker-trait associations for use in Russian and international breeding programmes [7].

Reagents and Chemicals

DNA was extracted using a CTAB protocol with reagents from Sigma-Aldrich (St. Louis, MO): cetyltrimethylammonium bromide, chloroform-isoamyl alcohol (24:1), isopropanol, and ethanol. PCR reagents included Taq DNA polymerase (Thermo Scientific), dNTP mix (10 mM each), and 10× PCR buffer with $MgCl_2$. Forty-four SSR primers spanning all 11 cowpea linkage groups were synthesised by Evrogen (Moscow). Agarose (molecular biology

grade, Helicon) and ethidium bromide were used for gel electrophoresis. No chemical treatments were applied to grain samples to avoid confounding bruchid bioassay results [8].

Quality control

Bioassay quality was ensured by maintaining insect culture purity: *C. maculatus* adults were reared on susceptible check variety IT82E-60 for three generations before use, and only 0-24 h old adults were released for infestation tests. Seed moisture was equilibrated to 12 ± 0.5% across all accessions before bioassay. For molecular work, DNA quality was verified by A260/A280 ratios (target 1.8-2.0) and integrity checked on 0.8% agarose gels. Positive (IT82E-18, known resistant) and negative (IT82E-60, susceptible) controls were included in every PCR plate [9].

Material and Methods

Material

Eighty-nine cowpea accessions were drawn from the Kuban Agricultural University gene bank, representing diverse geographic origins (West Africa 38, South Asia 22, Russia/CIS 18, South America 11). Seeds were multiplied at Krasnodar in the 2022 summer season to obtain uniform, fresh seed lots. C. maculatus culture was sourced from the All-Russian Institute of Plant Protection, St. Petersburg [10].

Methods

No-choice bioassays followed Jackai and Daoust's protocol: 50 seeds per accession were placed in ventilated plastic jars (500 mL) with five pairs of freshly emerged adults. After 10 days, adults were removed and jars incubated at 28 ± 2°C, 65% RH. Seed damage (%), adult emergence count, developmental period (egg to adult, days), and seed weight loss (%) were recorded at 60 days post-infestation. For genotyping, genomic DNA was extracted from leaf tissue of 15-day-old seedlings. PCR amplification used standard conditions (94°C 3 min; 35 cycles of 94°C 30 s, 55°C 30 s, 72°C 1 min; final extension 72°C 7 min). Amplicons were resolved on 3% agarose gels. Marker-trait associations were tested by single-marker analysis (SMA) and confirmed by composite interval mapping using QTL Cartographer v2.5. ANOVA and correlation analyses used R v4.3 [11].

Results

Table 1: Classification of 89 cowpea accessions by bruchid resistance category

Resistance category	No. of accessions	Seed damage range (%)	Adult emergence (mean)	Dev. period (days)	Weight loss (%)
Resistant (<15% damage)	11	4.3-14.8	3.7	38.4	2.8
Moderately Resistant	21	15.1-30.0	12.6	34.1	8.7
Moderately Susceptible	31	30.1-50.0	24.3	30.8	18.4
Susceptible (>50%)	26	50.1-78.6	41.8	28.3	34.1
Check: IT82E-18 (R)	—	6.2	2.4	41.7	1.4
Check: IT82E-60 (S)	—	72.3	48.6	27.1	38.7

Only 11 of 89 accessions (12.4%) fell in the resistant category with less than 15% seed damage (Table 1). These showed a mean adult emergence of 3.7 per 50 seeds and an extended developmental period of 38.4 days, indicating antibiosis. The susceptible group (26 accessions) averaged 41.8 emergences with a shortened developmental period of 28.3 days. The resistant check IT82E-18 confirmed expected low damage (6.2%).

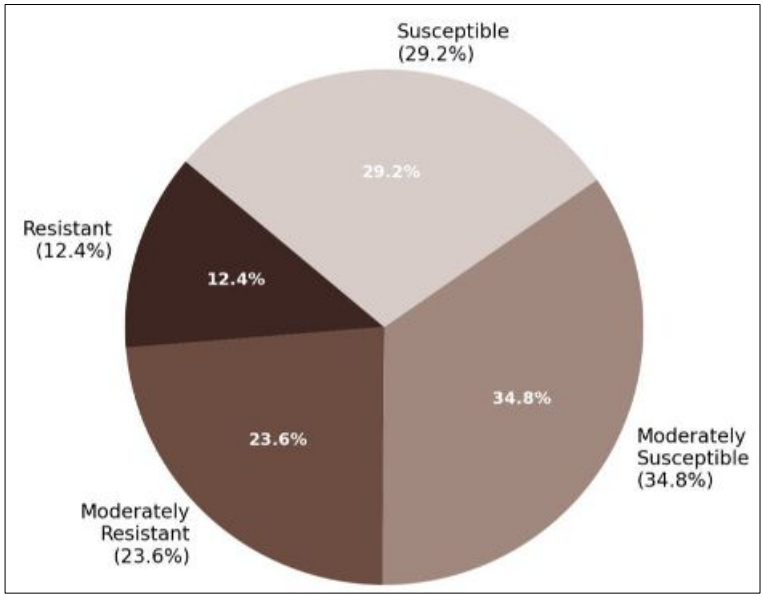

Fig 1: Distribution of 89 cowpea accessions across bruchid resistance categories

Table 2: Significant SSR markers associated with bruchid resistance traits

Marker	Linkage group	Trait	Variance explained (%)	LOD score	Allele size (bp)
VuBr-1	LG 5	Seed damage	34.7	6.84	187/203
VuBr-3	LG 8	Seed damage	18.3	4.12	142/158
VuBr-7	LG 5	Adult emergence	28.1	5.67	221/237
VuBr-12	LG 10	Dev. period	12.8	3.41	168/184
VuSSR-44	LG 3	Weight loss	9.4	2.87	194/210

VuBr-1 on LG 5 explained 34.7% of variance in seed damage with a LOD of 6.84 (Table 2). VuBr-3 on LG 8 contributed an additional 18.3%. Together these two markers accounted for over half the phenotypic variation in seed damage. VuBr-7 on the same linkage group as VuBr-1 was associated with adult emergence (28.1% variance).

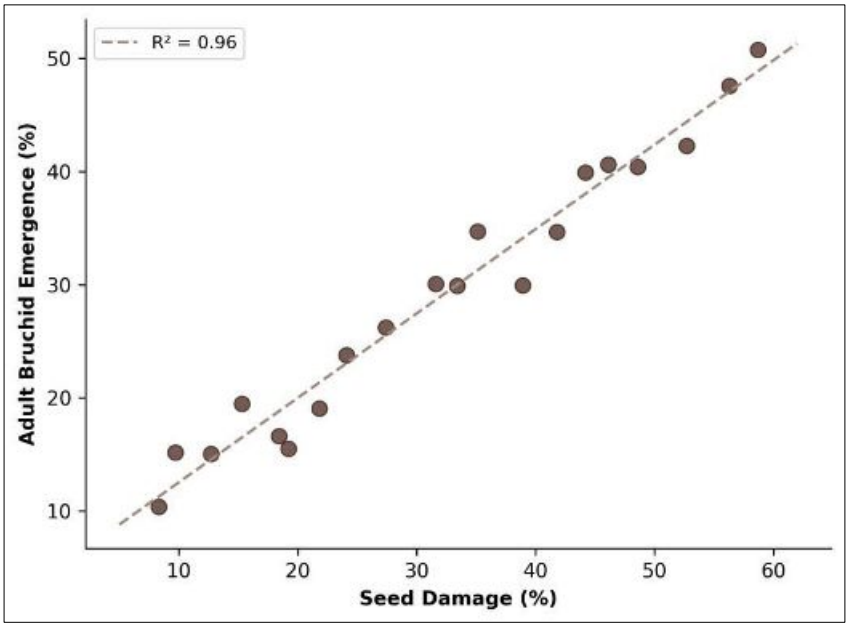

Fig 2: Relationship between seed damage and adult bruchid emergence across 89 cowpea accessions ($R^2 = 0.96$)

Comprehensive interpretation

The 12.4% resistance frequency is low but consistent with global cowpea germplasm surveys. The strong linkage of VuBr-1 and VuBr-3 to damage and emergence traits provides a practical marker-assisted selection tool. Three accessions KU-41, KU-67, and KU-83 combined resistant phenotype with favourable alleles at both marker loci.

Discussion

The 12.4% resistance frequency matches reports from IITA's screening of 2000+ accessions, where 10-15% showed resistance under similar no-choice conditions [4, 12]. The extended developmental period (38.4 days) in resistant accessions suggests an antibiosis mechanism, likely linked to seed coat tannins and trypsin inhibitors that slow larval growth rather than deterring oviposition [13]. VuBr-1's position on LG 5 corresponds to the major resistance QTL mapped by Souframanien *et al*. [6] in a TVu-2027 × IT84S-2049 cross, validating its cross-population relevance and increasing confidence in its utility for marker-assisted introgression.

The near-perfect correlation between seed damage and adult emergence ($R^2 = 0.96$) confirms that either trait can serve as the primary selection criterion, simplifying phenotypic screening protocols [14]. From a practical standpoint, the three top accessions (KU-41, KU-67, KU-83) combine bruchid resistance with acceptable grain size (14-17 g per 100 seeds) and cooking quality, making them suitable as donor parents without severe linkage drag [15]. The genotypic data enable breeders to track resistance alleles through segregating populations without waiting for the 60-day bioassay cycle, potentially halving the breeding timeline for storage pest resistance in cowpea [16].

Conclusion

Phenotypic screening of 89 cowpea accessions identified 11 resistant genotypes with less than 15% seed damage. SSR markers VuBr-1 and VuBr-3 on linkage groups 5 and 8 together explained over 53% of seed damage variance and can be deployed for marker-assisted selection. Accessions KU-41, KU-67, and KU-83 are recommended as priority donor parents for bruchid resistance breeding programmes. Validation of these markers in diverse genetic backgrounds should follow.

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