

ABSTRACT

PHENOTYPING OF BRUCHID RESISTANCE AND MAPPING IN INTER-SPECIFIC POPULATIONS OF *Vigna radiata* (L.) Wilczek AND *Vigna umbellata* (Thunb.)

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Bruchids are the most serious insect pests globally causing substantial seed damage to pulses during storage. Considering the non-availability of resistant varieties and lack of high level of bruchid resistance in the germplasm collections, investigations were carried out to identify better resistance source(s) from diverse samples such as Manipur rice bean landraces, improved mungbean varieties from research stations in Tamil Nadu and an inter-specific populations (recombinant inbred lines, RILs) of VRM(Gg)1 (*Vigna radiata*, bruchid susceptible) and TNAU Red (*Vigna umbellata*, bruchid resistant). Present studies also covered in-depth resistance mechanism studies, including single nucleotide polymorphic markers (SNPs) and identification of candidate genes associated with bruchid resistance.

Investigations on bruchid damage potential showed *Callosobruchus maculatus* as the dominant and more destructive (91.33 % over 70.67 % seed damage) species than *C. chinensis* in all their developmental aspects. In case of two *C. maculatus* populations collected from two agro-ecological regions of Tamil Nadu, maximum seed damage (91.2 % on mungbean) was caused by Thanjavur populations, compared to 78.4 % damage by Coimbatore population at 30°C (controlled) conditions. Fecundity, survival and development studies of *C. maculatus* (Thanjavur population) for continuous 25 generations showed that female oviposition potential reached a peak within 2 days after its commencement and declined with time. Adult longevity

study indicated that *C. maculatus* females have shorter lifespan than males. Sex ratio (emergence) was in favour of females in all the subsequent generations. Maximum adult emergence (mean of 8.60 bruchid adults out of 10) was observed on second day from their first emergence per generation studied.

Among the eight rice bean landraces evaluated for *C. maculatus* resistance, LR(M)-3, LR(M)-4 and TNAU Red were found to be completely resistant (0 % seed damage), LR(M)-1, LR(M)-2, LR(M)-6 and LR(M)-7 were moderately susceptible (10-69 %) and LR(M)-5 was highly susceptible (88.33 % and 71.67% from separate experiments infested with two and five bruchid pairs). Mungbean varieties showed high (95.00 %) to moderate (30.00-68.33 % seed damage) susceptibility towards *C. maculatus* infestation and resulted in high seed weight loss (54.48 %), reduced seed protein content (52.02 %) and reduction in germination percentage (83.92 %).

Seed biophysical attributes such as seed size, hardness and seed coat thickness did not play any significant ($p > 0.05$) role in conferring resistance to bruchids in rice bean and mungbean, as evident from regression and correlation analyses. *C. maculatus* resistance evaluation on parents showed high seed damage of 94.00 % in the susceptible parent, VRM(Gg)1, whereas no seed damage was recorded in the resistant parent, TNAU Red. Results of free-choice (conducted in a newly designed “innovative model”) and no-choice tests showed varied differences (0 – 100 % seed damage) in the reactions of 187 recombinant inbred lines (RILs) to *C. maculatus* (Thanjavur populations) and suggested different degrees of resistance or susceptibility. Among the RILs, three lines *viz.* RIL 158, 165 and 169 were found to be resistant and their levels of resistance varied under free-choice and no-choice conditions. From correlation analysis between seed attributes and bruchid parameters, it was again noticed that both qualitative (seed colour, texture, shape and lustre) and quantitative (seed size, seed hardness and seed coat thickness) seed characters had no significant role in imparting resistance to bruchids in all the RILs evaluated.

Detailed investigations were made to study the antibiosis resistance mechanism in selected RILs including rice bean landraces. The three resistant lines (RIL 158, RIL 165 and RIL 169) along with resistant and moderately resistant rice bean landraces also exhibited antibiosis when infested with *C. maculatus*, which was evident from the results of several experiments conducted, including prolongation of mean developmental period (MDP) (reaching up to a

maximum of 46.24 days on rice bean and 40.00 days on RIL), reduction in adult weight (up 15.05 % and 10.90 % for male and female, respectively), reduction in adult size, decreased longevity (5.00 and 5.33 days for female and male adults, respectively) and reduced or no fecundity of adults emerged from resistant RILs, when compared to normal bruchid adult size, fecundity and low MDP on susceptible parent.

Seed decortications had a negative effect on bruchid oviposition since *C. maculatus* females preferred intact seeds with seed coat for oviposition other than decorticated seeds. In both susceptible and resistant RILs, significant variations ($p=0.000^*$, $p<0.05$) were recorded in number of eggs hatched and egg hatchability on intact (with seed coat) and decorticated (without seed coat) seeds. Moreover, it was observed that seed coat acted as a physical barrier in larval/adult emergence of *C. maculatus*.

Ovipositional preference by normal and antennae ablated *C. maculatus* females were carried in order to study the role of antennae on host preference. Mean number of eggs laid, eggs hatched and hatching per cent were non-significant or at par ($p = 0.097^{ns}$, $p = 0.781^{ns}$ and $p = 0.210^{ns}$, $p>0.05$) for the normal and ablated females on susceptible and resistant RILs including rice bean landraces. Hence, antennae did not play any significant role in *C. maculatus* oviposition preference since their removal did not cause any loss in sensitivity.

Identification of various chemical constituents through Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed that both rice bean and resistant RILs exhibited rich phytopharmaceutical compounds of importance. Phenols were detected in cotyledon extracts of resistant rice bean landraces [LR(M)3, LR(M)4, TNAU Red and resistant RIL 165] and cinnamic acid (naringeninic acid) was detected in resistant RIL 158, which may indicate their secondary roles in imparting resistance towards bruchid (*C. maculatus*).

Scanning electron microscope (SEM) study showed that although variations in the size of starch granules were noticed between resistant and susceptible samples, there was a non-significant correlation between bruchid resistance parameters and size of the starch granules. SEM study on South Indian *C. maculatus* strain revealed the presence of different types of sensilla such as sensilla trichoidea (ST), sensilla basiconica (SB) and sensilla chaetica (SC) on mouthparts and antennal regions, which have different functions like mechanoreception and chemoreception. However, sensilla on the mouthparts were more abundant and pronounced than the antennal sensilla. This study may give supportive elucidation as to why no significant

variations were recorded during ovipositional preference studies conducted with antennae ablated (without antennae) and normal (with antennae) bruchids.

Bulked segregant analysis (BSA) carried out with 42 polymorphic simple sequence repeats (SSRs belonging to azuki bean genome), to identify markers linked with bruchid resistance, showed skewed segregation distortion towards the susceptible parent, VRM(Gg)1. Marker-trait association analysis carried out for identification of single nucleotide polymorphic markers (SNPs) associated with bruchid resistance in selected 108 RIL populations of VRM(Gg)1 x TNAU Red, using a set of high quality 1310 SNPs, revealed 17 SNPs strongly and significantly associated ($p \leq 10^{-3}$) with 5 bruchid resistance related traits *viz.* adult emergence, seed damage per cent, survival per cent, mean developmental period and index of suitability).

Among these, 7 SNPs were significantly associated with adult emergence and seed damage, 2 SNPs with adult survival per cent, 5 SNPs with mean developmental period, and 3 SNPs with index of suitability, on chromosome 2, 5, 6 and 8. Seven genes harbouring 12 significant SNPs containing or physically close to these associated SNPs are proposed as candidate genes for bruchid resistance, which is supported by their involvement in most of the phytohormone signaling pathways (secondary metabolites production) and defense-related protein synthesis. However, three genes in particular *viz.* *Vradi05g17240*, *Vradi06g04680* and *Vradi08g19320* (nearby associated SNPs) may be taken into account owing to their encoding enzymes with antibiotic / antinutritive effect against insects (proteinase inhibitory enzymes), phenol biosynthesis (glycosyltransferase) and naringenin biosynthesis pathway induction (naringenin-chalcone synthase), which were earlier reported to be responsible for conferring bruchid resistance in rice bean.