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Development of bruchid resistant genotypes in mungbean through introgression of wild genotypes

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Abstract
Vigna radiata (L.) wilczek, commonly known as greengram widely cultivating in India. Greengram is good source of protein and other cosmetic uses. The resistance source is available in wild genotypes of Vigna species. The *Vigna umbellata* having good source for bruchids durable resistance. The bruchid *Callosobruchus maculatus* (L.) was done by feeding assay in laboratory conditions. Resistance to bruchids was identified in the F_1 derivatives of *V. radiata* x *V. umbellata*. Out of 187 rills, the Rill - 158, Rill-165 and Rill- 169 showed 100 percent resistance against bruchid attack. All the other plants showed varying the level of resistance and susceptibility against bruchids. So, the rill -158, rill -165 and rill- 169 showed 100 percent resistance can be utilized for the development of bruchid resistant genotypes in greengram for future greengram improvement breeding programme.

Keywords: Greengram, rice bean, interspecific hybridization, bruchids, resistance

Introduction
Vigna radiata (L.) wilczek, commonly known as greengram or mungbean is the most widely distributed species among the *Vigna* species cultivated species. Grains are very quickly destroyed by the pulse beetle. Now a days, pulse beetle infestation has started field itself. 100 percent grains loss due to more population of pulse beetle. Climate also desirable for production of pulses beetle. It possessed certain added features compared to other pulses. It is relatively drought tolerant and well adapted to a range of soil conditions including light soils and can thrive even under limited irrigation, moreover, it is suited for crop rotation and crop mixtures (Baldev, 1988 [1] and Sadaphal, 1988) [8]. However, this crop is suffering from the yield due to pulses beetle infestation. Grain quality and germination also affected by pulses beetle's infestation. In Tamil Nadu it is cultivated nearly 2. Lakhs hectares with a lowest production. Besides management factors the prime cause for the low productivity can be ascribed to the inherently low yielding potential of the cultivars coupled with susceptibility to diseases.
The varietal breeding program taken up among the varieties had resulted only with limited success as far as yield improvement is concerned. The basic reason for limited success had been due to the limited variability is high among the parents used for hybridization in most of the studies. There had been always possibility of improving the crop by incorporating wild genes to the cultivated species always successfully achieved the targeted genes and also eradicating the problems uncounted with already existing genotypes. Crossing can be done for need based utilizing the primary, secondary and tertiary gene pools of this crop can result in tremendous improvement in yield. Primary gene pool when not in useful then we go for secondary and tertiary gene incorporation into agronomically good genotypes. The *Vigna umbellata* has durable resistant genes against bruchids. When we use this *Vigna* species for improvement of greengram to develop bruchids resistant genotypes in greengram.
Selection of parents from the diverse species for hybridization program is likely to generate superior transgressive segregants. The introgressed materials developed through wide crosses can also contribute as genetic reservoirs for novel genes apart from contributing to the improvement of yield and yield components.

Materials and Methods

To generate variability through interspecific hybridization involving *Vigna radiata* with *Vigna umbellate*, evaluate and characterize interspecific hybrids and studying the segregants for yield and yield components and to assess the pest and disease reaction of the parents, hybrids and selected F_2 crosses. For screening for bruchid resistance (*Callosobruchus* spp.), interspecific crosses between *V. radiata* with *V. umbellata* the bruchids ovipositional preferences and non-preferences were studied in the parents and its hybrids. One hundred seeds from these interspecific crosses was placed in cloth bags and 5 pairs of adult bruchids obtained from the maintained culture which were one day old were released into each of the bags. The experiments were replicated twice adopting factorial CRD. Observations were made on the number of eggs laid per seed on 24, 48, 72, 96,120 and 144 hrs after release. The mean number of eggs laid per seed each day was calculated and the number of adults emerged and seeds damaged as per cent were recorded. Data were analyzed using Excel package**.**

Results

In this cross combination bruchids were laid eggs on the seeds surface. In F1s some of the plants seeds were not damaged. The parent greengram completely damaged by the bruchids and another parent *V. umbellata* not damaged by the bruchids. In F1s most of the plants seeds showing damaged even full of bruchids released to the container except three plants namely rill no - 158, rill no - 165 and rill no - 169. In these rills, egg laying was not in its seeds. The bruchid damaged in the parental seeds varied from 0.0 percent in *V. umbellata* and *V. radiata* 100 .00 percent (Fig 1.). In this cross, 0.0 percent seed damaged to 100 percent seed damage in F_1 is presented in the table 1.

Table 1: Screening of bruchids in *V. radiata x V. umbellata* derivatives in F2 segregants.

Plant Name	No. of seeds	Number of pairs of insects released	No of eggs laid on the seed	No. of days for counting after bruchids released	No. of seeds damaged	% of seeds damaged
P1	20	2 pairs	58.0	25	20	100
P2	20	2 pairs	65.0	25	20	100
P3	20	2 pairs	68.0	25	20	100
P4	20	2 pairs	80.0	25	19	95
P5	20	2 pairs	55.0	25	20	100
P6	20	2 pairs	75.0	25	18	90
P7	20	2 pairs	48.0	25	17	85
P8	20	2 pairs	55.0	25	18	90
P9	20	2 pairs	62.0	25	20	100
P10	20	2 pairs	64.0	25	20	100
P11	20	2 pairs	58.0	25	20	100
P12	20	2 pairs	53.0	25	20	100
P13	20	2 pairs	75.0	25	20	100
P14	20	2 pairs	81.0	25	20	100
P15	20	2 pairs	75.0	25	20	100
P16	20	2 pairs	63.0	25	20	100
P17	20	2 pairs	70.0	25	20	100
P18	20	2 pairs	45.0	25	20	100
P19	20	2 pairs	49.0	25	18	90
P20	20	2 pairs	69.0	25	18	90
P21	20	2 pairs	62.0	25	18	90
P22	20	2 pairs	48.0	25	17	85
P23	20	2 pairs	59.0	25	19	95
P24	20	2 pairs	62.0	25	18	90
P25	20	2 pairs	80.0	25	18	90
P26	20	2 pairs	55.0	25	19	95
P27	20	2 pairs	75.0	25	19	95
P28	20	2 pairs	48.0	25	19	95
P29	20	2 pairs	55.0	25	18	90
P30	20	2 pairs	62.0	25	16	80
P31	20	2 pairs	64.0	25	15	75
P32	20	2 pairs	58.0	25	18	90
P33	20	2 pairs	53.0	25	19	95
P34	20	2 pairs	75.0	25	18	90
P35	20	2 pairs	80.0	25	18	90
P36	20	2 pairs	55.0	25	18	90
P37	20	2 pairs	75.0	25	20	100
P38	20	2 pairs	48.0	25	20	100
P39	20	2 pairs	55.0	25	18	90
P40	20	2 pairs	62.0	25	18	90
P41	20	2 pairs	80.0	25	18	90
P42	20	2 pairs	55.0	25	20	100
P43	20	2 pairs	75.0	25	20	100
P44	20	2 pairs	48.0	25	18	90
P45	20	2 pairs	55.0	25	18	90
P46	20	2 pairs	62.0	25	18	90
P47	20	2 pairs	64.0	25	20	100
P48	20	2 pairs	58.0	25	17	85
P49	20	2 pairs	53.0	25	18	90

P50	20	2 pairs	75.0	25	20	100
P51	20	2 pairs	80.0	25	20	100
P52	20	2 pairs	55.0	25	20	100
P53	20	2 pairs	75.0	25	20	100
P54	20	2 pairs	48.0	25	20	100
P55	20	2 pairs	55.0	25	20	100
P56	20	2 pairs	62.0	25	17	85
P57	20	2 pairs	64.0	25	18	90
P58	20	2 pairs	58.0	25	20	100
P59	20	2 pairs	53.0	25	17	85
P60	20	2 pairs	75.0	25	18	90
P61	20	2 pairs	80.0	25	20	100
P62	20	2 pairs	55.0	25	20	100
P63	20	2 pairs	75.0	25	20	100
P64	20	2 pairs	48.0	25	20	100
P65	20	2 pairs	55.0	25	20	100
P66	20	2 pairs	62.0	25	20	100
P67	20	2 pairs	64.0	25	17	85
P68	20	2 pairs	58.0	25	18	90
P69	20	2 pairs	53.0	25	20	100
P70	20	2 pairs	75.0	25	20	100
P71	20	2 pairs	80.0	25	20	100
P72	20	2 pairs	80.0	25	20	100
P73	20	2 pairs	55.0	25	20	100
P74	20	2 pairs	75.0	25	20	100
P75	20	2 pairs	48.0	25	20	100
P76	20	2 pairs	55.0	25	20	100
P77	20	2 pairs	62.0	25	17	85
P78	20	2 pairs	64.0	25	20	100
P79	20	2 pairs	58.0	25	18	90
P80	20	2 pairs	53.0	25	20	100
P81	20	2 pairs	75.0	25	20	100
P82	20	2 pairs	80.0	25	17	85
P83	20	2 pairs	55.0	25	18	90
P84	20	2 pairs	75.0	25	20	100
P85	20	2 pairs	48.0	25	20	100
P86	20	2 pairs	55.0	25	20	100
P87	20	2 pairs	62.0	25	20	100
P88	20	2 pairs	64.0	25	20	100
P89	20	2 pairs	58.0	25	20	100
P90	20	2 pairs	53.0	25	17	85
P91	20	2 pairs	75.0	25	17	85
P92	20	2 pairs	80.0	25	18	90
P93	20	2 pairs	55.0	25	20	100
P94	20	2 pairs	75.0	25	20	100
P95	20	2 pairs	48.0	25	20	100
P96	20	2 pairs	55.0	25	20	100
P97	20	2 pairs	62.0	25	20	100
P98	20	2 pairs	64.0	25	20	100
P99	20	2 pairs	58.0	25	17	85
P100	20	2 pairs	53.0	25	18	90
P101	20	2 pairs	75.0	25	20	100
P102	20	2 pairs	80.0	25	20	100
P103	20	2 pairs	55.0	25	20	100
P104	20	2 pairs	75.0	25	20	100
P105	20	2 pairs	48.0	25	20	100
P106	20	2 pairs	55.0	25	20	100
P107	20	2 pairs	62.0	25	17	75
P108	20	2 pairs	64.0	25	17	75
P109	20	2 pairs	58.0	25	18	90
P110	20	2 pairs	53.0	25	20	100
P111	20	2 pairs	75.0	25	20	100
P112	20	2 pairs	80.0	25	20	100
P113	20	2 pairs	55.0	25	20	100
P114	20	2 pairs	75.0	25	20	100
P115	20	2 pairs	48.0	25	20	100
P116	20	2 pairs	55.0	25	17	75
P117	20	2 pairs	62.0	25	17	75
P118	20	2 pairs	58.0	25	18	90
P119	20	2 pairs	58.0	25	20	100
P120	20	2 pairs	53.0	25	20	100
P121	20	2 pairs	75.0	25	20	100
P122	20	2 pairs	80.0	25	20	100

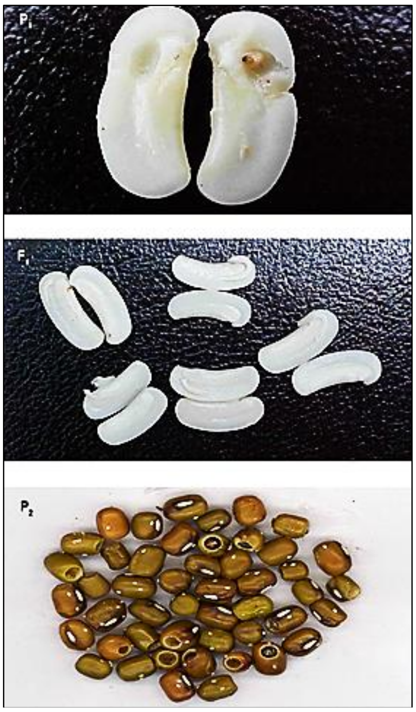
P123	20	2 pairs	55.0	25	20	100
P124	20	2 pairs	75.0	25	20	100
P125	20	2 pairs	48.0	25	17	85
P126	20	2 pairs	55.0	25	17	85
P127	20	2 pairs	62.0	25	18	90
P128	20	2 pairs	64.0	25	20	100
P129	20	2 pairs	58.0	25	20	100
P130	20	2 pairs	53.0	25	20	100
P131	20	2 pairs	75.0	25	20	100
P132	20	2 pairs	80.0	25	20	100
P133	20	2 pairs	55.0	25	20	100
P134	20	2 pairs	75.0	25	17	85
P135	20	2 pairs	48.0	25	17	85
P136	20	2 pairs	55.0	25	18	90
P137	20	2 pairs	62.0	25	20	100
P138	20	2 pairs	64.0	25	20	100
P139	20	2 pairs	75.0	25	20	100
P140	20	2 pairs	48.0	25	20	100
P141	20	2 pairs	55.0	25	20	100
P142	20	2 pairs	62.0	25	20	100
P143	20	2 pairs	64.0	25	17	85
P144	20	2 pairs	75.0	25	17	85
P145	20	2 pairs	48.0	25	18	90
P146	20	2 pairs		25	20	100
P147	20	2 pairs	75.0	25	20	100
P148	20	2 pairs	48.0	25	20	100
P149	20	2 pairs	55.0	25	20	100
P150	20	2 pairs	62.0	25	20	100
P151	20	2 pairs	64.0	25	20	100
P152	20	2 pairs	75.0	25	17	85
P153	20	2 pairs	48.0	25	17	85
P154	20	2 pairs	55.0	25	18	90
P155	20	2 pairs	62.0	25	20	100
P156	20	2 pairs	64.0	25	20	100
P157	20	2 pairs	75.0	25	20	100
P158	20	2 pairs	48.0	25	NIL	0.0
P159	20	2 pairs	75.0	25	20	100
P160	20	2 pairs	48.0	25	20	100
P161	20	2 pairs	55.0	25	17	85
P162	20	2 pairs	62.0	25	17	85
P163	20	2 pairs	64.0	25	18	90
P164	20	2 pairs	75.0	25	20	100
P165	20	2 pairs	48.0	25	NIL	0.0
P166	20	2 pairs	55.0	25	20	100
P167	20	2 pairs	62.0	25	19	95
P168	20	2 pairs	64.0	25	18	90
P169	20	2 pairs	75.0	25	NIL	0.0
P170	20	2 pairs	48.0	25	18	90
P171	20	2 pairs	55.0	25	19	95
P172	20	2 pairs	62.0	25	17	85
P173	20	2 pairs	64.0	25	18	90
P174	20	2 pairs	75.0	25	20	100
P175	20	2 pairs	48.0	25	20	100
P176	20	2 pairs	55.0	25	20	100
P177	20	2 pairs	62.0	25	20	100
P178	20	2 pairs	64.0	25	20	100
P179	20	2 pairs	75.0	25	20	100
P180	20	2 pairs	48.0	25	17	85
P181	20	2 pairs	55.0	25	19	95
P182	20	2 pairs	62.0	25	18	90
P183	20	2 pairs	64.0	25	20	100
P184	20	2 pairs	75.0	25	20	100
P185	20	2 pairs	48.0	25	20	100
P186	20	2 pairs	55.0	25	20	100
P187	20	2 pairs	62.0	25	20	100

Discussion

The present investigation was assessed in introgressed genotypes derived from V.*radiata x V.umbellata* for the bruchids resistance. Bruchids (*Callasobruchus macculatus*) is a very serious post-harvest pest of greengram. There is no resistant source for this pest across the greengram germplasm as reported by Tomooka *et al*. (2000) [7], Chakrabarty (2003) [2] and Kalyan and Dadhich (1999) [3]. However, Kashiwaba *et al*. (2003) [4] had reported that some unidentified compounds contained in the cotyledon of *Vigna umbellata* has an inhibitory effect on the growth of bruchids. The *umbellata* having the biochemicals play a major role to avoid the hatching of eggs in the seed surface and hatched larvae enter into inside of the seed but not develop fully and not survive. Among the *Vigna* species *V. umbellatta* only species having the bio chemicals against bruchids at high level of resistance. Pandiyan *et al.* (2006) [6].

The hybrids of the cross *V. radiata x V. umbellata* exhibited certain level of infestation in many of the plants, only three plants showed 100 percent as compared to other hybrids. This result supports the resistant finding of Kashiwaba *et al.* (2003) [4] that *V. umbellata* posses' resistance to bruchids. As high level of resistance was observed in the three plants of F_1 hybrid of V.*radiata* x V. *umbellata*, the screening studies were also continued in the F_2 generation of lesser population (Fig-1). The results revealed that the segregants of *V. V. radiata* x *umbellata* exhibited more resistance in three plants. According to the recovery of sufficient plant population could be recovered in the F_2 generation selection should be postponed to later generations for fixing the resistance by adopting breeding procedure like single seed descent method or bulk method or pedigree or backcross methods. The resistance could be maintained by continuing selection till large number of populations is achieved. Similar attempt for transferring bruchids resistance from *V. umbellata* into *V. radiata* has been reported Umamaheshwari (2002) [5] and Tomooka *et al.* (2000) [7]. Tomooka *et al.* (2000) [7] and Pandiyan *et al* (2006) [6] suggested that species level germplasm collection is an efficient means for revealing many new resistance accessions and it may also be effective to explore other types of novel genes.

Green gram plant with infected pods in the field



Adult Bruchid beetle

Fig 1: P1, F1 P2-Larva, Pupa on the seeds of green gram

Conclusion

Considering the potential economic importance and genetic erosion of wild species, collection, conservation and evaluation of the wild relatives of crop species deserve greater attention. Even in the smaller population of F_2 segregants, there was resistance reaction registered by *V. radiata* x *V. umbellata* hence selection for resistance may be employed at later generation when plant population is high.

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