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Short Communication

Inheritance of bruchid resistance and morphological traits in greengram

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Abstract

Feeding assay of F_2 and F_3 seeds, derived from a cross between bruchid resistant wild greengram Sub2 and a bruchid susceptible high yielding cultivar B1 revealed that bruchid resistance in greengram is dominant and governed by a few major genes (probably two) with some modifiers. A single dominant gene is responsible for the production of purple coloration in the hypocotyl region at the seedling stage of greengram. Involvement of two or more genes along with the effects from environment was observed for the traits like leaf lobation, leaf vein colour, seed lusture and seed shape. No particular seed coat colour or phenotypic traits was found to be associated with the bruchid resistance. Significant and positive correlation was there between seed weight and bruchid susceptibility.

Key words: Bruchid, morphology, greengram, resistance

Bruchid (*Callosobruchus chinensis* L.) is one of the most devastating storage pests of greengram and other *Vigna* species. The adult female bruchids deposit eggs on the seed coat. The larvae bore into the seed and hollow out the interior as they feed. After pupation, the adults emerge and deposit eggs on sound seeds starting a new life cycle. The cycle from egg to adult requires only three to four weeks. If uninterrupted, infestation may continue until all seeds in the storage area are destroyed. The damaged seeds are unsuitable for human consumption and for other commercial and agricultural uses due to presence of an odour and low germination rate. Almost 10 % of the total greengram production is completely lost due to attack of *C. chinensis* during storage condition [1]. Wild form of greengram especially *Sublobata* is superior to the cultivated variety in respect of bruchid resistance [2]. However, a major constraint in using wild greengram in cultivar development was the linkage drag of undesirable traits like pod dehiscent. In the present study, inheritance of bruchid resistance and its association with morphological characters of greengram was unveiled.

A *Sublobata* (Sub2) line having bruchid resistance was used as a pollen source during crossing with a susceptible cultivar of greengram, B1. The hybridity of F_1 seedlings was detected by the presence of the dominant markers of the pollen parent i.e., purple hypocotyl pigmentation at seedling stage and the presence of lobbed leaves. Seeds from individual F_1 plant were harvested separately. A total of 350 F_2 plants were raised from 12 F_1 plants, out of which harvesting was possible in 336 plants, remaining 14 F_3 plants germinated but died after two-leaved stage. This experiment was conducted during February to April, 2012. Data were recorded on twenty morphological characters of parental lines, hybrids and the F_2 plants following the guidelines [3] for the conduct of test for Distinctness, Uniformity and Stability (DUS) on greengram [*Vigna radiata* (L.) Wilczek].

The traits observed were as follows: 1. Hypocotyl: anthocyanin colouration; 2. Time of flowering: early (<40 days), medium (40-50 days), late (>50 days); 3. Growth habit: spreading, non-spreading;

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4. Plant type: determinate, indeterminate; 5. Stem colour: green, green with purple splashes, purple; 6. Stem pubescence: absent, present; 7. Leaf lobation: absent, present; 8. Leaf shape: deltoid, ovate, lanceolate, cuneate; 9. Vein colour: green, greenish purple, purple; 10. Petiole colour: green, green with purple splashes, purple; 11. Pod pubescence: absent, present; 12. Pod position: above canopy, intermediate, not visible; 13. Plant height: short (<50 cm), medium (50-70 cm), long (>70 cm); 14. Pod colour: brown, black; 15. Curvature of mature pod: straight, curved; 16. Mature pod length: short (<8 cm), medium (8-10 cm), long (>10 cm); 17. Seed colour: yellow, green, brown, mottled; 18. Seed lustre: shiny, dull; 19. Seed shape: oval, drum-shaped; 20. 100 seed weight: small (<3 g), medium (3-5 g), large (>5 g).

Bruchids were reared continuously in glass jars (10 inch) by using fresh gram (*Cicer arietinum*) seeds as their food source. Fifty fresh seeds each from Sub2 and B1 and 50 F_3 seeds harvested from each of the 336 F_2 plants were kept in separate small plastic vials. Five pairs of freshly emerged adults of *C. chinensis* (L.) were released for 24 to 30 hours in each vial where the male and female insects were differentiated by structural differences in antenna; males having pectinate type (comb like) and females having serrate type (saw like) of antenna. All the vials were kept in incubator at 30º C temperature and 70% relative humidity. The infested seeds were studied daily under a stereoscopic binocular. Observations were made on the duration of egg, larval and pupal stage and emergence of adults following the procedure of Singh and Pandey [4]. The whitish appearance of the egg shell was an indication of the larva's successful penetration of the seed. Thirty days after insect introduction, the number of damaged seeds was recorded. The damaged F_3 seed, from which an adult insect had emerged, was considered as the 'susceptible' and an undamaged F_3 seed, from which no adult insect had emerged, was considered as 'resistant'. On the basis of damaged and un-damaged F_3 seed number, the susceptible and resistant F_2 plants were detected respectively. The goodness of fit of the expected ratio in F_2 generation was tested by using chi-square (χ^2) test. The test for independence of two factors was also done. The observed ratio was considered to fit the expected ratio if the χ^2 value had a corresponding probability greater than or equal to 0.05. The null hypothyesis (r = 0) was tested through the application of t test, $t=r[(n-2)/(1-r^2)]^{1/2}$ where, r = correlation coefficient and n=total number of observations. This t value was tested against the table value of t [5] for (n-2) degrees of freedom.

In the present investigation, out of 350 F_2 plants, at seedling stage, 79 did not have the anthocyanin pigment (purple) at the hypocotyl region while 271 had it at seedling stage. Hence, segregation of 3:1 :: pigmented : non-pigmented held good (Table 1). So, the hypocotyl pigmentation character can be considered as a dominant character controlled by single gene. Earlier, Sen and Ghosh [6] opined that purple anthocyanin pigment in the hypocotyl was monogenetically dominant over green hypocotyl. A monogenic inheritance for purple anthocyanin pigmentation was reported by Pathak and Singh [7], Verma [8]. From the bruchid feeding assay of F_3 seed lots of 336 F_3 plants, 99 F_2 plants were found to be completely resistant (0% seed damage in insect feeding assay) against *C. chinensis* (L.) and 28 plants were found to be completely susceptible (80-100% seed damage). The independence of two factors was tested through the application of χ^2 test with Yate's correction [9] and concluded that the two factors are independent of each other. So, hypocotyl pigmentation is not linked with bruchid resistance in greengram.

Trifoliate leaf lobation character is dominant over entireness of leaves *i.e.*, non-lobbed. The shape of the terminal leaflet of non-lobbed trifoliate leave was ovate type while cuneate type of terminal leaflet was found in case of lobbed ones. Among 350 F_2 plants, 240 had lobation in trifoliate leaves and the remaining 110 F_2 plants had non-lobbed leaves. The assumed 3:1 segregation ratio did not hold good (Table 1). Moreover, other known ratios regarding gene interaction also did not fit here which might be due to the small population size and / or the effect of two or more loci with environmental effects. Our observation was against earlier observation that the leaf-lobe was a monogenically inherited character with (i) complete dominance [10] and (ii) with incomplete dominance [6]. Leaf lobation and bruchid resistance also showed independent assortment as confirmed by χ^2 analysis. The purple colouration in vein was observed as a dominant character. In the F_2 population, 173 and 174 plants had greenish purple and purple veins respectively but the assumed 9:7 segregation ratio did not hold good as per χ^2 test (Table 1). Again, it can be concluded that the two factors (bruchid resistance and leaf vein colour) were inherited independently.

There were 45 lots of yellow seeds, 133 lots of green seeds, 59 lots of brown seeds and 99 lots of mottled seeds. No particular seed colour was found to be linked with the bruchid resistance in greengram. Among two different types of seed lustres, 110 lots were of shiny seeds and 226 lots of dull seeds, and among two different types of seed shapes, 236 lots were of oval-shaped and 100 lots of drum-shaped seeds. Probably, more than one gene was involved in these cases. Chen and Liu [11] opined that the seed lustre of greengram was controlled by more than two genes. But Sen and Ghosh [6] opined that the rough (dull) seed coat was monogenically dominant over the glossy type. Dharmasena and Subasinghe [12] found that small seeds and glossy seed coats of greengram to be associated with higher degree of resistance against *Callosobruchus* species. Hard, large and heavy seeds of greengram were preferred by *C. chinensis* for oviposition [13]. In our study, bruchid resistance in greengram was not linked with the seed lustre as well as seed shape as confirmed from the χ^2 test (Table 1). So, selection of bruchid resistant genotypes with consumer preferred shiny seeds can be made from the successive generations. B1 produced at an average of 35 pods per plant where Sub2 produced about 20 pods per plant. Most of the resistant as well as susceptible F_2 plants produced 6 to 10 pods. Only two resistant segregants produced equivalent or greater number of pods per plant than that of B1. The number of seeds harvested per plant ranged from 41 to a maximum of 412. The hundred seed weight ranged from 1.21 g to 3.12 g from different F_2 plants. Sub2 had smaller seeds with average 1.94 g weight while B1 had 2.71 g. Unlike pods per plant or seeds per plant, hundred seed weight showed almost a normal distribution where a significant portion of segregants possessed mid-value of two parents. Correlation coefficient (r) between the hundred seed weight of F_2 plants and percentage of damaged seeds (PDS) was 0.24. So, it can be concluded that there remains a significant and positive correlation between greater seed weight and bruchid susceptibility. Southgate [14] also suggested that small seeds had higher bruchid resistance than larger seeds which supports our observation too. James *et al.* [15] found that the resistance to *C. maculatus* in Australian wild greengram was negatively associated with seed size. A significant positive correlation between seed size of *V. nepalensis* Tateishi and Maxted and PDS by bruchids was found by Somta *et al.* [16] as we found in our case. The result of feeding assay of F_3 seeds by *Callosobruchus chinensis* (L.) are presented in the Fig. 1. The PDS ranged from 0% to 100% in the F_2 generation. The frequency distribution of PDS in the present study in a F_2 population showed a skewed distribution towards the resistant parent Sub2. This indicates that a few dominant genes may be involved in resistance to the bruchid along with some minor modifier genes. The validity of the assumed 13:3 ratio was tested with the help of χ^2 test. As the calculated χ^2 value (0.91) was smaller than the table value under 0.05 *P* (Table 1) hence, the assumed 13:3 segregation ratio held good.

Table 1. Segregation pattern for different traits

Traits	Total no. of F_2 plants	Classification	Observed values	Ratio tested	Observed χ^2 value	P value
Hypocotyl purple pigmentation at seedling stage	350	Present Absent	271 79	3:1	1.101	0.05
Leaf lobation	350	Present	240	3:1	7.715	0.05*
		Absent	110			
Leaf vein colour	350	Greenish purple	173	9:7	5.76	0.05*
		Purple	174			
		Green	3**			
Seed lustre	336	Dull	226	3:1	10.73	0.05*
		Shiny	110			
Seed shape	336	Oval	236	3:1	4.064	0.05*
		Drum-shaped	100			
Bruchid resistance	127	Resistant	99	13: 3	0.91	0.05
		susceptible	28			

*significant; **not considered in the calculation

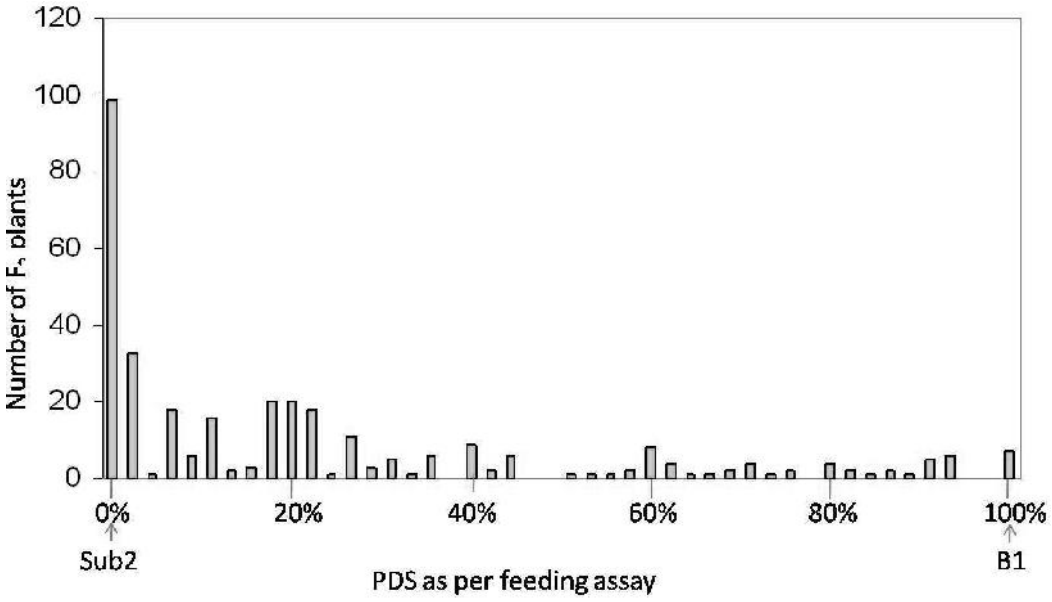

Fig. 1. Frequency distribution of percentage of damaged seeds (PDS) caused by *Callosobruchus chinensis* (L.) in F_2 population (based on F_3 seeds) derived from a cross between *Vigna radiata* (L.) Wilczek (B1) and *Vigna radiata* var. *sublobata* (Sub2)

So, we can accept the fact that two genes are interacting for expression of a single character *i.e.* bruchid resistance. Also the Fig. 1 showed that the F_2 population skewed towards the resistant parent Sub2. So, it can be said that two dominant genes are involved for the *C. chinensis* (L.) resistance in wild greengram *V. radiata* var. *sublobata* (Sub2), where either of them cannot confer the resistance alone. In wild greengram accession TC1966, bruchid resistance was controlled by a single dominant gene [17, 18]. Young *et al.* [19] and Kaga and Ishimoto [20] found a single major gene responsible for resistance to *C. chinensis* in greengram. Sun *et al.* [21] found that the bruchid resistance in a greengram cultivar from India was controlled by one dominant gene called *Br2*. Several researchers have suggested a more complex mode of bruchid resistance in *Vigna* species. Somta *et al.* [22] suggested that the resistance to bruchid in greengram was controlled by a major gene with varying dominant expression and with the presence of modifiers.

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