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Short Note

Tannins in Resistance to Bruchid, *Callosobruchus maculatus* Fab. (Bruchidae: Coleoptera)

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Cultivated *Vigna radiata* accessions, wild *Vigna umbellata* accessions and non-edible leguminous seeds were evaluated for their resistance to the bruchid, *Callosobruchus maculatus* and the possibility of using them as resistant donors. All the seeds except *Leucaena leucocephala* were susceptible to oviposition by bruchids. But *V. radiata* accessions supported normal emergence of bruchids while *V. umbellata* accessions terminated their development at different larval instars. The tannin content of *V. umbellata* accessions was also significantly higher (2.48 to 4.24 mg/g) when compared to *V. radiata* accessions (1.66 to 2.14 mg/g). In the case of non-edible legumes, development of bruchids was arrested at the seed coat itself while the first instar grubs were about to penetrate seed coat. Hence seed coat tannin content of non-edible legumes was estimated which was the highest (4.13 to 13.02 mg/g) compared to other groups of legumes.

Key words: *Vigna radiata*, *Vigna umbellata*, non-edible legumes, tannins, bruchid resistance

The coevolutionary relationships between bruchids, *Callosobruchus maculatus* Fab. (Bruchidae: Coleoptera) and legumes is so strong that the bruchids have evolved special means of dealing with large quantities of potentially toxic substances and antinutritional factors in the seeds of legumes. (Southgate, 1979). Proteinase inhibitors (Zhu-Salzman, 2003), tannins (Lattanzio *et al.,* 2005), saponins (Duhan *et al.,* 2001), lectins (Vasconcelos and Oliveira, 2004) and phytic acid (Chitra *et al.,* 1995) have been reported as factors responsible for resistance against bruchids. Tannins are phenolic compounds of higher molecular weight (500 – 4000 Da) found in leaves, bark, seed, wood, *etc*. of plants and bound to proteins to form tannin-protein complexes. They are closely associated with plant defense mechanism against insects (Hassanpour *et al.,* 2011). Hedin *et al.* (1988) observed that there is a significant correlation between tannin content and bruchid resistance. In studies conducted by Dabire-Banso *et al.* (2010), cowpea variety *viz.,* IT86D-716 showed higher amounts of flavonoids, tannins, *etc*. and consequently exhibited lesser preference by the pod sucking bug, *Clavigralla tomentosicollis.* The effective role of seed coat tannins in deterring, poisoning or starving the bruchid larvae that feed on the seeds has also been suggested by Lattanzio *et al.* (2012). The present paper deals with the evaluation of three groups of leguminous seeds *viz.,* cultivated green gram accessions, wild species belonging to the genus *Vigna* and non edible legumes to find possible sources of resistance for use in breeding programmes.

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Materials and Methods

The insect used for this study were obtained from culture maintained continuously on *Vigna radiata* (var. CO 6) at the Biocontrol Unit of Tamil Nadu Agricultural University, Coimbatore, India following the procedure of Strong *et al.* (1968). The insects were maintained at a temperature of 30 ± 5$_o$C and 70 ± 5% relative humidity throughout the period of study.

Three groups of leguminous seeds were used for the study *viz.,* cultivated green gram (*Vigna radiata*) accessions, wild rice bean (*V. umbellata*) accessions and wild non -edible legumes. The green gram accessions were obtained from National Bureau of Plant Genetic Resources, New Delhi and *V. umbellata* accessions from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. Seeds of non-edible legumes *viz.,* Humming bird tree (*Sesbania grandiflora* L.), Balmota (*Sesbania sesban* Merr.), Horse tamarind (*Leucaena leucocephala* Lam.), soapnut (*Acacia concinna* DC.), Aila (*Acacia caesia* W.) and Lebbeck tree (*Albizia lebbeck* L.) were collected in and around TNAU campus, Coimbatore. The green gram cultivar CO 6 was used as check in entire study.

The tannin content of the sample was estimated by Folin-Denis Method (Sadasivam and Manickam, 1996) and expressed as mg/g of sample. Six numbers each of *V. radiata* accessions, *V. umbellata* accessions and non edible legumes were subjected to intensive no-choice test (Gibson and Raina, 1972) under laboratory conditions along with a control (CO 6). Twenty five seeds of each leguminous seed were confined separately in polythene bags of dimensions 9 x 6.5 cm and three pairs of freshly emerged adults of *C. maculatus* were released. Each entry was replicated thrice and the setup was left undisturbed for three days under room temperature after which the no. of eggs laid was visually observed. Later, the per cent survical, mean developmental time and index of suitability were worked out using formulae (Howe, 1971).

$$\text{Percentage survival} = \frac{\text{No. of adults emerged}}{\text{No. of eggs laid}} \times 100$$

$$\text{Developmental time} = \frac{d_1a_1 + d_2a_2 + d_3a_3 + \ldots + d_na_n}{\text{Total number of adults emerged}}$$

where, d_1 = day at which the adults started emerging (1^{st} day)

a_1 = number of adults emerged on d_1^{th} day

$$\text{Suitability Index (SI)} = \frac{\text{Log (per cent survival)}}{\text{Mean developmental period}}$$

A one way ANOVA was used and the means were compared based on least significant difference (LSD) at P = 0.05 using AGRES package. A two-tailed Pearson's correlation coefficient (R) was used to correlate the relationship between various parameters using SPSS 10.0 package.

Results and Discussion

All the green gram accessions tested were susceptible for egg laying and there was no significant difference in the survival percentage between different accessions (Table 1). Prolongation of developmental period to an extent of 2.5 to 4.12 days was noticed in the accessions and subsequently the suitability index revealed considerable differences. The accession LM 103 was the least susceptible (0.0493) followed by COGG 912 (0.0496) while the control (CO 6) was the most susceptible (0.0582). Maximum tannin content was also noticed in LM 103 (2.14 mg/g) and the minimum in CO 6 (0.67 mg/g).

Table 1. Development of *Callosobruchus maculatus* and tannin content of green gram (*V. radiata*) accessions

Accession No.	Eggs* (No./ 25 seeds)	Survival (%)**	Mean developmental period* days)	Suitability Index	Tannin content* (mg/g)
LM 103	38.00 (6.20)b	35.10 (36.33)	31.33 (5.64)a	0.0493	2.14 (1.62)a
PLS 276-1	41.67 (6.49)c	38.42 (38.31)	29.72 (5.50)a	0.0534	2.03 (1.59)b
PLS 262	41.00 (6.44)bc	39.98 (39.22)	30.46 (5.56)a	0.0526	1.91 (1.55)c
ML 192-1	46.00 (6.82)d	35.49 (36.56)	30.02 (5.52)a	0.0516	1.84 (1.53)d
IC 39412	33.00 (5.79)a	38.39 (38.29)	31.04 (5.62)a	0.0511	1.82 (1.52)d
COGG 912	45.33 (6.77)d	35.34 (36.47)	31.17 (5.63)a	0.0496	1.66 (1.47)e
CO 6	38.33 (6.23)bc	38.35 (38.27)	27.21 (5.26)b	0.0582	0.67 (1.08)f
LSD (5%)	3.50	4.64	1.70	-	0.04
CV (%)	4.94	7.10	3.21	-	3.76

All values represent mean of three replications; * Values in parentheses are square root transformed values; **Values in parentheses are arcsine transformed values; Means followed by a common letter in a column are not significantly different by LSD (P=0.05)

As in the case of cultivated accessions the wild accessions of *V. umbellata* posed no problem for oviposition by the bruchids (Table 2). But none of the accessions permitted successful completion of life cycle. Further, in all the accessions dead larval stages at different instars were found inside the cotyledons. Similar observations on intermittent mortality of grubs was earlier reported by Srinivasan and Durairaj (2007). The tannin content was also more in these wild accessions (2.48 to 4.24 mg/g) when compared to cultivated green gram accessions (0.67 mg/g). However, the tannin content in the cotyledons did not in any way affect the oviposition by bruchids as evident from the egg laying tendencies.

Table 2. Development of *Callosobruchus maculatus* and tannin content in rice bean (*V. umbellata)* accessions

Accession No.	Eggs* (No./ 25 seeds)	Survival (%)**	Mean developmental period* days)	Suitability Index	Tannin content* (mg/g)
LRB 111	50.00 (7.11)b	Nil	Nil	Nil	4.24 (2.18)a
TNAU UMG	37.00 (6.12)c	Nil	Nil	Nil	3.98 (2.12)a
LRB 85	13.67 (3.76)bc	Nil	Nil	Nil	3.31 (1.95)b
LRB 173	21.33 (4.67)a	Nil	Nil	Nil	2.67 (1.78)c
LRB 40-1	21.33 (4.67)a	Nil	Nil	Nil	2.67 (1.78)c
LRB 292	57.00 (7.58)	Nil	Nil	Nil	2.48 (1.73)c
CO 6	39.67 (6.34)bc	28.41 (29.11)	30.90 (5.60)	0.0670	0.67 (1.08)d
LSD (5%)	3.50	4.64	1.70	-	0.31
CV (%)	4.94	7.10	3.21	-	6.12

All values represent mean of three replications; * Values in parentheses are square root transformed values; **Values in parentheses are arcsine transformed values; Means followed by a common letter in a column are not significantly different by LSD (P=0.05)

Oviposition by *C. maculatus* varied widely in the non-edible leguminous seeds (Table 3). *Leucaena leucocephala* seeds were devoid of eggs while *Sesbania sesban*, *Acacia concinna* and *A. caesia* exhibited very low levels of egg laying. However, in all the seeds the grubs were dead at the seed coat itself.

The tannin content ranged from 1.66 to 2.14 mg/ g in *V. radiata* accessions, 2.48 to 4.24 mg/g in *V. umbellata* accessions and 4.13 to 13.02 mg/g in the seed coats of non-edible legumes while the cultivar recorded 0.67 mg/g. The presence of dead larval instars inside the cotyledons of *V. umbellata*

Table 3. Development of *Callosobruchus maculatus* in non-edible legumes and their seed coat tannin contents

Non-edible legume	Eggs* (No./ 25 seeds)	Survival (%)**	Mean developmental period* days)	Suitability Index	Tannin content* (mg/g)
Sesbania grandiflora	47.33 (6.92)d	Nil	Nil	Nil	13.02 (3.68)a
Sesbania sesban	1.33 (1.35)a	Nil	Nil	Nil	12.17 (3.56)b
Leucaena leucocephala	0.00 (0.71)a	Nil	Nil	Nil	9.73 (3.20)c
Acacia concinna	3.00 (1.87)a	Nil	Nil	Nil	5.98 (2.55)d
Acacia caesia	1.58 (2.00)a	Nil	Nil	Nil	5.83 (2.52)d
Albizia lebbeck	19.00 (4.42)b	Nil	Nil	Nil	4.13 (2.15)e
CO 6	40.33 (6.39)c	43.88 (41.48)	29.74 (5.50)	0.0552	0.67 (1.08)f
LSD (5%)	4.55	-	-	-	0.46
CV (%)	16.85	-	-	-	3.66

All values represent mean of three replications; * Values in parentheses are square root transformed values; **Values in parentheses are arcsine transformed values; Means followed by a common letter in a column are not significantly different by LSD (P=0.05)

implies that tannins have the ability to arrest the development of bruchids. The reasons for such premature death were earlier explained by Swain (1977) who inferred that tannins affect the growth of insects by imparting an astringent taste affecting the palatability leading to low consumption levels or form complexes with protein resulting in reduced digestibility or act as enzyme inactivators which in turn lead to growth retardation and death. Lattanzio *et al.* (2005) observed that stored cowpea seeds with lesser bruchid damage possessed 13 times higher levels of seed coat tannins. Thus the edible legumes permitted successful completion of life cycle by the bruchids, wild *Vigna* spp. permitted partial development and the non -edible legumes killed the bruchid before entering the larval stage indicating the role of tannins in the resistance mechanisms. With advances in the field of genetic engineering it is possible to increase the tannin content in the cultivars by using wild leguminous plants as resistant donors without affecting their nutritional qualities.

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