Journal of Food Legumes 21(4): 262–265, 2008

Biochemical basis of resistance to stored grain pest (*Callosobruchus chinensis*) in different *Vigna* species

A.M. MISAL, J.V. PATIL and U.D. CHAVAN

Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722, Maharashtra, India; E.mail: patiljvp@yahoo.co.in

Abstract

Alpha α-amylase inhibitor, trypsin inhibitor, total phenols and tannic acid had positive correlation with resistance to bruchid in different *Vigna* species. No seed damage, adult emergence, loss in seed weight and high seed hardness were found in ricebean. However, mungbean was found to be most preferred host for oviposition followed by urdbean. Mungbean (TARM 18) and urdbean (PU 9205-11-3) genotypes recorded minimum number of damaged seeds and loss in seed weight. Maximum reduction in germination was noticed in mungbean and urdbean whereas it was negligible in ricebean.

Key words: Biochemical basis, Bruchid, Storage study, *Vigna* species

Pulses form the major constituent of Indian diet. Efforts have been made to achieve a major breakthrough in pulse production so as to meet the domestic requirements of the pulses. However, increasing pulses has to be complemented with reducing the losses caused by the insect pests during storage. India produces around 13-15 million tonnes of pulses annually and about 8.5% of this production is lost during post-harvest handling and storage (Anonymous 2004). Among the insect pests, bruchid is the most serious storage insect pest of pulses. To overcome the problems of stored grain pests, pulse breeders are required to develop new varieties possessing inherent resistance to them. It has been reported that some of the pulses are resistant to bruchid beetle due to the presence of α–amylase inhibitor, trypsin inhibitor, polyphenol and tannin content in the seed coat (Ishimoto and Kitamura 1989). Activities of certain oxidative enzymes like peroxidase and polyphenol oxidase are also responsible to protect seeds from insect pests (Nicholson *et al.* 1986). Therefore, the present study was undertaken to assess biochemical basis of resistance to stored grain pest in different *Vigna* species.

MATERIALS AND METHODS

Pure seeds of mungbean (*Vigna radiata*) and urdbean (*Vigna mungo*) were obtained from the Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. The seeds of ricebean (*Vigna umbellata*) genotypes were procured from the AICRP on Under Utilized Crops, Mahatma Phule Krishi Vidyapeeth, Rahuri. The biochemical constituents were estimated by using standard methods such as α–amylase inhibitor activity (Rajendran and Thayumanavan 2000), trypsin inhibitor activity (Kakade *et al.* 1969), protein content by Micro-Kjeldahl method (Jackson 1971), total phenols by Bray and Thorpe (1954), tannic acid by Sadashivam and Manickan (1992), and peroxidase and polyphenol oxidase by Kumar and Khan (1982).

For evaluation of resistance in mungbean, urdbean and ricebean against bruchid, a laboratory experiment was conducted for a period of 40 days. The initial culture of pulse beetle (*Callosobruchus chinensis*) was obtained and pure culture was prepared for experimentation. The seeds of mungbean, urdbean and ricebean were dried and fumigated with aluminum phosphate separately to avoid the residual infestation of pulse beetle. Hundred gram seeds were placed in five jars of one kg capacity for mungbean, urdbean and ricebean to which 0, 1, 2, 4 and 8 pairs of freshly emerged pulse beetles were released separately. Moisture content, oviposition, number of adult emerged, loss in seed weight, number of damaged seeds, reduction in germination, 100-seed weight, and seed hardness were determined by using standard methods of AOAC (1990) and germination count was measured by ISTA method (Anonymous 1985). The statistical analysis, correlation studies and path analysis were performed using standard methods of Snedecor and Cocharan (1967) and Dewey and Lu (1959).

RESULTS AND DISCUSSION

The mean values of biochemical constituents studied in mungbean, urdbean and ricebean are presented in Table 1. Mungbean contained higher protein than urdbean and ricebean. Mungbean cv. Kopergaon had 28.5% protein followed by AKM 8802 (26.1%) while urdbean cv. TAU 2 recorded 24.9% protein content. The results indicated preference for high protein genotypes by bruchid. The α–amylase inhibitor activity was high in ricebean (25.6 mmol of maltose formed/10 m) followed by urdbean (25.2 mmol /10 m). Ricebean genotypes had the highest trypsin inhibitor potential than urdbean and mungbean genotypes. Similarly, total phenols, tannic acid and peroxidase activity were at higher level in ricebean genotypes than in urdbean and mungbean. Ishimoto and Kitamura (1989) and Naik and Jadhav (2002) stated that seed α–amylase inhibitor protein plays a protective role against bruchid. Kashiwaba *et al.* (2003) reported similar results related to bruchid in ricebean. High trypsin inhibitor activity is reported to relate with resistance to bruchid beetle. Total phenols and tannic acid also play important role in pulses for resistant to bruchid. These constituents are known to make complex with the digestive enzymes, dietary proteins, minerals

Table 1. Biochemical constituents of mungbean, urdbean and ricebean genotypes in relation to stored grain pest

Genotype	Protein (%)	α-Amylase inhibitor activity (μmol of maltose formed/10 min)	Trypsin inhibitor activity (units/ml/10 min)	Total phenol (mg/100g)	Tannic acid (%)	Polyphenol oxidase activity (A/min/mg of soluble protein)	Peroxidase activity (A/min/mg of soluble protein)
Mungbean							
PM 2	20.8	15.3	74.0	32.5	1.10	2.48	3.00
PM 9339	21.3	13.5	72.0	37.4	1.06	3.07	2.92
AKM 8802	26.1	13.2	56.5	25.0	0.96	2.41	2.48
TARM 18	19.8	16.0	93.2	57.5	1.28	5.97	2.15
Kopergaon	28.5	12.7	53.0	22.5	0.74	6.48	5.12
Mean	23.3	14.1	69.7	35.0	1.03	4.08	3.13
Urdbean							
TAU 1	21.5	24.5	64.5	35.0	1.21	3.45	5.02
TAU 2	24.9	22.6	59.5	32.5	1.02	5.89	3.36
TAU 4	19.8	25.6	67.3	42.5	1.11	5.43	2.66
PU 9503-8	17.4	27.2	68.8	47.5	1.45	3.57	3.14
PU 9205-11-3	16.6	26.0	70.2	55.0	1.30	5.31	3.50
Mean	20.0	25.2	66.1	42.5	1.22	4.73	3.54
Ricebean							
RBL 36	21.4	28.4	71.5	57.5	1.70	3.95	7.26
RBL 50	22.1	25.0	78.2	65.0	1.75	3.50	6.56
RBL 99	20.3	25.1	78.5	60.0	1.98	2.91	5.57
LRB 199	21.6	25.0	82.5	52.5	1.90	3.25	7.01
LRB 202	23.1	24.5	79.7	50.0	1.95	4.15	7.15
Mean	21.7	25.6	78.1	57.0	1.86	3.55	6.71
S.E.	2.97	5.49	10.15	12.93	0.38	1.30	1.80
CD at 5%	8.92	16.48	30.45	38.79	1.15	3.90	5.41

Table 2. Direct and indirect effects of biochemical constituents on bruchid resistance in mungbean and urdbean

Character	α-Amylase inhibitor activity (μmol of maltose formed/10 m)	Trypsin inhibitor activity (unit/ml/10 m)	Total phenol (mg/100 g)	Tannic acid (%)	Polyphenol oxidase activity (A/m/mg of soluble protein)	Peroxidase activity (A/m/mg of soluble protein)	Protein (%)	Correlation coefficient (r)
Mungbean								
α-Amylase inhibitor	-0.231	0.326	-0.005	0.032	-0.007	0.322	0.129	0.566*
Trypsin inhibitor	-0.152	0.494	-0.006	0.051	0.012	0.361	0.106	0.866*
Total phenol	-0.177	0.472	-0.007	0.047	0.047	0.345	0.121	0.848*
Tannic acid	-0.115	0.390	0.005	0.065	-0.036	0.355	0.123	0.777*
Polyphenol oxidase activity	0.006	0.021	-0.001	-0.008	0.274	-0.218	-0.048	0.025
Peroxidase activity	0.143	-0.034	0.004	-0.044	0.115	-0.520	-0.158	-0.803
Protein	0.147	-0.257	0.004	-0.039	0.605	-0.402	-0.204	-0.687*
Urdbean								
α-Amylase inhibitor	0.081	0.206	0.180	0.132	-0.019	-0.242	-0.016	0.323
Trypsin inhibitor	0.055	0.303	0.225	0.122	-0.034	-0.267	-0.021	0.384
Total phenol	0.053	0.248	0.275	0.248	-0.021	-0.160	-0.028	0.615*
Tannic acid	0.023	0.079	0.146	0.466	0.057	0.029	-0.027	0.775*
Polyphenol oxidase activity	0.014	0.095	0.053	-0.246	-0.109	-0.178	0.005	-0.366
Peroxidase activity	-0.053	-0.216	-0.118	0.036	0.052	0.374	-0.004	0.070
Protein	-0.025	-0.123	-0.150	-0.246	-0.011	-0.033	0.052	-0.537*

*Significant at 5%

Table 3. Effect of treatments on bruchid resistance on stored grains of mungbean, urdbean and ricebean

Treatment (Pair of bruchid)	Moisture (%)	Initial germination (%)	No of damaged seeds (%)	Loss in seed weight (%)	Germination after emergence of first generation of bruchid	Reduction in germination (%)	Ovipo-sition	No. of adults emerged
Mungbean								
0	8.40	91.40	0.00	0.00	90.60	0.86	0.00	0.00
1	8.24	91.30	3.20	1.71	82.05	10.12	15.85	56.30
2	8.44	91.25	8.10	4.56	75.14	16.94	20.85	131.30
4	8.45	91.50	13.95	9.20	69.45	24.28	32.90	180.00
8	8.40	91.50	26.29	21.93	59.60	35.44	46.90	272.00
Urdbean								
0	8.62	91.94	0.00	0.00	90.80	1.02	0.00	0.00
1	8.56	91.45	3.72	2.57	86.00	5.76	18.25	50.70
2	8.56	91.40	10.05	7.26	80.40	12.12	26.75	118.20
4	8.60	91.90	17.50	13.67	75.05	18.67	36.75	162.55
8	8.65	91.65	25.80	17.54	68.02	25.53	52.75	257.65
Ricebean								
0	8.61	93.20	0.00	0.00	92.75	0.80	0.00	0.00
1	8.59	93.65	0.00	0.00	92.00	0.91	12.20	0.00
2	8.70	92.70	0.00	0.00	92.12	0.85	22.00	0.00
4	8.62	92.40	0.00	0.00	92.14	0.85	28.80	0.00
8	8.67	93.10	0.00	0.00	92.42	0.90	36.80	0.00
S.E. ±	0.12	0.76	9.17	6.99	10.47	11.00	15.83	76.99
C.D. at 5%	0.37	2.29	NS	NS	31.41	NS	NS	NS

NS=Non significant

Table 4. Mean performance of different parameters for evaluating resistance in mungbean, urdbean and ricebean genotypes at the maximum population of bruchid

Genotype	Ovipo-sition	No. of adults emerged	Loss in seed weight (%)	Seed hardness (kg/cm^2)	100-seed weight (g)	Reduction in germination(%)	No. of resistant seeds (%)
Mungbean							
PM 2	40.75	261.75	22.00	4.22	3.30	29.15	76.50
PM 9339	47.00	281.75	23.68	4.30	3.66	39.36	73.25
AKM 8802	52.75	300.00	25.10	3.82	4.15	43.92	71.25
TARM 18	37.50	87.00	11.26	4.80	3.19	7.92	87.00
Kopergaon	56.50	429.50	18.15	3.40	4.66	56.88	60.75
Mean	46.90	272.00	22.04	4.11	3.79	35.45	73.75
Urdbean							
TAU 1	42.50	304.75	19.20	5.10	5.13	28.57	70.50
TAU 2	50.25	395.00	27.00	5.02	5.76	35.96	65.50
TAU 4	41.75	239.75	18.20	5.32	5.50	26.52	75.00
PU 9503-8	40.00	194.50	12.10	5.62	4.72	19.84	79.00
PU 9205-11-3	38.00	154.25	11.20	5.72	4.56	16.78	81.00
Mean	42.50	257.65	17.54	5.36	5.13	25.53	74.20
Ricebean							
RBL 36	38.00	-	-	6.30	5.16	1.33	100
RBL 50	35.00	-	-	6.50	5.10	0.88	100
RBL 99	37.00	-	-	6.25	6.18	0.79	100
LRB 199	32.00	-	-	6.12	5.27	0.53	100
LRB 202	42.00	-	-	6.47	5.07	1.06	100
Mean	36.80	-	-	6.33	5.36	0.92	100
S.E.	6.58	-	-	1.57	0.83	17.76	13.60
CD at 5%	19.76	-	-	4.73	2.52	NS	NS

NS=Nonsignificant

and vitamins and reduce their bioavailability. The phenolic compounds and enzymes related to their metabolism mostly peroxidase and polyphenol oxidase are widely implicated in resistance to infection of insect (Jambunathan *et al.* 1986). Tannin is a condensed polyphenolic compound and it acts as defense mechanisms in plants against insects, pathogens and herbivores. The tannins were highly toxic to bruchid larvae in cowpea (Boughdad *et al.* 1986). Ricebean, urdbean and mungbean followed decreasing trend for these constituents and the same order of resistance to bruchid was observed. Peroxidase and polyphenol oxidase are known to accelerate the oxidation of phenolic compounds which in turn are responsible for resistance against biotic stresses. The peroxidase activity of ricebean genotypes was significantly higher than urdbean and mungbean. A positive correlation between peroixidase activity and resistance to injury and diseases as well as better storability has been reported (Aluko and Oghodu 1986). The α–amylase, total phenol, peroxidase and protein showed negative direct effect while trypsine inhibitor, tannic acid and polyphenol oxidase showed positive direct effect on bruchid resistance in mungbean. The α–amylase, trypsin inhibitor, total phenol, and tannic acid showed significantly positive correlation while protein showed significantly negative correlation (Table 2). The biochemical constituents studied showed direct positive effect on resistance to bruchid except polyphenol oxidase. Total phenol and tannic acid showed significantly positive correlation while protein showed significantly negative correlation with pest resistance.

Evaluation of mungbean, urdbean and ricebean seeds against freshly emerged pulse beetles showed that as the number of pairs of pulse beetle increased, the number of damaged seeds, seed weight loss, reduction in germination, oviposition and adult emergence increased in urdbean followed by mungbean (Table 3). In ricebean, there were no damaged seeds, no seed weight loss and no adult emergence. These observations indicate that ricebean is resistant to bruchid during storage as also reported earlier by Ramzan *et al.* (1990), Asrof *et al.* (1999) and Obaidullah *et al.* (2003). On the basis of seed hardness, seed weight, resistant seed percentage and oviposition, seed weight loss and germination reduction, ricebean was found highly resistant to bruchid (Table 4) while mungbean cv. Kopergaon and urdbean cv. TAU 2 were highly susceptible during storage.

REFERENCES

Aluko R and Oghadu GH. 1986. Analysis of eggplant varieties for enzyme related to their organoleptic properties. Tropical Science 26: 163-171.

Anonymous. 1985. International rules for seed testing (Rules and Annexes). Seed Science and Technology 13: 299-513.

AOAC. 1990. Official Methods of Analysis 15th edn. Association of Official Analytical Chemists, Washington DC.

Ashraf M, Sohail M, Akhtor M and Ali M. 1999. Varietal resistance of pulses of *dhora* (*Callosobruchus chinensis*). Journal of Agricultural Research Lahore 29: 81-86.

Boughdad A, Gillon Y and Gagnepain C. 1986. Influence des tannins condenses du tegument des feves (*Vicia faba* L.) Su le development larvaire de C maculates (F.) Entomological Experimental Application 42: 125-132.

Bray HG and Thorpe WV. 1954. Methodology in Biochemistry and Analytical 1: 27-57.

Dewey DR and Lu KH. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. Agronomy Journal 9: 515-518.

Ghosal TK, Dutta S, Senapati SK and Deb DC. 2004. Role of phenol contents in legume seeds and its effect on the biology of *Callosobruchus chinensis*. Annual Plant Protection Science 12: 425-475.

Ishmoto M and Kitamura K. 1989. Growth inhibitory effect of α-amylase inhibitor from kidney bean (*Phaseolus vulgaris* L.) on three species of bruchids (*Coleoptra: Bruchidae*) Applied Entomology 24: 281-286.

Jackson ML. 1971. Soil chemical analysis. Prentice hall of India (Ltd.), New Delhi.

Jambunathan R, Butler LG, Bandopadhyay R and Mughogho KK. 1986. Phenol concentration in grain, leaf and callus tissue of mold resistant and mold susceptible sorghum cultivars. Journal of Agriculture and Food Chemistry 34: 425-429.

Kakade ML, Simons N and Liener IE. 1969. An evaluation of natural versus synthetic substrates for measuring the antitryptic activity of soyabean sample. Cereal Chemistry 46: 518.

Kashiwaba K, Tomooka N, Kaga A, Han OK and Vaughan DA. 2003. Characterization of resistance to three bruchid species (*Callosobruchus* spp. *Coleoptera, Bruchidae*) in cultivated rice bean (*Vigna umbellata*). Journal of Economical Entomology 96: 206-213.

Kumar KS and Khan PA. 1982. Peroxidase and polyphenol oxidase in excised *ragi* (*Eleusine corocana* cv. PR-202) during senescence Indian Journal of Experimental Biology 20: 412-416.

Naik RM and Jadhav SS. 2002. Biochemical basis of resistance to stored grain pest in rice bean (*Vigna umbellata*). Indian Journal of Agricultural Biochemistry 14: 57-58.

Nicholson RI, Butler LG and Asquith TN. 1986. Glycoproteins from *colltrotrichum greaminicola* that binds phenols: implication from survival and virulence of phytopathogenic fungi. Phytopathology 76:1315-1318.

Obaidullah M, Haque MA, Sardar MA, Islam MZ and Biswas M. 2003. Host preference of pulse beetle *Callosobruchus chinensis* L. to different legume seeds. Bangladesh Journal of Agricultural Science 30: 37-41.

Rajendran R and Thayumanavan B. 2000. Purification of an a-amylase inhibitor from seeds of little millet (*Panicum sumatrens* Roth). Journal of Plant Biochemistry and Biotechnology 9: 89-94.

Ramzan M, Chabal BS and Judge BK. 1990. Storage losses to some commonly used pulses caused by pulse beetle *Callosobruchus maculatus*. Journal of Insect Science 3:106-108.

Sadasivam S and Manickam A. 1992. Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd, New Delhi. pp.189-191.

Sendecor GW and Cochran WG. 1967. Statistical methods. 6th edn. Oxford and IBH Publ. Co. Calcutta. pp. 172.