BIOCHEMICAL BASIS OF RESISTANCE TO STORED GRAIN PEST (*Callosobruchus chinensis*) OF DIFFERENT *VIGNA* SPECIES

By

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AMOL MANIKRAO MISAL

Reg. No. 20008

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413722, DIST-AHMEDNAGAR, MAHARASHTRA STATE (INDIA)

In partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

SEED TECHNOLOGY

DEPARTMENT OF AGRIL. BOTANY

POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI-413722,
DIST-AHMEDNAGAR,
M. S., INDIA

2005

Dedicated to
My
Beloved
Aai and Baba
&
Atul

......AMOL

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2005

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place: - M.P.K.V., Rahuri.
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This is to certify that the thesis entitled, "**BIOCHEMICAL BASIS OF RESISTANCE TO STORED GRAIN PEST (*Callosobruchus chinensis*) OF DIFFERENT *VIGNA* SPECIES**" submitted to the faculty of agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist-Ahmednagar, Maharashtra, India, in partial fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY in AGRICULTURAL BOTANY (Seed Technology)** embodies the results of a piece of bona-fide research work carried out by **SHRI. AMOL MANIKRAO MISAL**, under the guidance of **Dr. J.V. Patil,** Senior Sorghum Breeder, Mahatma Phule Krishi Vidyapeeth ,Rahuri and that no part of the thesis has been submitted for any other degree, diploma or publication.

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Place: MPKV, Rahuri
Dated: / /2005

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Place : MPKV, Rahuri
Date : 15/04/2005

(A.M. Misal)

CONTENETS

LIST OF TABLE

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

@	:	At rate of
C.D.	:	Critical difference
et al.,	:	et alli
Fig.	:	Figure
g	:	Gram (s)
kg	:	Kilogram(s)
cm	:	Centimeter(s)
i.e.	:	That is
R.H.	:	Relative humidity
S.E.	:	Standard error
Spp.	:	Species
Viz.	:	Namely
^{0}C	:	Degree Celsius
%	:	Per cent
APS	:	Ammonium persulphate
HCL	:	Hydrochloric acid
hr	:	Hour
M	:	Molar
mM	:	Milli molar
mA	:	Milli Ampere
min.	:	Minute(s)
ml	:	Millilitre
NaOH	:	Sodium hydroxide
1N	:	One normal
No.	:	Number
PAGE	:	Polyacrylamide Gel Electrophoresis
pH	:	Negative logarithm of the hydrogen ion concentration
Rm	:	Relative migration

rpm	:	Revolutions per minute
SDS-PAGE	:	Sodium Dodecyl sulphate polyacrylamide gel electrophorus
TCA	:	Trichloroacetic acid
TAMED	:	N, N, N; N; tetramethylethylendiamine
Tris	:	Tris hydroxymethylaminomethane
μl	:	Microlitre
V	:	Volts
μg	:	Microgramme (s)
BAPNA	:	Benzeyl-DL-arginine-P-nitroanilide
nm	:	Nanometer
DNSA	:	3-5, Dinitro salicylic acid
mm	:	Millimeter

ABSTRACT

BIOCHEMICAL BASIS OF RESISTANCE TO STORED GRAIN PEST (*Callosobruchus chinensis*) OF DIFFERENT *VIGNA* SPECIES

By

Amol Manikrao Misal

2005

Research Guide	:	Dr. J.V. Patil Senior Sorghum Breeder, MPKV, Rahuri

The present investigations entitled "Biochemical basis of resistance to stored grain pest (*Callosobruchus chinensis*) of different *Vigna* species" was designed and executed to evaluate the level of bruchid resistance of different genotypes of green gram, black gram and rice bean and to know the biochemical factors responsible for resistance to bruchid.

For the above studies three species of *Vigna* viz. green gram (PM-2, PM-9339, TARM-18 and Kopergaon), black gram (TAU-1, TAU-2, TPU-4, PU-9503-8 and PU-9205-11-3) and rice bean (RBL-36, RBL-50, RBL-99, LRB-199 and LRB-202) were taken.

Green gram was found to be most preferred host for oviposition followed by black gram and rice bean. The green gram genotypes Kopergaon and AKM-8802 having lustrous green colour bold seeds, which may favoured the insect for oviposition. The black gram genotypes TAU-1 and TAU-2 recorded highest oviposition.

Highest number of adult emergence, damaged seeds and loss in seed weight were recorded in green gram genotypes as compared to black gram. However no adult emergence, damaged seeds and loss in seed weight were found in case of rice bean. The green gram and black gram genotype TARM-18 and PU-9205-11-3 recorded minimum number of damaged seeds, loss in seed weight was found to be relatively resistant to bruchid than the rest.

Highest seed hardness was recorded in rice bean followed by black gram and it was least in green gram. Maximum reduction in germination percentage was noticed in green gram and black gram, where as it was negligible in rice bean. This may be due to destruction of embryo as evident by adult emergence from respective species. In green gram and black gram more seed weight relates to more susceptibility to bruchid in all genotypes.

In green gram and black gram, most of the related parameters to bruchid resistance were found to be strongly negative with resistance to seed except seed hardness. Maximum amount of positive direct effect was shown by the parameter seed hardness, which indicates that they are the principle component of seed resistance to bruchid.

Biochemical parameter like ∝-amylase inhibitor, trypsin inhibitor, total phenols and tannic acid had positive correlation with resistant seed, which indicates that they might be involved in the resistance to bruchid.

Electrophoresis studies for seed protein profile and peroxidase isozyme pattern of green gram, black gram and rice bean genotypes under study were unique. Genotypic differences were revealed by the presence or absence of particular band in electrophorograms. The qualitative differences were observed in the banding pattern of green gram, black gram and rice bean seed storage protein and peroxidase isozyme, indicating variation among these *Vigna* species. Unique bands present in these species might be used as marker for accurate and precise identification of genotypes, which showed genetic variation, might be used as probes to mark genotype. TARM-18 in green gram, PU-9205-11-3 in black gram and RBL-99 in rice bean showed differential banding pattern than other four genotypes.

Abstract contd... **A.M. Misal**

The seed borne fungi like *Alternaria alternara, Fusarium monoliforme, Aspergilus* sp., *Penicillium* sp. and *Rhizopus stolonifer* were common in green gram, black gram and rice bean. The lowest incidence of seed born fungi was recorded in green gram (TARM 18), black gram (TPU 4) and rice bean (RBL 50).

Pages: 1 to 187

INTRODUCTION

1. INTRODUCTION

Pulses, being a rich and important source of protein (20-30 %), form the major constituent of diet for masses in India. It offers the major practical mean of solving malnutrition in our country. In India, 23.00 million hectares of land is under different pulses cultivation with an annual production of 14.00 million tonnes (Anonymous, 2004). Against the dietary recommendations of 105 g of pulses per day per capita only 47 g are available. Efforts are being made to achieve a break through in pulse production so as to overcome this problem. However, growing more pulses alone is not enough but reducing losses both quantitatively and qualitatively due to the attack of pest and microorganism during storage is also essential.

Despite of insecticide treatment, pulses suffer a great deal of damage during storage due to insect pest. Among the insect pest, bruchids appear to be the most serious one and a number of species belonging to genus *Callosobruchus* causes a considerable damage. The genus *Callosobruchus* includes a number of economically important species that attack stored pulses throughout the world but *Callosobruchus maculatus* (Fab.), *Callosobruchus chinensis* (Linn) and *Callosobruchus analis* (Fab.) are more important.

According to the FAO reports, India produces around 11.1 million tonnes of different pulses per year; out of this nearly 8.5 percent are lost during post harvest handling and storage (Anonymous, 2004). To overcome this problem, the plant breeder in India and around the world are engaged in producing a new varieties of pulses rich in protein as well as possessing inherent resistance to insect attack.

The genetic resistance is one of the most important approaches of integrated pest control. Painter (1951) was among the pioneers, who advocated the concept of host plant resistance. The basic aim of varietal pest control is to reduce the losses caused by insect pests. This aim can only be achieved when no insect infestation occurs and the plant varieties succeed in escaping their attack. Generally, a new variety does suffer the insect attack but may sustain less damage than the susceptible variety because in some way, it is comparatively more resistant. This resistance may be attributed to some intrinsic as well as extrinsic properties of the pulse variety in question interacting with specific insect species.

The commonly used pulses in India are green gram, black gram, pigeonpea, chickpea, lentil and cowpea. Out of these green gram and black gram from genus *Vigna* are used in daily consumption as source of protein. These pulses are infested by pulse beetle (*Callosobruchus chinensis*) and causes substantial economic and nutritional losses. Apart from this traditional pulses, the under utilized pulse rice bean (*Vigna umbellata*) is reported as resistant to bruchid (Chatterjee and Dana, 1979).

In common bean, it has been reported that the resistance to bruchid beetle is largely because of presence of ∝ -amylase inhibitor, a seed protein that is toxic to larva. Ishimoto and Kitamura (1989) demonstrated that a seed ∝-amylase inhibitor protein in several genotype of common bean plays a protective role against bruchid pest. The ∝-amylase inhibitor strongly inhibited the larval midgut ∝-amylase.

In other crops, proteases inhibitor such as trypsin has been reported (Chen and Mitcheli, 1973) to impart resistance towards many pest and diseases. Trypsin inhibitors are distributed widely in legume seeds; it causes adverse effect on protein digestion when ingested by animal or insect. Similarly the polyphenol content has been shown to strongly inhibit the activities of extracellular enzymes produced by microorganisms. The activities of certain oxidative enzymes like peroxidase and polyphenol oxidase has been reported to protect crop from certain insect species (Nicholson *et al.*, 1986). Several studies indicated that dihydroxy phenols are desirable for resistance to pest and diseases however these must be oxidized to be effective. The oxidation of phenol is catalyzed by peroxidase and polyphenol oxidase enzyme.

Electrophoresis of total soluble protein and isozyme are used for the identification of variation in the genotypes of green gram, black gram and rice bean.

In the present investigation, study of biochemicals imparting resistance to attack of pulse beetle in different *Vigna* species *viz., Vigna mungo, Vigna radiata* and *Vigna umbellata.* For this investigation, green gram, black gram and rice bean was taken with following objectives.

1) To evaluate the levels of resistance against pulse beetle (*Callosobruchus chinensis*) in seeds of green gram (*Vigna raidata*), black gram (*Vigna mungo*), and rice bean (*Vigna umbellata*).
2) To study α-amylase and trypsin inhibitor activities
3) To study the protein banding of green gram, black gram, and rice bean.
4) To study the seed mycoflora associated with green gram, black gram, and rice bean.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The present investigation on "Biochemical basis of resistance to stored grain pest (*Callosobruchus chinensis*) of different *Vigna* species" was undertaken with objectives to evaluate the level of resistance to pulse beetle (*Callosobruchus chinensis*) in seed of green gram, black gram, and rice bean varieties, biochemical characters responsible for bruchid resistance, electrophoretic studies for total soluble protein and isoenzyme banding pattern to identify the characteristic band of genotype, and seed mycoflora associated with green gram, black gram and rice bean. Literature on these aspects has been reviewed and given here under with following subheadings.

1] Evaluation of levels of resistance to the bruchid
2] Biochemical parameters
3] Electrophoresis studies
4] Seed mycoflora study

2.1 Evaluation of levels of resistance to the bruchid

The relative resistance of some varieties of broad bean (*Vicia faba* L.) to *Callosobruchus maculatus L.* is solely due to difficulties encountered by the hatching larvae and by the inability to penetrate through the seed coat. No differences in development were noticeable on decorticated seeds of the different varieties. The thickness of the seed coat reported to be the only limiting factor, and *Vicia faba* seed coats were apparently non-toxic to larvae. This was demonstrated by the high survival of larvae penetrating *Vicia faba* seed coats. Podoler and Applebaum (1968) suggested that selection of broad bean varieties with thicker seed coat would significantly reduce infestation by *Callosobruchus chinensis* during storage.

Jakhmola and Singh (1971) concluded that adult females showed a preference for oviposition in varieties with large seeds and adults reared on varieties with large seed were larger than those from smaller seeds.

Yadav and Pant (1975) studied the germination of seeds of pulses infested singly with eggs of *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) and showed that due to infestation of *Callosobruchus maculatus the* germination of pigeon pea seeds 30 days after oviposition was only 6 percent while it was completely lost in case of green and black gram.

Dabi *et al.*, (1978) studied ten varieties of black gram, *Phaseolus mungo* L. for their relative resistance to *Callosobruchus maculates* L. The varieties were compared on the basis of percent seed infestation, percent loss in weight, and number of adult emerged and quality of food consumed. Varieties Krishna and PLS-364 were found less susceptible having < 20 % infestation while G-31 and K-5 proved to be highly susceptible with > 60 % infestation. None of the varieties tested were immune to the attack of *Callosobruchus maculatus*; however, there were appreciable differences in their susceptibility to the pest.

Chatterjee and Dana (1979) reported that the seeds of minor legume rice bean (*Vigna umbelluta*) were absolutely free from attack by *Callosobruchus chinensis* (L) even under artificial infestation.

Southgate (1979) stated that bruchid resistance might be the result of one more four causes: (1) the seed may be too hard for newly hatched larva to penetrate; (2) the seed may physically be too small or of an inconvenient shape for the larvae to reach full size; (3) the seed may contain to little food to support a larva; and (4) the seed may contain toxin or other substance in cotyledons or it's enveloping seed coat that inhibits the development of larvae.

seed may contain toxin or other substance in cotyledons or it's enveloping seed coat that inhibits the development of larvae.

Hassan and Mudithir (1982) studied the effect of various levels of damage caused to pulse seeds during storage by *Callsobruchus maculatus* (F.) on germination and observed decrease in germination as number of holes per seed increased.

Patil and Jadhav (1982) screened pea varieties in laboratory to find out their relative susceptibility to *Callosobrochus maculatus.* The variety "Wai early" was preferred more for ovipoistion while, Bonneville was found to suffer the maximum percent loss in grain weight (10.70). The percent grain infestation was also more in case of Bonneville (45.70). The percent loss in weight and percent grain infestation was the lowest in case of B.M. Kunjeer. No relationship was reported between the oviposition preference and percent grain infestation and percent loss in grain weight.

Fujii (1984) studied the effect of bean water content on production of the active form in *Callosbruchus maculatus.* High larval density caused an increase of water content of beans through larval metabolism. However, bean water content influenced the production of the active form only when combined with artificial temperature rise. The results showed (i) beans with high water contents (16.2 and 22.1 %) create a favorable condition for the production of the active form. (ii) The active form does not emerge from dry beans with water contents of 8.7 and 12.4 percent. (iii) The increased water content of beans during larval growth facilitates the production of the active form and (iv) decrease of bean water content prevents the production of the active form. It was also shown that bean water content and temperature are closely related and work, in one sense, in a complementary manner for the production of the active form.

Singh and Sharma (1984) reported that the grain damage and loss of germination due to infestation by *Callosobruchus maculatas* were significantly higher in the varieties ML-5, G-55 and Shining Moon No.1 of a mung bean (*Vigna radiata*) than in the varieties T-9 and Mash 1-1 of mash bean (*Vigna mungo*). The proportion of damaged grain varied from 43.55 to 57.77 in mung and from 7.97 to 10.20 percent in mash. The corresponding losses in germination after storage for 5 months were 47.53 to 70.60 percent and 14.13 to 19.76 percent.

Thiery (1984) studied seed coat hardness of *Phaseolus vulgaris* L., *Phaseolus lunatus* L., *Phaseolus coccineus* L. and *Lablab purpureus* L. and observed that the ability of *Acanthoscelides obtectus* (Say.) larvae to penetrate *Phaseolus vulgaris* was strongly affected by seed coat hardness. Comparisons between the four species suggest differences in the chemical properties of the seed coat of *Phaseolus lunatus* seeds.

Sehgal and Sachdeva (1985) screened eight new varieties of chickpea against the attack of *Callosobruchus maculatus* in order to evaluate the susceptibility to this pest. The beetle laid eggs on all varieties but the ovipositional preference was not related to the susceptibility of particular variety for further development.

Twelve varieties of mung bean (*Vigna radiata*) were studied for seed characteristics contributing to resistance to *Callosobruchus* sp. by Dharmasena and Subasighe (1986). They reported that varieties with small seeds and glossy seed coat were shown to be associated with a higher degree of resistance than large seed with dull surface. Female bruchid find it more difficult to lay their egg on the highly convex and shiny surface of small seed.

The growth and development of bruchid (*Callosobruchus maculatus*) on rice bean (*Vigna umbellata*) and 16 genotypes of cowpea (*Vigna unguiculata*) were evaluated by Sandha *et. al.*, (1987) in laboratory experiment. The seed of genotype varied in size, colour, hardness and seed coat texture. In *Vigna umbellate,* larvae did not complete penetration into the cotyledon nor they complete their development, larvae death occurred in both decorticated and whole seed of *Vigna umbellata* indicating that neither the seed coat nor the cotyledon was responsible for resistance to the bruchid.

Ahmed *et al.* (1989) evaluated eighteen chickpea (*Cicer arietinum* L.) for their susceptibility to pulse beetle, *Callosobruchus maculatus* F. (Bruchidae) taking into account the number of undamaged seeds (resistance to bruchids), number of eggs oviposited (ovipositional preference), and number of emergence holes (adult survival) per 50 seeds. Resistance to bruchids appeared to be a more heritable trait than the other two damage characters. The number of emergence holes is a better indicator of seeds resistance than the number of eggs present on the seeds.

Baker *et al.* (1989) analysed fifteen lines of cowpeas, *Vigna unguiculata* (L.) Walpers for physical and chemical characteristics to study their relationship with resistance to *Callosobruchus maculatus* (F.). No significant differences were found ($P < 0.05$) when data for each characteristic were compared with *Callosobruchus maculatus* susceptible or resistant lines of cowpeas. However, numerous correlation coefficients between cowpea characteristics were statistically significant. Contrary to claims that elevated trypsin inhibitor levels may correlate with *C. maculatus* resistance.

Relative preference of the pest for different varieties of green gram was studied by Choudhary *et al.,* (1989). Green gram with larger seed size and thinner testa was preferred more as compared to small seed with thicker testa. Variety Kopergaon appeared to be the most preferred/susceptible and J-45 was least preferred and least susceptible for the development and survival of *Callosobruchus chinensis*.

Rasul *et al* (1989) tested varieties of grain legumes for their resistance to *Callosobruchus analis* under laboratory conditions. Mash- 59 (*Vigna mungo*), Mung- 6601 (*Vigna radiata*), Massor D/9-6 and 18-12 (lentils) were the least damaged varieties (5.68, 21.19, 3.33, 3.33 weight loss rest), while Mash-80 (*Vigna mungo*), Mung- 71-27 (*Vigna radiata*) and Precoz-355 (Lentils) were the most damaged (18.71, 28.45 and 7.33 % weight loss resp.).

Singal *et al.,* (1989) studied the effect of initial infestation of chickpeas, with one, two, three, four and five sexual pair of pulse bruchid, up to F_1 (one generation) and F_2 (two generation), a highly positive correlation (r=0.949) occurred between population in the F_1 and the number of pairs of beetle, but not up to F_2 generation. Significant correlations of r=0.921 and r=0.385 between grain damage (%) and final population also occurred for the F_1 and F_2 generations respectively, also have positive correlations (r=0.974 for F_1 and r=0.959 for F_2) between loss in grain weight. The coefficient of determination revealed that 97 % and 92 % variability in loss in weight was due to number of pairs of beetles initially released, their final population and percent grain damage for the F_1 and F_2 generation respectively.

Ten commonly grown cultivar of cowpea (*Vigna unguiculata*) Udaipur-1, Udaipur-2 PI-1309, PI 1414, P 456-157, IC-11352, Cs-152, Co-1 and Kanpur black (14/2-3) were evaluated for the extent of damage by and varietal resistance to (*Callosobruchus maculatus*) by Manoha and Yadava (1990) and observed that Udaipur-2 suffered the maximum loss of 44.97

percent in apparent weight, while Co-1 had the least damage of 16.25 percent. The loss in seed viability ranged from 9.0 on Co-1 to 49.34 percent on Udaipur-2. Udaipur-2 and Pl-1309 were most preferred for oviposition and adult development, while Co-1 was least preferred.

Ramzen *et al* (1990) reported damage caused by *Callosobruchus maculatus* to seeds of bengal gram (*Cicer arientinum*), black gram (*Vigna mungo*) black eyed cowpea (*Vigna unguiculata*), green gram (*Vigna radiata*), kabuli gram (*Cicer arientinum*), red and spotted bean (*Phaseolus vulgaris*), lentil, moth bean (*Vigna aconfifolia*), red gram (*Cujanus cajan*) and rice bean (*Vigna umbellata*) under natural storage condition in India. *Vigna unguiculata* (cowpea) had the greatest damage in terms of exit holes (69.2 %) and weight losses (34.5 %) followed by *V. acontifolia* (moth bean) (53.7 and 21.9 %), *Vigna radiata* (50.3 and 19.4 %). No damage to rice bean (*Vigna umbellata*) was recorded.

The growth and development of *Callosobruchus maculatus* on various pulse seed were studied in the laboratory. Shrivastava and Pant (1990) found that green gram (*Vigna radiata*), black gram (*Vigna mungo*), red gram (*Cajanus cajan*) and cowpea (*Vigna sinensis* / *Vigna unguiculata*) were the most susceptible to the pest. Lentil, Bengal gram (*Cicer arientinum)*, Pea, *Lathyrus sativus* and yellow/black-seeded soyabean were less preferred, while french bean (*Phaseolus vulgaris*) was unsuitable for development of the pest.

Singh and Singh (1990) tabulated data on seed damage, seed weight reduction and oviposition frequency for 13 cultivars studied. They reported that JU 78-3, RU-2, T-9 and JU 78-27 were free of *Callosobruchus chinensis* infestation.

Late ane Efovbokhan (1991) compared three new high yielding and earlier maturing cultivars (TVX-3236, JT-82D889 and

IT-82E18) with traditional cultivar for their resistance to *Callosobruchus maculatus*, prameters used for comparison were mean number of egg laid on the seeds of each cultivar, mean percentage adult emerge, mean daily adult emergence and mean percentage larval mortality. TVX-3236 and IT-82D889 demonstrated significant resistant to *Callosobruchus maculatus*. Compared with traditional cultivar, this cultivar caused both significant larval mortality and a reduction in oviposition, *Callosobruchus maculatus* showed significant preference for IT- 82 E18 laying significantly more eggs on it than on traditional cultivar. It was suggested that a seed coat oviposition deterrent factor (s) and a cotyledonal antibiotic factor (s) may be involved in conferring polygenic resistance in TVX- 3236 and IT-82D889.

Damage caused by *Callosobruchus maculatus* to four varieties of stored seed of *Vigna radiata* and five varieties of soybean were assessed. Osman *et al* (1991) stated that in *Vigna radiata* the number of bruchids was highest in cv. MG50-10A and lowest in V 1968. The damage after 3 months was 100 percent and therefore the germination rate could not be assessed. In soybean, the percentage of damaged seed was low for all varieties and germination rate was highest in cv. Palmetto.

Sixteen mung bean (*Vigna radiata*) genotypes were evaluated by Sarkar *et al* (1991) for resistance to stored product pest *Callosobruchus maculatus*, on the basis of healthy seed (resistance seed), number of egg deposited (oviposition preference) and total emergence hole (adult survival) for 50 seed. All 3 characters were highly heritable, but ovipositional preference was slightly lower in heritability than other 2. Resistance was negative function of adult survival and showing the importance of these characters for selecting bruchid resistant varieties.

In laboratory studies, 13 varieties of mung bean (*Vigna radiata*) were screened for resistance to *Callosobruchus maculates* and lowest feeding damage and adult emerge were recorded with Cv. PDM-14 (Ranganath and Ram 1992).

The characteristics of resistance to *Callosobruchus chinesis* in two mungbean (*Vigna radiata*) and one black gram (*Vigna mungo*) accession were investigated in a series of laboratory tests (Talekar and Lin, 1992). Oviposition and emergence of 1st generation of *Callosobruchus chinesis* were significantly reduced with pods of two mung bean accessions, (V-2709 and V-2802) compared with the susceptible check VC-1973A. The smaller seed size of the 2 resistant mung bean accessions and one resistant black gram accession, VM-2164 was not responsible for the resistance. Artificial seeds were prepared from mixture of seeds of resistant and susceptible accessions. As the concentration of resistant accession increased, the number of *Callosobruchus chinensis* adult emerging after feeding as larvae in such artificial seeds decreased. Adults that emerged from the artificial seeds made from mixture of resistant and susceptible accession were significantly higher than from the susceptible accession alone. The results indicated the possible presence of antibiotic factors in resistant accession.

In laboratory studies, Dhepe *et al* (1993) stated that the germination of different varieties of green gram (*Vigna radiata*), red gram (*Cajaanus cajan*) and soyabean were affected to varying degree by infestation with *Callosobruchus maculatus*.

Dogo Seck, (1993) studied seeds of 80 varietiesfrom the sanegal cowpea breeding programme collected for bruchid resistance in five replication and reported significant diference among the varieties in oviposition, progeny and bruchid emergence. The variety 58-57 which was largly grown in Senegal appeared highly susceptible. On the other hand, 6 varieties (59-12, 58-28, 66-50, 66-5, 58-16 DI and 59-26) showed a high level of resistance.

Seeds of 24 accessions of pigeonpea (*Cajanus cajan* (L.) Millsp.) and four other species of *Cajanus* (formerly *Atylosia*) were evaluated for their resistance to infestation by *Callosobruchus maculatus* (F.) (Dongre *et al.,* 1993). None of the pigeonpea accessions were resistant but resistance was evident in three species of *Cajnaus*. In *Cajanus platycarpus* most of the larvae failed to enter the hard seed coats but the few which did enter the seeds, developed normally. Adults did not emerged from seeds of *Cajanus scarabaeoides,* even though most of larvae entered seeds. In *Atylosia sericeus,* number of larvae entering the seeds as well as adult emergence was significantly reduced.

Seventy-five cultivated accessions and two wild progenitors of black gram (*Vigna mungo*) and green gram (*Vigna radiata*) were evaluated for their resistance to infestation by *Callosobruchus maculatus* (Dongre *et al.* 1996). None of the cultivated accessions either of black gram or green gram was reported to be resistant. However, resistance was evident in a wild progenitor of black gram (*Vigna mungo* var. silvestris). The mechanism of resistance of *Vigna mango* var. silvestris was examined and it was found to be larvae antibiosis expressed as reduced survival, longer developmental period and reduced body weight.

Several mung bean entries were screened for resistance to *Callosobruchus chinensis* (Sison *et al.* 1996). Results for both free choice and no-choice test showed that TC- 1966 was highly resistant, while Pagasa series (1, 3, 5 and 7) were susceptible. The life history of *Callosobruchus chinensis* was also studied on the susceptible variety Pag-asa-7. It was taken about

21.30 days for the bruchid to complete development (egg to adult development).

Chavan *et. al.,* (1997) studied ovipositional preference of *Callosobruchus chinensis* for 70 cowpea lines. The lines with rough seed surfaces were less preferred, resulting in a smaller percentage of grain infested with eggs and smaller number of egg for grain. No antibiosis effect in the viability of eggs was noticed. *Callosobruchus chinensis* distributed eggs uniformly on seed of different cowpea lines and oviposited a small number of eggs per grain. Brown, black gray and red colour seeds were more preferred than white coloured seeds.

Muhammad *et. al.,* (1997) evaluated eight different varieties of mung bean (*Vigna radiata*) (MB-26, MB-48, MB-55, MB-63, MB-66, MB-87, MB-246 and Kanti) for susceptibility to *Callosobruchus chinensis,* on the basis of the number of egg laid, duration of development of immature stages, percentage of adult emerge and weight loss due to damage by the pest. The strains MB-246 and Kanti were found to be highly susceptible, with 13.6 and 13 % loss in weight of seed respectively, strains MB-86, MB-26 and MB-66 were susceptible and strain MB-69, MB-48 and MB-55 were moderately susceptible. The size colour and protein content of seeds had no influence on the susceptibility of mung bean seed to *Callosobruchus chinensis.*

Rahman and Schmidt (1997) studied five different pulses *Lablab purpureus, Vigna radrata, Vigna unguiculata, Vicia faba* and two varieties of *Phasrolus vulgaris* for their susceptibility to *Callosobruchus phaseoli* using choice and non-choice test in laboratory. The bruchid showed various responses to seeds with regard to oviposition, larval development and adult emergence. Considering all aspects, an insect appeared to be adapted to seed of *Lablab Purpureus,* but did not develop in the seed of *Phaseolus vulgaris.*

Ming (1998) studied both artificial and natural infestation of *Callosobruchus chinensis* for identification of resistance sources of mung bean (*Vigna radiata*). Some 80 germplasm lines from Asia vegetable Research and Development Center (AVRDC) and 784 domestic germplasm lines were tested. The rate of damaged mung bean seed was used as an evaluation, into some 17 germplasm lines from AVRDC showed moderate resistant to immunity to *Callosobruchus chinesis* while only 3 landraces from Guangxi showed moderate resistance among domestic germplasm.

Nine different varieties of cowpea (*Vigna unguiculata*) were evaluated for their relative susceptibility to pulse beetle *Callosobruchus chinensis.* Based on oviposition preference, growth and damage as the main criteria of evaluation, cv. V-218 and EC-1705851 were found to be substantially resistant against bruchid infestation (Sharma *et al* 1998).

Ramanagoudar *et al.,* (1998) estimated seed parameters *viz.,* seed size, test weight, seed colour, protein content and tannin content in 23 genotypes of horse gram. These genotypes were screened for their relative susceptibility to pulse beetle (*Callosobruchus chinensis*) by artificial inoculation. The criteria considered were total number of adult emerged, level of seed infestation and percent seed weight loss, seed size and test weight. These parameters were positively correlated with relative susceptibility parameters.

Varieties of mung *[Vigna radiata]* (321, 6601, 141, No1 and 72), mash *[Vigna mungo]* (80, 48, 59, 118 and AARIM 117) and chickpea (86208, CM-72, 86221, 86037 and C-44) were tested for their relative resistance against *Callosobruchus chinensis* under controlled laboratory condition (25-30^{0}C) (Ashraf *et al.* 1999). Differences in oviposition, percent adult emerge, percent number of seed bored and percent weight loss caused by *Callosobruchus chinesis* were reported as significant. Among varieties, mung 141 (27.2 % wt loss), Mash AARIM 117 (27.5 % wt. loss) and chickpea C44 (13.7 weight loss) were relatively susceptible to *Callosobruchus chinensis* (L.) where as mung No1, Mash 59 and Gram 86037 appeared to be resistance.

Kalyan and Dadhich (1999) screened the ten varieties of green gram for reaction to *Callosobruchus chinensis* and reported that none of the varieties was completely resistant. R-288-8 and K 851 was found to be least susceptible. PDM-219, RMG-62, RMG-148, RMG-345 and RMG-343 to be moderately susceptible while Pusa 105 and PS 16 were highly susceptible to the pest.

Twenty six mung bean (*Vigna radiata*) varieties and accessions were screened for resistance to four bruchid species (Coleptera : Chrysomelidae). On the basis of percentage of seed damaged, all Australian commercial mung bean varieties tested were highly susceptible to strains of C*allosbruchus maculates* and C*allosobruches chinesis*, the 2 species that cause most damage worldwide to mung bean in storage. These accessions (TC-1966, ACC-23 and ACC-41) appeared to have bruchid resistance. Lambrides and Imrie (2000) reported all 3 varieties are member of the subspecies sublobata, and typically have wild mung bean characteristics of small seed size, and the presence of well-formed texture layer on the seed. These characters may act as oviposition deterrents. Consequently, these assays for determining resistance to bruchid infestation may not be suitable for identifying biochemical resistance of some mung bean genotypes.

The physical basis of the resistance of soyabean (PK-416, SL-295 and Punjab soybean No. 1) and cowpea (cowpea- 74 and cowpea-88) cultivars to *C. maculate* were studied by Harminder *et al.* (2001) under laboratory conditions. Seed size was positively correlated with oviposition preference. Cowpea-88 having large size seed and darker seed coat was most preferred for oviposition. The soybean cultivars received less number of eggs because of their shiny and convex seed surface and creamy seed coat. Punjab soyabean No. 1 had the hardest seeds followed by cowpea- 74, SL-295, PK-416 and cowpea-88. Seed hardness was negatively but non significantly correlated with percent adult emerge seed coat thickness was maximum in Punjab soyabean No. 1, followed by SL-295, Cowpea- 88, PK-416 and Cowpea-74. Cowpea-74 which had thinner seed coat compared to cowpea- 88 was most preferred by *Callosobruchus maculates.*

A laboratory experiment was conducted by Patro *et al* (2001) to study the effect of different levels of beetle (1 pairs, 2 pairs, 4 pairs, 8 pairs) of *Callosobruchus* sp. infestation on seed determination of pigeon pea (var. UPAS 120) and green gram (variety K-851) under ambient conditions (27± 4^0 C temperature and 84 ± 10 % relative humidity). The result indicated that both pigeon pea and green gram the level of bruchid infestation was directly proportional to population count and percent infestation but inversely proportional to germination percentage.

Shashi *et al.* (2002) evaluated seeds of sixty-three genotypes of cowpea, V*igna ungiculata* for their relative susceptibility to cowpea weevil, *Callosobruchus maculates* under artificial infestation, conditions based on several parameters, including development period, percent adult emerged, average female percent, growth index, percent loss in weight. None of the accessions was found to completely inhibit the development of

Callosobruchus maculatus. The accessions, however displayed different suitability for the development of pulse beetle genotype IC-58905, EC-240922A and NIC-1066 with growth indices 0.96, 1.06 and 1.30 respectively, were found to be superior to others in terms of bruchid resistance/ tolerance. Growth index of *Callosobruchus maculates* and percent loss in weight was positively correlated.

Umrao and Verma (2002) evaluated twenty pea varieties against *Callosobruchus maculates* for physical character (hardness, moisture and damaged grain) and fecundity, F_1 progeny and index susceptibility. The hardness of the grain showing significant negative correlation with F_1 progeny (-0.785) and index susceptibility (-0.984). It means the increase in hardness resulted decrease in growth and development and vice versa. The moisture content of the grain having significant positive correlation with fecundity (0.517) and index susceptibility (0.499). It means the increase in moisture content will certainly increase the growth of pest and vice versa. The weight loss and damaged grain showing significant positive correlation with F_1 progeny and index susceptibility. It means increase in fecundity, F_1 progeny and index susceptibility will certainly increase loss in weight and damaged seed and vice versa.

Kashiwaba *et al.*, (2003) evaluated the resistance of wild and cultivated rice bean (*Vigna umbellata*) to three bruchid species, *Callosobruchus chinensis*, *Callosobruchus maculates* F. and *Callosobruchus analis*. All but three accession of cultivated and all wild rice bean accessions tested, exhibited complete resistance to three bruchid species. The results revealed that a chemical compound contained in the cotyledon of rice bean has an inhibitory effect on the growth of these bruchid species.

Green gram was found to be most preferred host for oviposition followed by black gram and rice bean. Similar results have been reported by Obaidullah *et al.,* (2003), Ghosal *et al.,* (2004).

Chakrabarty *et al.,* (2004) studied the relative susceptibility of some mung bean (*Vigna radiata*) genotypes to pulse beetle and their correlation with different seed parameters. The susceptibility parameters, *viz.,* percentage of affected seeds, number of eggs laid, number of adult emerged and percent weight loss were found to have significant and positive correlation with seed weight but were negatively and significantly correlated with seed coat. The moisture and protein content of seeds were found to have no influence on susceptibility of mung bean to pulse beetle. High positive correlation between weight loss and number of adult emerged, percent adult emerged was reported by Babu (2000). Highly significant positive correlation between adult emerged with weight loss and damaged seed in mung bean were recorded by Khattak *et al.,* (1987).

2.2 Biochemical parameter

2.2.1 ∝- amylase inhibitor

The fourth protein in the PHA (phytohemagglutinin) family, ∝-amylase inhibitor is powerful inhibitor of mammalian and insect ∝-amylases. The α-amylase inhibitor generally do not affect endogenous α-amylases except in few noted instances and are not thought to be important in the regulation and mobilization of starch metabolism. When this protein was fed to insect larvae in an artificial seed system @ 0.2 per cent (w /w) was enough to inhibit the development of bruchid beetle larvae quite strongly (Ishimoto and Kitamura, 1989). The resistance of common bean seeds to cowpea weevil has been correlated to the content *of* higher levels of a- amylase inhibitor in seeds (Huesing *et al.,* 1991), The common been seed contains a glycoprotein that inhibit the activity of mammalian and insect a-amylases, but not a plant α-amylases. It is therefore, classified as antifeedant or seed defense protein (Moerno *et al.,* 1996). 'The effect of α-amylase inhibitor were tested against insect storage pest both in *vitro* against insect ∝-amylases and in *vivo* in insect feeding trials. It was found that ∝-amylase inhibitor was effective against the growth of larvae of storage pests like *Collosobruchus maculatus* and *Trifolium confusum* (Gatehouse *et al.,* 1986). Common bean, which are resistant to bruchid beetle, are known to contain high alpha amylase inhibitor protein and the gene responsible for this protein has been successfully tranfered in peas seeds. The level of alpha amylase inhibitor in pea seed was as high as in bean seed (Shade *et al.,* 1994, and Schroeder et al 1995).

Seeds of eight lines differing in storage pest resistance were analyzed for inhibitors of the enzymes porcine amylase, Bacillus amylase, bovine chymotrypsin and trypsin. Piergiuvinni *et al* (1994) observed that a broad variation among sample for all tested inhibitors. Principal component analysis indicated that resistant lines were characterized by high level of both antitryptic and anti amylasic activity. Moreover, high activity of a simple inhibitor class was typical of the bruchid (*Callosobruchus chinensis*) susceptible lines. Hence breeding for high content of this protein inhibitor could be an effective way of obtaining lines that are naturally resistant to storage pest attack. A limiting factor in this breeding strategy is the need to reduce the antinutritive activity before eating.

2.2.2 Trypsin inhibitor

The trypsin inhibitor activity in rice bean cultivars ranges from 15.30 to 28.04 TIU/ml (Singh *et al.,* 1985). The trypsin inhibitor activity in cowpea reported up to 21.1 TIU /mg dry matter (Valdebouze *et al.,* 1980) and 15.9 TIU/mg dry matter (Roman *et al.,* 1987).

The poor nutritive value of raw edible legume is usually attributed to the presence of large number of heat labile inhibitors (Liener and Kakade, 1980). The microwave and conventional cooking for 15 to 20 min. results in 92 to 97 per cent reduction of trypsin inhibitor in cowpea (Chung *et al.,* 1981). The trypsin inhibitors are widely distributed in legume and pulses seeds. Although their function in plants are obsecure, several roles have been proposed for them including control of protein hydrolysis and resistance to diseases and insects (Chen and Mitcheli, 1973). Certain inhibitors e.g. Bowman birk inhibitor from soybean and trypsin inhibitors from limabean in artificial seed system have adverse effect on burchid growth and development (Shade *et al.,* 1994). The bruchid beetle cause extensive damage to seeds of cowpea during storage, the resistant variety contained a significantly higher level of inhibitors, about twice as much as any other varieties. Insect feeding trials in which various protein fractions were added to a basic meal confirmed the antimetabolic nature of cowpea trypsin inhibitor and the albumin protein of cowpea (containing trypsin inhibitor) at a level of 10 per cent was toxic to larvae of bruchid beetle (Gatehouse, 1979). The cowpea trypsin inhibitor is an effective antimetabolite of bruchid beetle in insect feeding trials (Gatehouse and Boulter, 1983). Besides *Bacillus thuriengiensis* (Bt) gene alternative insect resistance gene such as serine proteinase inhibitor gene have been transfered in tobacco (Hilder *et al.,* 1987). In recent years gene transfer to confirm protection against insect-pest *of* seeds in post harvest storage have been attempted, the cysteine proteinase inhbitor was found to be toxic to cowpea weevil (Murdock *et al.,* 1988).

Fifteen lines of cowpea *Vigna unguiculata* (L.) walper were analyzed for physical and chemical characteristic to study relationship with resistance to *Callosobruchus maculatus.* Richard *et al* (1989) observed no significant difference (P<0.05) when data for each character were compared between *Callosobruchus maculatus* susceptible and resistant lines of cowpea. However numerous correlation coefficients between cowpea characters were statistically significant contrary to claims that elevated trypsin inhibitor level may correlate with *C. maculatus* resistance. Trypsin inhibitor activities in seed did not differ significantly between resistant and susceptible lines nor was the level of trypsin inhibitor correlated to *C. maculatus* developmental time or mortality

Trypsin inhibitor content was determined in the seeds of 21 cowpea (*Vigna unguiculata*) lines (10 known to be resistant to *Callosobruchus chinensis and* 11 susceptible), while fatty acid composition was examined in 58 lines (11 resistant and 47 susceptible). Piergiovanni *et al* (1990) observed wide range in trypsin inhibitor content but these were not related to level of resistance. Total unsaturated fatty acid content, however, were associated with resistance level, being the lowest in resistant lines six fatty acid varieties differing significantly between resistant and susceptible lines were identified.

Seeds of cowpea (*Vigna unguiculata*) line, TVU-2027 is moderately resistant to the bruchid *Callosobruchus chinensis,* larvae exhibit higher mortality and take longer time to develop in TVU-2027 than in more susceptible seed. Claim has been made that an elevated level of cowpea trypsin inhibitor of CPTI confers resistance to TVU-2027 (Zhu *et al.* 1994). To test this hypothesis CPTI was isolated and purified and its impact on *Callosobruchus chinensis* evaluated using an artificial seed system. CPTI at dietary level up to 2 percent caused no significant increase in mortality of *Callosobruchus chinensis* and only slight delays in within seed developmental time. These results do not support the hypothesis that CPTI is the basis of resistance in TVU- 2027.

Five green gram varieties *viz.* Mg-161, M-3, Co-2, Pusa-8793 and Pusa baisakhi were analyzed for phytic acid, tannic acid and trypsin activity. Philip and Prema (1998) observed significant varietal variations in tannic (310 to 400 mg), phytic acid (201.33 to 265.33 mg) and trypsin inhibitor activity (55.74 to 97.70 TIU/mg).

Seeds of various wild and cultivated pulses (grain legumes) express varied degrees of resistance to damage by *Callosobruchus maculatus* , a major pest of stored pulse. Seeds were analyzed for trypsin/chemotrypsin inhibitor (qualitatively and quantitatively) and protein profiles (SDS PAGE) to study the chemical basis of resistance to bruchid infestation. Ignacimathu *et al* (2000) reported that seeds of the wild pulses *Dunberia fearuginca Neonotonia wightii, Trphrosia cavorensis, Cajanus albican's* and *Vigna bourneaus* differ from other wild germplasm as well as cultivars having characteristically higher levels of trypsin / chrmotrypsin inhibitors. SDS-PAGE analyses of stored seed protein of *Dolichos lablab* and *Rhyncorea canareveal,* the presence of high level of non nutrient chemical such as vicilins, amylase inhibitors, and lectins in quantities that may impart resistance to *Ç maculatus.*

Naik and Jadhav (2002) studied ∝-amylase inhibitor, trypsin inhibitor and chlorogenic acid content of rice bean (*Vigna umbellata*) and mung bean seed meal showed significant differences. All the three parameters were positively correlated with resistance offered to storage grain pest. The in vitro alpha amylase inhibitor potential of rice bean when tested against procine pancreatic alpha amylase was almost twice as that of mung bean. Trypsin inhibitory potential and chlorogenic acid content was also significantly higher in rice bean which is least attacked by stored grain pest.

2.2.3 Crude protein

Rice bean is rich is protein content which is quite comparable to other major pulses. The crude protein in rice bean has been reported to vary from 16 to 27 per cent depending upon cultivars (Kuizuma and Miura, 1974, Gupta, 1982a, Singh *et al.* 1980 and Kapoor *et al.* 1972). The crude protein content of major pulses like cowpea, chickpea and mung bean has been reported upto 24.6 per cent, 19.5 per cent and form 22 to 25 per cent respectively (Kaul, 1971 and Singh *et al.,* 1968).

Umrao and Verma (2003) studied protein composition of twenty pea genotypes for preference of *Callosobruchus chinensis* Linn. It was found that low protein content (21.51, 21.87, 22.06, 22.07, 22.09 and 22.47 per cent respectively) genotypes (DMR-29, HPMR-186, HtJP-2, KPMR-347, KPMR-327 and HuDP-19) were least susceptible while higher protein content (24.24, 23.72, 23.61, 23.53, 23.42 and 23.24 percent) genotypes (HuP-13, MDP-3, MDP-1, KPMR-389, KPMR-157 and KPMR-397 respectively) were highly susceptible to pulse beetle. The relationship of grain protein with the infestation (Fecundity, F_1 progeny and index susceptibility was positive and significant. It means the increased protein content of pea genotypes certainly increase the infestation.

2.2.4 Total phenols and Tannic acid

Farkas and Kiroly (1962) and Fernanadez and Health (1989) reported that responses of plants to pathogens are characterized by the early accumulation of phenolic compounds at the infection site and that limited development of pathogen a result of rapid (hypersenistive) cell death. It is thought that rapid accumulation of phenols may result in the effective isolation of the pathogen at original site of ingress.

Phenolic compounds are oxidised to highly reactive quinones, which are effective inhibitors of enzymes having sulfhydral groups thereby preventing metabolic activities of host and parasite cells (Rubin and Artsikhovskaya, 1963).

The phenolic compounds (phenolic acid, flavonoids) and enzymes related to their metabolism (perioxidase, polyphenoloxidase and phenylalanine ammonialyase) are widely implicated in resistance to infection by plants to different insects, pathogenic bacteria, fungi, or Viruses (Panda, 1979), Jambunathan *et al.* 1986). Phytoalexin, Cajanone are the phenolic compounds in the roots of pigeonpea have the ability to inhibit completely the growth of *Fusarium udum* (Preston, 1977). The phenolic compounds flavan-4ols were responsible for mold resistance in sorghum (Jambunathan *et al.,* 1986). The wheat cultivars resistant to loose smut had a great number of phenolic compounds than susceptible cultivars (Gupta *et al.,* 1978).

Phenolic compounds are widely distributed in higher plant. Their concentration is usually higher in resistant than susceptible genotypes of different crop plants (Arora and Wagle, 1985).

Matern and Kneusel (1988) reported that the defensive strategy of plants exist in two stages. The first is assumed to involve the rapid accumulation of phenols at the site of infection, which function to slow the growth of the pathogen and to allow for the activation of Secondary strategies that would more thoroughly restrict pathogen. Secondary response will involve the activation of specific defenses such as de nova synthesis of phytoalexin or other stress related substances. Thus sequence of events in a defense response can be thought to include host cell death and necrosis, accumulation of toxic phenols, modification of cell walls by phenolic constituents or physical barriers such as appositions or papilae and finally the synthesis of specific antibiotic such as phytoalexin fungitoxic substances which inhibit the growth of the micoorganisms pathogenic to plants.

Haque *et al* (1992) screened 150 genotypes of *Vigna mungo* for susceptibility to *Callosobruchus maculatus* at 30^0 C and 70 % R.H. The genotype SBG No. 3 and UH 83-2 were least susceptible (3.0-3.5 % wt. loss) while Pu-26 and UH 132-46 were the most susceptible. Smoothness and thickness of seed coat and size and mineral content of the seed were positively correlated with susceptibility. Protein, crude fiber and phenolic contents appeared to impart resistance. Hatchability of eggs, survival, numbers of F1 adult and growth index were significantly greater in highly susceptible genotypes. The net reproductive rates and population increases per weak on the genotypes UH 82-46, Pu-26, SBG No. 3 and UH 83-2 were 60.0, 43.9, 22.14 and 40.3 and 101, 87, 60 and 78 % respectively.

The association between some physical characteristic and tannic content of cowpeas and their susceptibility to infestation by bruchid *Callosobruchus maculatus* was investigated. Oigianghe and Ongbinde (1996) reported that the physical characteristic were coal colour and text, seed height, length and width and thickness of the seed coat. The dimensional parameters showed a significant (PC< 0.05) correlation with the number of egg laid with height accounting for about 70 percent of variance. The same parameter accounted for 77 percent of variance in the number of F_1 progeny. The tannic acid content, however, become increasingly with the growth of larvae to adulthood. The tannic acid content for 14.3 and 39.9 percent for the variance in the number of F_1 progeny and percentage adult emergence respectively.

Marconi *et al.* (1997) reported that significant positive correlations were found between seed resistance to bruchids and trypsin inhibitor and tannic acid in V. Vexillata.

2.2.5 Peroxidase and polyphenol oxidase

The results obtained from the analysis of five varieties of Bringal indicated that the round green was best for storage and resistance to injury and diseases having high peroxidase activity (Aluko and Oghadu, 1986; Karla et al., 1986). *Pernosclerospora sorghi* cause downey mildew, a major problem in sorghum production, the resistance sorghum line (QL-3), resistant to P. sorghi because of high peroxidase and polyphenol oxidase in it (Bhawanishankar Gowda et al., 1989). The resistance variety of cotton to *Alternaria macrospora* was due to higher levels of peroxidase and polyphenol oxidase activity in it (Bhashakaran et al., 1975). Department de biologia, chil reported the possible role of peroxidase in lignincation during germination and early development and in repair of seed injury before and during germination.

2.3 Electrophoresis studies

Electrophoretic varation was used to identify yellow mosaic resistant, tolerant and susceptible lines at early stages of plant growth. Five enzyme system, i.e. peroxidase (po), Catalase (Cat), Polyphenoloxidase (Ppo), Esterase (Est) and ribonuclease-1 (Rnase) were studied (Malik *et al.* 1996). Po bands number 5, 7, 8 and 12 in 6 day old catyledons, Cat 1, 2, 3 and 4 in 3, 6 and 12 day old tissue. Ppo 6, 7, 9 in 3 and 6 day old tissues, Est 1, 3, 5, 6 and 8 in sand 6 day old seedling and Rnase 1, 2, 3, 4 and 5 in 3, 6 and 12 day old tissues were the most prominent bands which can be used as biochemical marker for identification of resistant, tolerant and susceptible soyabean lines at these specific stages.

SDS-polacrylamide gel electyrophoresis of total seed proteins of 37 mungbean genotypes evidenced for the pressure of 23 polypeptide bands dispersed over five zones, A to E within a molecular weight range of 17.4 to 75.0 kD (Naik and Kole, 2001). The banding patterns exhibited polymorphism for the presence of a band of 62.4 kD, B_1 or two bands present jointly (61.0 and 58.2 kD), B_2 + B_3, indicating absence of gene (s) involved in post translational dissociation in the genotypes without these two bands. Another band of 30.2 kD, D_2 (D_{2a}) had a high molecular weight variant (D_2^{β}) of 31.3 kD. This is the first report on a detailed electrophoretic banding pattern of mung bean based on a large number of Indian cultivars.

2.4 Seed Mycoflora study

Highest counts of Fusarium monoiliforme from seeds of Vigna radiata were detected by agar plate method and *Fusarium semitectum* by standard blotter method (Baghel *et al*, 1985).

Seed borne fungi of mungbean were detected by standard blotter and agar method (Singh *et. al.*, 1973). The fungi found were *Aspergillus flavus, Aspergillus niger, Chaetomium olivaccus, Fusarium oxysporum, Penicillium ctrysogenum, Rhizopus orhizus, Rhizoctonia bataticola* and *Sporotrichum* sp.

Bilgrami *et al* (1976) reported a large number of fungi associated with mung bean and udid bean seed in storage. The frequency of *Aspergils flavcs* was the highest.

Saxena and Sinha (1977) reported *Alternaria alternata, Fusarium monoliforme, Macrophomina phaseolina, Curvularia lunata* and *Apsergillus niger* associated with the black gram seed.

Seed borne fungi of black gram were detected by standard blotter and agar method reported *Aspergillus niger, Curvularia* sp. *Fusarium equiseti, Fusarium oxysporum, Penicillium* sp. and *Phoma glomerata* were reported to be associated with black gram seed (Singh *et al.,* 1977). Reduction in seed germination and seedling vigour resulted from infection with some of these fungi.

Rashid *et al* (1983) examined 15 seed samples of five *Vigna radiata* cultivar. The isolates identified were *Alternaria alternata, Colletotrichum dematium, Curvularia lunata, Fusarium equiseti, Fusarium semitectum* and *Macrophomina phaseolina. Curvularia lunatus* were most prevalant followed by *Fusarium equiseti.*

Green gram varieties J-781, Phule M-1 Pusa Vaishakhi and S-8 were tested under lab condition for the presence of seed borne fungi by a standard blotter paper and agar plate method by Patil *et al* (1990). He recorded 18 species of fungi belonging to 14 genera. Out of which 8 fungi viz. *Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Curvularia geniculata, Fusarium moniliforme, Fusarium oxysporum, Macrophomina phaseolina, Rhizoctonia bataticola* were observed to be pathogenic.

Teggi and Hiremath (1990) isolated 11 species of fungi from seeds of 4 varieties of *Vigna radiata.* The fungi *Alternaria alternata. Cladosponum fulvam, Fusarium moniliforme, Aspergillius flavus, Aspergillus niger* and *Trichothecium roseum* were predominant.

Samples of *Vigna radiata* seed from different locations of Madhya Pradesh yielded, isolates of 18 fungi among which *Aspergillus* sp., *Botrytis cinerea, Penicillium* sp., *Fusarium* sp. and *Macrophomina Phaseolina* were predominant (Thakur *et al.*,1990).

Sharma and Ray (1991) reported fungi viz. *Aspergillius flavus, Aspergillus niger, Aspergillus terreus, Fusarium moniliforme, Fusarium roseum, Penicillium chrsogenum Rhizopus oryzae* were predominant on *Vigna radiata* seeds.

Ahmed (1993) reported *Alternaria, Cladosporium, Fusarium, Curvalaria* species to be predominant during seed storage of *Vigna mungo.*

Raut and Ahire (1998) detected 14 fungi from the seed of 10 cultivars of *Vigna radiata* from different localities in Madhya Pradesh. These were *Aspergillus* sp., *Curvularia lunata, Fusarium* sp. and *Macrophomina phaseolina,* detected in all parts of seeds.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation entitled "Biochemical basis of resistance to stored grain pest (*Callosobruchus chinensis*) of different *Vigna* species" was conducted at Department of Agricultural Botany and Seed Technology Research Unit of Mahatma Phule Krishi Vidyapeeth, Rahuri. The details of material used and methods followed have been given in this chapter.

3.1 Materials

Pure seeds of green gram (*Vigna radiata*) and black gram (*Vigna mungo*) genotypes required for the present investigation were obtained from the Pulses Improvement Project, M.P.K.V., Rahuri. Seeds of rice bean (*Vigna umbellata*) genotypes were obtained from AICRP on Under Utilized Crop Plant Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. Salient features of the genotypes used for study are given in Table 1.

Table 1: Salient feature of green gram, black gram, and rice bean genotypes.

Sr. No.	Genotype	Evolved at	Salient feature
Green gram			
1	PM-2	MPKV, Rahuri	Stable, high yielding
2	PM-9339	MPKV, Rahuri	Bold seeded, high yielding, resistant to powdery mildew
3	AKM-8802	Dr. P.D.K.V., Akola	High yielding
4	TARM-18	Dr. P.D.K.V., Akola and BARC	Small seeded, high yielding, powdery mildew resistant
5	Kopergaon	Nagpur	Stable, high yielding bold seeded
Black gram			
1	TAU-1	Dr. P.D.K.V., Akola	High yielding, Bold seeded
2	TAU-2	Akola and BARC	Early maturity, bold seeded, high yielding

Sr. No.	Genotype	Evolved at	Salient feature
3	TPU-4	Rahuri and BARC	Bold seeded early maturity, high yield
4	PU-9503-8	Promissing genotype MPKV, Rahuri	Bold seeded, resistant to powdary mildew
5	PU-9205-11-3	Promissing genotype MPKV, Rahuri	Bold seeded, resistant to powdary mildew
Rice bean			
1	RBL-36	PAU, Ludhiana	High yield
2	RBL-50	PAU, Ludhiana	High yield
3	RBL-99	PAU, Ludhiana	High yield
4	LRB-199	PAU, Ludhiana	High yield
5	LRB-202	PAU, Ludhiana	High yield

3.2 Methods

3.2.1 Evaluation of level of resistance to bruchid

The experiment was conducted to evaluate the level of resistance of seeds of green gram, black gram and rice bean against pulse beetle (*Callosobruchus chinensis*) under ambient laboratory conditions for a period of 40 days (i.e. only to study the first generation of the *Callosobruchus chinensis*) during October-November 2002.

3.2.1.1 Maintenance of colony of Pulse beetle

The initial culture of pulse beetle (*Callosobruchus chinensis*) was obtained from the storage godown of seed cell, Mahatma Phule Krishi Vidyapeeth, Rahuri.

To initiate culture, ten pair of adult pulse beetle were isolated and released into healthy seed of local green gram genotype and kept in 32 x 22.5 cm cylindrical jar. The top of jar was covered with muslin cloth, secured firmly by rubber band. After emergence of new adult, the beetles were introduced into green gram seed kept in a series of cylindrical jar for building up a homogenous

PLATE1: Male and Female of pulse beetle

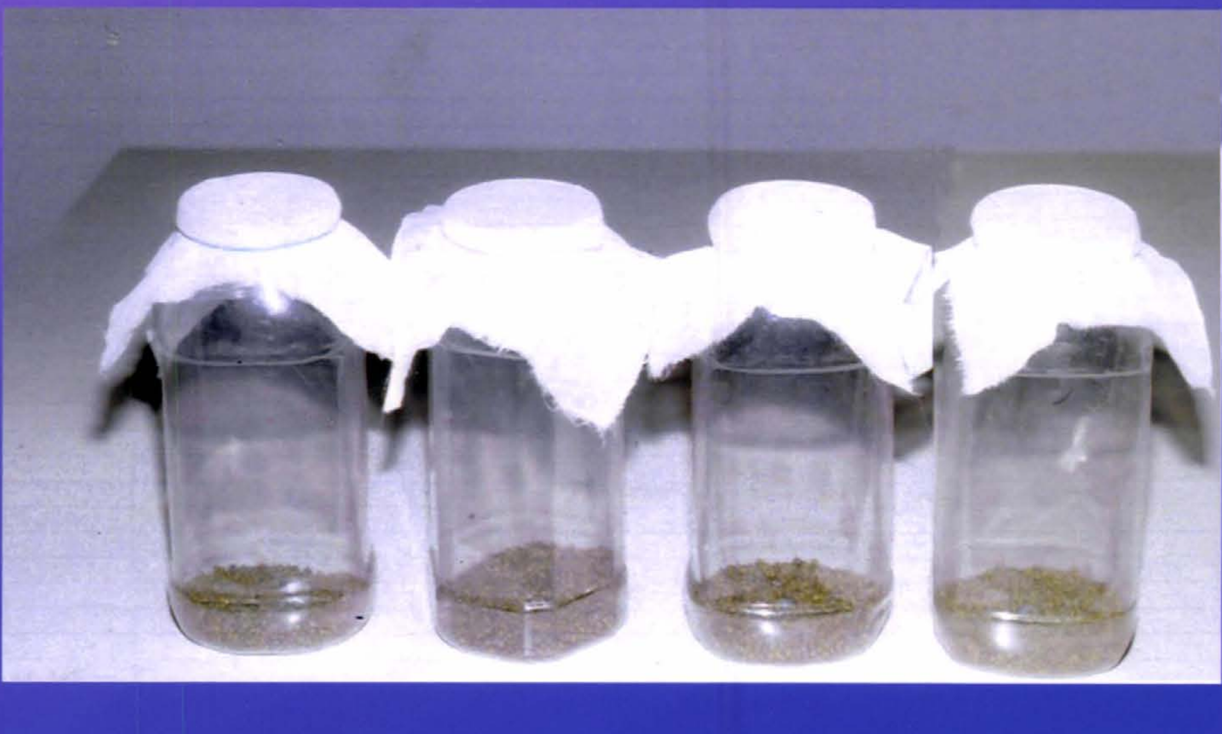


PLATE 2 : Maintainance of colony of pulse beetle

population. Density of population per jar was standardized to prevent crowding and to avoid the risk of producing less reproductive forms (Sano, 1967). Permanent colony was maintained as per the procedure described by Strong *et al.,* (1986). Adult of uniform age were used from this colony for the experiment. The sex of adult had been determined by the characters given by Halstead (1963). These studies were conducted at 25± 1°C temperature and 65-70 per cent relative humidity.

3.2.1.2 Artificial infestation of pulse beetle

The seeds of green gram, black gram and rice bean obtained were dried in bright sunlight in the month of May 2002 for three days and then carefully fumigated with aluminum phosphate separately to avoid the residual infestation of pulse beetle. About one kg capacity plastic jar (32 x 22.5 cm) was used for experiment.

Seed samples form each crop (100 g) was placed in 5 jars of one kg capacity to which 0, 1,2,4 and 8 pair of freshly emerged pulse beetles were released separately.

3.2.1.3 Experimental details

Design	**: Completely Randomized Block Design**
Replication	: Four
Treatment	: Five
T_1	: No release of *Callosobruchus* (control)
T_2	: One pair of *Callosobruchus*
T_3	: Two pairs of *Callosobruchus*
T_4	: Four pairs of *Callosobruchus*
T_5	: Eight pairs of *Callosobruchus*

3.2.1.4 Observations recorded

3.2.1.4.1 Percent moisture content of seed (Before start of experiment)

The seed moisture content was determined on working seed sample taken from each treatment. The seeds were grinded in the grinding mill in such a way that at least 30 percent of grinded material was passed through sieve of 4 mm mesh. Five-gram grinded seed sample of each treatment was taken in four replications and placed in the box and distributed evenly over bottom surface. It was dried in hot air over at 130 $\pm$ 1^0C temperature for 60 minutes (Anonymous, 1985). Before taking the sample the weight of the empty box with its lid was taken. After drying, the sample was taken out and the box was placed immediately in desiccators to cool for 30-48 min. The weight of box with sample and lid was noted.

Moisture content was calculated on weight basis and expressed in percentage by using formulation.

$$\text{Moisture content (\%)} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

Where,

M_1 = Weight of box with lid (g)

M_2 = Weight of box with lid and sample before drying (g)

M_3 = Weight of box with lid and sample after drying (g)

3.2.1.4.2 Initial germination (%)

Seeds of each treatment in four replications were taken and the germination percentage was determined by using between papers (BP) method as per the procedure suggested by ISTA (Anonymous, 1985).

Hundred seeds were counted randomly from each treatment. They were placed on wet germination paper and covered by other wet germination paper. The rolls of the paper were placed in the germinator at temperature 25±1^{0}C and 75 % relative humidity. After eight days the normal and abnormal sprouted seeds were counted and germination percentage was calculated on the basis of normal seedling. The data in respect of seed germination in all treatments were tabulated and mean percentage was worked out.

3.2.1.4.3 Oviposition

After seven days of oviposition, beetles were removed from each treatment. The fifty seeds were taken randomly from each treatment, observed with naked eye and total number of eggs laid was counted. The counter part of seeds was kept undisturbed in the jar till the emergence of beetle.

3.2.1.4.4 Number of adult emerged

Total number of adult insect emerged were recorded on each alternate day from the date of first emergence of adult up to 20 days.

3.2.1.4.5 Loss in seed weight (%)

The initial weight at the beginning and final weight at the termination of the experiment was recorded and from this, the percent loss in seed weight was worked out

$$\text{Percent loss in seed weight} = \frac{\text{Loss in seed weight}}{\text{Initial seed weight}} \times 100$$

3.2.1.4.6 Number of damaged seed (%)

After completion of experiment, 100 seeds were taken from each treatment randomly. The seeds showing exit holes were taken as infested. Such seeds were counted as infested and the percent seed damaged were calculated.

3.2.1.4.7 Germination after emergence of first generation of bruchid

The germination test of seeds was conducted after 40 days i.e. after emergence of first generation of bruchid by using between paper method as per ISTA standard (Anonymous, 1985) in seed germinator at 25±10C temperatures and 75 % relative humidity. Four replications for each treatment were maintained for germination of each genotype of green gram, black gram and rice bean. Observation on germination was recorded after 7 days. The seedling, which showed normal growth, undamaged shoot and root were considered as normal and the percentage, was worked out using the formula

$$\text{Percent germination} = \frac{\text{Number of normal seedling}}{\text{Total number of seeds}} \times 100$$

3.2.1.4.8 Reduction in germination (%)

The initial germination percentage of each treatment was recorded. The reduction in germination percentage after emergence of first generation of bruchid was calculated by the following formula (Singh and Sharma, 1985).

$$\text{Percent germination loss} = \frac{\text{GC-CD}}{\text{GC}} \times 100$$

Where,

GC : Initial germination percent

GD : Germination percentage after emergence of first generation of bruchid.

3.2.1.4.9 100 seed weight (g)

Hundred seed were randomly taken from each genotypes of green gram; black gram and rice bean and hundred seed weight were taken on mono pan Micro Analytical Balance (Mettler).

3.2.1.4.10 Seed Hardness (kg/cm^2)

The hardness of the seed was measured with the help of hardness tester (IEC- 5 Table hardness tester) manufactured by M/s Indian Equipment Corporation Bombay (India). Hardness was expressed as seed crushing strength in kg/cm^2. For each genotype hardness of 10 seed in four replications was measured and average was work out.

3.2.2 Biochemical parameters

The biochemical such as enzyme inhibitor and enzyme activity, crude protein, and phenols were analyzed in all the five genotypes of each of green gram, black gram and rice bean. Following eight biochemical parameters were studied.

1. α- amylase inhibitor activity
2. Trypsin inhibitor activity
3. Protein content
4. Total phenols
5. Peroxidase activity
6. Polyphenol oxidase activity
7. Tannic acid

Laboratory analysis was carried out at Department of Agricultural Botany and Seed Technology research unit MPKV, Rahuri.

3.2.2.1 α - amylase inhibitor

The α-amylase inhibitor was extracted and inhibitory assay performed as per the method given by Rajendran and Thayumanavan (2000).

Reagents

1. 0.02 M Sodium phosphate buffer (pH 6.9)

A. 0.04 M Na_2HPO_4: 7.12 g of Na_2HPO_4 was dissolved in 1000 ml distilled water.

B. 0.04 M NaH_2PO_4: 6.24 g of NaH_2PO_4 was dissolved in 1000 ml distilled water.

97.2 ml of reagent A and 152.5 ml of reagent B were mixed thoroughly. The pH of the solution was adjusted to 6.9 and volume made to 500 ml with distilled water.

2. 0.02 M sodium phosphate buffer (pH 6.9) containing 0.3 M NaCl

A. 0.04 M Na_2HPO_4: 7.12 g of Na_2HPO_4 was dissolved in 1000 ml distilled water.

B. 0.04 M NaH_2PO_4: 6.24 g of NaH_2PO_4 was dissolved in 1000 ml distilled water.

97.5 ml of reagent A and 152.5 ml of reagent B were mixed thoroughly and 1.75 g sodium chloride was added. The pH of the solution was adjusted to 6.9 and volume made to 500 ml with distilled water.

3. DNSA-reagent (3-5 Dinitro salicylic acid)

One gram of DNSA was dissolved in 20 ml of 2 N NaOH and 50 ml water. Thirty gram of sodium potassium tartarate was added and final volume was made to 100 ml.

4. One percent starch solution

One gram soluble starch was dissolved in 100 ml of 0.02 M phosphate buffer (pH 6.9) containing 0.3 M NaCl.

5. Standard maltose solution (1 mg/ml)

This was prepared by dissolving 100 mg maltose in 100 ml distilled water.

Procedure

1. Calibration of standard curve for maltose

Standard maltose solution (1mg/ml) 0, 0.2, 0.4...2.0 ml was pipette corresponding to 0, 0.2, 0.4...2.0 mg in 25 ml flask. Total volume was made to 2 ml by adding required quantity of distilled water, 2 ml of DNSA reagent was added and the flasks were kept in boiling water bath for 10 min. Cooled under running tap water and the final volume was made to 25 ml by adding distilled water. The absorbance was read at 560 nm wavelength on Spectronic -20.

2. Extraction of ∝-amylase inhibitor

Seeds were initially powdered in grinding machine and crude protein extracts were prepared by further grinding the fine powder (@ 5 ml g^{-1} powder) in 20 mM sodium phosphate buffer (pH 6.9) in pestle and mortar. The extract was centrifuged at 10,000 rpm for 10 min. at room temperature and the supernatant discarded. The pellet was suspended in 5 ml of 20 mM sodium phosphate (pH 6.9) containing 300 mM NaCL, vortexed for 30 min. and centrifuged at 10,000 rpm for 20 min at room temperature. The supernatant was incubated at 70^{0}C for 20 min. to inactive endogenous proteases and β- amylases. The precipitated protein was removed by centrifuge at 10,000 rpm for 10 min. and the supernatant (crude extract) was used for calibrating alpha-amylase inhibitor potential.

3. Estimation of ∝- amylase inhibitor activity

The ∝-amylase activity was assayed by quantifying the reducing sugar (maltose equivalent) liberated with soluble starch as substrate. Inhibitor extracts (0.5 ml) were pre incubated for 30 minutes at 37^0 C. Control, without the addition of enzyme and inhibitor were run simultaneously. After incubation, 0.5 ml substrate (1 % soluble starch in amylase extraction buffer) was added. The reaction was carried out at 37^0 C for to 10 min. The reaction was stopped by adding 1 ml DNSA reagent followed by heating in boiling water in water bath for 5 min. The volume in each tube was made up to 25 ml with distilled water and the colour developed was read at 560 nm.

One unit of ∝-amylase activity is defined as the amount that liberated one μg of maltose under the assay condition. One unit of ∝-amylase is defined as the amount that decreased the amylase activity by one unit.

3.2.2.2 Trypsin inhibitor

Trypsin inhibitor assay was performed as per the method of Kakade *et. al.* (1969).

Reagents : Following reagents were used for calibration of Trypsin inhibitor activity.

1. Tris buffer (0.05 M, pH 8.2) containing 0.08 M $CaCl_2$

6.05 g tris (hydroxymethyl) aminocethane and 2.94 g $CaCL_2$ $2H_2O$ were dissolved in 900 ml distilled water. The pH was adjusted to 8.2 and the volume brought to 1 liter with water.

2. BAPNA substrate solution

30 mg Benzyl-DL-arginine-P-nitroanilide was dissolved in 1 ml dimethyl sulfoxide and diluted to 100 ml with the tris buffer pre warmed to 37^0C. The BAPNA solution was freshly prepared daily and kept at 37^0C while in use.

3. Stock trypsin solution

Accurately weighted trypsin 4 mg (3 x crystallized, salt free) was dissolved in 100 ml of 0.001 M HCl. This solution was stored in refrigerator.

4. 0.001 M HCl

Concentrated hydrochloric acid (0.09 ml) was diluted to 1000 ml with distilled water.

5. Acetic acid (30 %)

Acetic acid 30 ml was diluted to 100 ml with distilled water.

Trypsin standard curve

The stock trypsin solution (0.2 to 1.0 ml) was pipette into a triplicate set and the final volume of each tube adjusted to 2 ml with the phosphate buffer. The tubes were kept in a water bath at 37 ^{0}C. In one test tube 1 ml 30 % acetic acid was added to serve as a blank. The reaction was terminated after 10 minutes by adding 1 ml of 30 percent acetic acid to each tube. After thoroughly mixing, the absorbency of each solution was measured at 410 nm against the appropriate blank.

Preparation of sample

Seeds were grinded and passed through 100-mesh sieve and extracted with 10 volume of petroleum ether at room temperature. One gram of the meal was suspended in 19 ml water, and the pH of the suspension adjusted to 7.6. After mechanical shaking for 1 hr., the suspension was centrifuged at 5000 rpm for 20 min. and 1 ml of the supernatant was diluted to 10 ml distilled water.

Trypsin inhibitor activity

Inhibitor extract (1 ml) was pipette into a triplicate set of test tubes and final volume adjusted to 1 ml with water; one ml of the stock trypsin solution was added to each of the tubes. The test tubes were placed in water both at 37 ^{0}C. One ml of 30 percent acetic acid was added to one of the tube to serve as blank. To each tube 7 ml of BAPNA solution was added previously warmed to 37 ^{0}C, and exactly 10 min. late, the reaction was terminated by adding 1 ml of 30 percent acetic acid. After thorough mixing, the absorbency of each solution was measured at 410 nm against the appropriate blank.

Expression of activity

One trypsin unit (TIU) is arbitrarily defined as an increase of 0.01 absorbency units at 410 nm per 10 ml of the reaction mixture under the condition used herein. Trypsin inhibitor activity was expressed in terms of trypsin units inhibited (TIU).

3.2.2.3 Protein content

The nitrogen content of seed was determined by using Micro-Kjeldahl Method (Jackson, 1971). The protein content (%) was worked out by multiplying the nitrogen content by the factor 6.25 (Anonymous, 1975).

The seed samples were powdered in grinding mill and known quantity (0.20 g) was digested in a mixture of concentrated H_2SO_4 (30 %) in 1:1 proportion. The acid extract was used for determination of nitrogen content of grain in percent.

The crude protein percentage was calculated as follows.
Protein content (%) = N content (%) x 6.25 factor

3.2.2.4 Peroxidase and polyphenol oxidase activities

Reagents

a) Phosphate buffer – 0.1 M (pH 7.0)
b) Pyragallol - 0.01 M
c) Hydrogen peroxide - 0.005 M

A. Peroxidase activity

The peroxidase activity was essayed as per the procedure given by Kumar and Khan (1982) with slight modifications.

Enzyme assay

One gram of germinated seeds was macerated with 10 ml of 0.1 M phosphate buffer (pH 7.0) in pre chilled mortar and pestle. The homogenate was centrifuged at 14,000 rpm at 4^0C for 30 min. and the supernatant was used as a source of enzyme.

The reactions mixture contained 2 ml of phosphate buffer (0.1 M, pH 7.0), 1 ml of 0.01 M pyragallol, 1 ml 0.005 M hydrogen peroxide and 0.2 ml enzyme extract. The absorbency was measured immediately after mixing the enzyme extract with mixture at 420 nm on spectronic- 20 for 3 min. after 30 seconds enzyme activity was expressed as $\Delta A/min/mg$ of soluble protein.

B. Polyphenol oxidase activity

The polyphenol oxidase activity was assayed in the same supernatant used for peroxidase as per the method given by Kumar and Khan (1982) with slight modifications.

Reaction mixture for polyphenol oxidase contained 2 ml of 0.1 M phosphate buffer (pH 7.0); 1 ml of 0.01 M pryagallol and 0.2 ml enzyme extract. The absorbance was measured at 420 nm on spectronic 20 for 3 min. after 30 seconds enzyme activity was expressed as $\Delta A/min/mg$ of soluble protein.

3.2.2.5 *Total phenols*

Total phenols content was determined by using Folin Ciocalteu reagent as per the method of Bary and Thorpe (1954).

Reagents

1. 80 % Ethanol
2. Folin Ciocalteu –reagent (FCR)
3. 20 % Na_2CO_3
4. Standard solution of catechol: 100 mg catechol was dissolved in 100 ml of distilled water further diluted 10 times for working standard.

Extraction of phenols

One gram of sample was grind in mortar and pestle by adding 10 ml of 80 % ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. and the supernant was saved. The extraction was reported and supernatants were pooled. The ethanol was evaporated by heating 60^0C on water bath and the volume of residual extract was made to 5 ml by adding distilled water. The extract was again centrifuse at 10,000 rpm for 20 min. supernatant was collected and used for colour development.

Colour development

The aliquot (0.2 ml) in triplicate were taken in the test tube and the volume was adjusted to 3 ml. In each tube 0.5 ml folin. Ciocaltea reagent was added. After three min. 2 ml of 20 per cent Na_2CO_3 solution was added and mixed thoroughly. The test tubes were placed in boiling water bath for one minute. After cooling the test tube, the absorbance was measured against blank.

Preparation of standard curve

Standard solution of catechol reagent (0.2 to 1 ml) was taken into triplicate set of test tubes and the final volumes in each test tube was made up 3 ml with distilled water. Then 0.5 ml of folin - ciocalteu reagent was added. After 3 min, add 2 ml of 20 % Na_2CO_3 solution was added in each tube and mixed thoroughly. The test tubes were placed in a boiling water bath for 1 min and cool it. The absorbance was measured at 650 nm against blank. The standard curve was plotted. By using standard curve the concentration of phenols in the test sample was calculated and express in mg phenols/100 g sample.

3.2.2.6 Tannic acid

The tannic acid was estimated by a method described by Sadashivam and Manickan (1992).

Reagents

1. Folin Denis reagents

100 g sodium tungstate and 20 g phosphomolybdic acid was disolved in 750 ml distilled water and 50 ml phosphoric acid. The mixture was reflexed for 2 hr in hot water bath and the volume was made up to one liter.

2. Sodium carbonate solution

35 g of anhydrous sodium carbonate was dissolved in 100 ml of distilled water at 70-80^0 C and cooled overnight.

3. Standard tannic acid solution

100 mg of tannic acid was dissolved in one liter of water (i.e. one ml containing 0.1 mg of tannic acid.)

Extraction of tannins

The powdered 0.5 mg was taken into 250 ml canical flask containing 75 ml of distilled water and mixed well. The flask was heat gently and boil for 30 min. the extract was centrifuge at 2000 rpm for 20 min and the supernatant was collected in 100 ml volumetric flask and volume was made up.

Determination

1 ml aliquot of the sample extract was taken in to 100 ml volumetric flask containing 75 ml water. Five ml of Folin Denis reagent, 10 ml of sodium carbonate solution was added. The mixture was diluted to 100 ml and shaked well. The absorbency was read at 760 nm after 30 min.

Preparation of standard curve

Aliquot of the standard tannic acid solution (0 to 10 ml) was taken into 100 ml volumetric flask containing 75 ml of distilled water. To this 5 ml Folin- Denis reagent and 10 ml sodium carbonate solution was added in each of the volumetric flask was added and make up volume 100 ml with water. The solution was mixed well and absorbency was measured after 30 min at 760 nm against blank.

Calculation

$$\text{Tannins as Tannic acid \%} = \frac{\text{mg of tannic acid x Dilution x 100}}{\text{ml of sample taken x wt. of sample x 1000 for colour development}}$$

3.2.3 Electrophoretic Studies

3.2.3.1 Electrophoresis of total soluble protein

Sodium dodecyl sulphate polyarylamide gel electrophoresis (SDS-PAGE) on 6-8 seeds was conducted in two replications as per the procedure described by Dadlani and Varier (1993). The method is described as below.

Apparatus and equipments

The Atto AE 6210 slab gel cast with Atto AE 6220 Dual slab chamber unit was used for electrophoresis. It consists of 160 x 160 mm glass plate size, notched glass plates with 1 mm spacer, 1 mm gasket, buffer volume 480 ml + 450 ml , chamber dimension (WEH) 21.1 x 19.6 x 20.5 cm and weight 2-3 kg.

Chemicals

The following chemicals (Analytical grades) were used for preparation of different stock solution.

1. Acrylamide "Electrophoresis" Grade
2. Bis-acrylamide "Electrophoresis" Grade
   (N, N, Methylene-bis-acrylamide)
3. Tris-hydroxy methyl amino methane (Tris)
4. Glycine
5. Hydrochloric acid (HCl)
6. Sodium dodecyl sulphate (SDS)
7. 2-Mercaptoethanol
8. Ammonium persulphate
9. TEMED (N, N, N, N – tetramethylethelene diamine)
10. Methanol
11. Glacial acetic acid
12. Trichloro acetic acid
13. Coomassic brilliant blue
14. Methyl green

Reagents

1. Stock buffer for running/resolving Gel (1.875 M Tris-HCl buffer, pH 8.8)

22.69 g of Tris-hydroxymethl aminomethane (Tris) was dissolved in 50 ml of distilled water. The pH of solution was adjusted to 8.8 by adding conc. HCl dropwise and the final volume made upto 100 ml. Solution was stored in a refrigerator.

2. Stock buffer for stocking gel (0.6 M Tris HCl buffer, pH 6.8)

7.26 g of Tris was dissolved in 50 ml of distilled water and pH adjusted to 6.8 by adding conc. HCl drop by drop. Distilled water was added to make 100 ml. Solution was stored in a refrigerator.

3. Stock SDS solution (10 %)

10 g SDS was dissolved in distilled water with constant stirring and gentle heating. Volume was made to 100 ml with distilled water.

4. Ammonium persulphate (5 %)

It was prepared fresh just before preparation of gel by dissolving 0.5 gm Ammonium persulphate in 10 ml of distilled water.

5. Stock protein extraction solution

2 g SDS and 10 mg methyl green in 10.4 ml 0.6 M Tris HCl buffer (pH 6.8), 7.9 ml distilled water and 10 ml glycerol solution was added, warm gently and mix well.

6. Electrode (Tank) buffer (SDS-Tris glycine pH 8.3)

Dissolved 3 g Tris, 14.12 gm Glycine and 1 gm SDS in one litre distilled water. This solution was stirred well and adjusts pH 8.3.

7. Fixing solution (15 % TCA)

150 g of Trichloroacetic acid was dissolved in distilled water and final volume was made to 1 litre.

8. Staining solution

Ten ml of 1 % comassie blue prepare in methanol was added to 100 ml of 15 % TCA solution. Staining solution was filtered 2-3 times before use.

9. 30 % Acrylamide for stocking/resolving gel

Dissolve 7.5 g acrylamide and 0.1 g bis acrylamide were dissolved in distilled water. Volume was made to 50 ml. Solution was kept in brown colour bottle at refrigerator.

10. 30 % Acrylamide for running gel

30 g acrylamide and 0.8 g bis acrylamide was dissolved in distilled water and make up volume 100 ml.

Sample preparation

About 6-8 seeds of each genotype were homogenized using mortar and pestle to get fine powder. It was transferred separately in 1.5 ml eppendorf tubes. 1 ml freshly prepared protein extraction solution was added to each tube. The sample was left for 2 hrs at room temperature and kept in refrigerator for overnight. Next day the tubes were heated in a boiling water bath for 10 min, cooled and centrifuged at 10.000 rpm 20 min. at constant temperature of $4^{0}C$. The supernatant was used immediately for electrophoresis.

Preparation of gel

Clean and dry gel plates were assembled in the cassette as per the instruction manual. A gel thickness of 1.00 mm was used.

1. Preparation of separating gel

Following solution were mixed

Tris buffer pH 8.8	12.0 ml
Water	7.4 ml
30 % running gel acrylamide	20.00 ml
5 % Ammonium persulphate	0.4 ml

10 % SDS	0.4 ml
TEMED	0.04 ml

(TEMED was added just before pouring of gel)

All the reagents were mixed well and poured between the glass plates assembled in the cassette. A (Pasteur pipette or syringe needle was used to pour the gel mixture).

Care was taken so that no air bubble is trapped in the solution. Filled the gel cast up to the level mark on the front support i.e. about 3 to 4 cm from top. The gel solution was overlayed with distilled water and allowed the gel to set (It took about 30 to 60 min.).

2. Preparation of stocking gel

After polymerization of running gel the water layer was poured off from the top and blotted the gel surface with filter paper. The following stacking gel mixture was poured carefully over polymerized running gel

Tris buffer pH 6.8	1.50 ml
Water	6.00 ml
30 % stacking gel acrylamide	2.00 ml
5 % Ammonium per sulphate	0.8 ml
10 % Sodium dodecyl sulphate	0.1 ml
TEMED	0.04 ml

(TEMED was added just before pouring of gel)

After pouring the stacking gel solution, immediately inserted the comb having required number of wells (14, 24) between the glass plates taking due care not to trap air bubble. Allowed the gel solution to polymerize (it take 15-30 min.) After polymerization of gel, the comb was carefully removed from the gel without disturbing the shape of wells and washed the wells with tank buffer or distilled water. Removed the gasket, rinsed the sample wells, with electrophoresis buffer with the use of pipette.

Added 450 ml of electrode buffer into lower tea buffer tank of the chamber up to the level mark. The gel sandwich cassette was fixed into the electrophoresis unit as per the design of the equipment. An aliquot of 20 µl was loaded to each well with the help of microsyrings. The gel plates were fixed over cathode chamber cab heat exchanger with clamps and screws. The upper tank was filled with the electrode buffer i.e. 450 ml tank buffer. Two-three drops of bromophenol dye (tracking dye) were added to the electrode buffer in the upper tank.

The electrophoresis apparatus were connected to the power supply with the anode connected to reservoir and cathode to upper reservoir. Adjusted the voltage to 220 V and current 22 mA till the sample migrate into the running gel and subsequently the current was increased to 30 mA till the tracking dye reached to the bottom of the gel.

The power supply was turned off and disconnected the power leads and removed the cover as soon as tracking dye reached the bottom of the gel.

Fixing and staining of gel

Gently taken out the gel sandwich from the chamber, peeled of the glass plate gently by prizing the glass plate with spatula. Cut off the side edges of the gel along with a spatula from those sides loading of sample was done. Gradually peeled off the gel with spatula. Immersed the gel into the plastic tray containing standing solution to remove excess stain from 3-12 hrs.

The band intensity was assessed visually by placing the gel over Trans illuminator and recorded as dense, medium, light and weak bands.

The banding pattern of protein was considered as 'finger print' of the genotype. The genotypes were separated into different groups according to the total number of bands and their intensity and presence or absence of specific bands.

The relative migration (Rm values) of each bands were calculated was follow

$$\text{Rm value: } \frac{\text{Distance migrated by the protein bands from the origin (cm)}}{\text{Distance migrated by tracing day (cm)}}$$

The Rm values for each genotype were recorded separately.

3.2.3.2 Electrophoresis of Peroxidase isozyme

Polyacrylamide gel electrophoresis (PAGE) was undertaken as per the procedure described by Santha et al (1997). The method is presented below.

Apparatus

The Atto AE 6210 slab gel cast with Atto AE 6220 Dual slab chamber unit was used for electrophoresis.

The extraction buffer and stock solution for running and stacking gel, electrode buffer and staining solution required for electrophoresis studies were prepared as described below.

Extraction buffer / (Tris-cl buffer 50 mM, pH 7.6)

Tris- Cl (0.788 gm) was dissolved in 50 ml distilled water. PH was adjusted to 7.6 and 100 ml volume was made with distilled water.

Stock solutions

A. Tris glycine electrode buffer stock solution pH 8.3

Tris	6.00 gm
Glycine	28.8 gm
Distilled water to	1000 ml

Working standard

The tris-glycine electrode buffer solution was diluated in 1:9 ratio with distilled water.

B. Tris-Chloride buffer stock solution pH 8.9.

HCl 1 N	48.00 ml
Tris	36.6 gm
TEMED	0.23 ml
Distilled water to	100 ml

C. Tris chloride buffer stock solution, pH 6.7

HCl 1 N	48.00 ml
Tris	5.98 gm
TEMED	0.46 ml
Distilled water to	100 ml

D. Resolving gel acrylamide stock solution

Acrylamide	28.00 gm
N^1N^1 Methylene bis-acrylamide	0.74 gm

Made volume up to 100 ml with distilled water. Stored in a dark bottle at -4^0 C.

E. Ammonium persulphate

0.1g of ammonium persulphate was dissolved in 100 ml of distilled water. This solution was made fresh every time.

F. Staining solution

Incubate the gel in 0.6 M sodium acetate buffer (pH 5.4) containing 0.5 % O- dionisidine HCl for 30 min. at room temperature. Transfer the gel to 0.1 M hydrogen peroxide until visible bands develop.

Enzyme extraction

The sample (Germinating seeds) was grinded in a pre chilled pestle and mortar in 50 mM Tris –cl (pH 7.6) buffer in the ratio of 1: 2 (w/v). All this procedure was carry out at 4^0 C temperature and centrifuged at 14,000 rpm for 10 min at 4^0C.

Preparation of gel

Clean and dry gel plates were assembled in the cassette as per the instruction manual. A gel thickness of 1.3 mm was used.

1. Preparation of resolving / separating gel

Following solutions were mixed.

Tris chloride buffer solution, pH 8.9	5 ml
Resolving gel acrylamide solution	10 ml
Distilled water	25 ml
Ammonium persulphate solution	300 ml

All the reagents were mixed well and poured between the glass plates assemble in the cassette.

Care was taken so that no air bubble is trapped in the solution. Allowed the gel to set. It takes about 15-30 min.

2. Preparation of stacking gel

After polymerization of running gel, poured off the water layer from the top and blotted the gel surface with filter paper. The following stacking gel mixture was poured carefully over polymerized running gel.

Tris chloride buffer stock solution pH 6.7	2.5 ml
Resolving gel acrylamide solution	3.1 ml
Distilled water	14.1 ml
Ammonium per sulphate	300 ul

After pouring the stacking gel solution immediately inserted the comb between the glass plate taking due care to trape air bubble.

After polymerization of gel the comb was carefully removed from the gel without disturbing the shape of wells and wash the well with tank buffer.

Sample preparation and running the gel

Glycerol (10 µl) and bromophenol blue solution (4 µl) was added in 100 µl of sample extract and mixed thoroughly. The sample solutions were loaded in the well of gel using micropipette. After loading the sample, apparatus was connected to power pack and for the first 20-30 min the gel was run at 30 mA until the tracking dye reached the separating gel. After that current strength was increased to 60 mA until the bromophenol dye reaches the bottom of the gel. It was conducted at 4^0C temperature.

After completing the gel, the gel cassette was carefully removed and gel was incubate in 0.6 M sodium acetate buffer (pH 5.4) containing 0.5 % O-dianisidine for 30 min at room temperature. The gel was transferred to 0.1 M hydrogen peroxide until visible bands developed.

3.2.4 Detection of seed mycoflora

The ISTA'S standard blotter paper test as described by Neergoard (1977) was used for detection of external seed born fungi of green gram, black gram and rice bean. The protocol of standard blotter paper method used was as given below.

1. Blotter was soaked in distilled or sterilized water and place in three layers in transparent petriplates (plastic) after draining off excess of water.
2. A fix number of seeds i.e. 20 per plate were placed equidistant from one another under aseptic condition like wise 400 seeds were plated.
3. After plating the seeds, the petriplates were incubated at 20 ± 2^0C under ultra violet/fluorescent light with an alternate cycle of 12 hr light and 12 hr darkness in an incubation room for 7 days.
4. The seeds were examined on 8^{th} day under stereo binocular microscope.

5. The fungi were identified mostly on the basis of morphological characters of conidia, fruiting bodies (structures) and condiophores.
6. The fungi present on the seed were also examined under binocular research microscope for confirmation.
7. The per cent incidence of different seed borne fungi associated with seeds was recorded.

3.3 Statistical analysis

The statistical analysis of the data was carried out by the standard method (Chochran and Cox 1957) and critical differences (CD) were calculated whenever, the results were significant at 5 per cent level of significance. The data were analyzed as per C.R.D.

3.4 Correlation studies

Simple correlation among bruchid resistance, different characters, and biochemical parameters were calculated as per formula suggested by Snedecor and Cocharan (1967).

$$r = \frac{\Sigma(X_1-\overline{X_1})(X_2-\overline{X_2})}{\sqrt{\Sigma(X_1-\overline{X_1})^2 \times \Sigma(X_2-\overline{X_2})^2}}$$

Where,

X_1 and X_2 are the characters one and two respectively.

The significance of correlation coefficient was tested against 'r' values given by Fisher and Yates (1963) at (n-2) degree of freedom at 5 per cent and 1 per cent level of significant.

3.5 Path Analysis

Path coefficient analysis as suggested by Dewey and Lu (1959) was used to partition the correlation coefficient in direct and indirect effects. The first step in path analysis is to prepare the path diagram based on cause and effect relationship. In the present diagram based on cause and effect relationship. In the present study, path diagram was prepared by taking resistant seed as effect i.e. the function of various components X_1, X_2, X_3 and these components show following type of association with each other.

In path diagram the resistant seeds is the result of X_1, X_2, X_3 and X_4 some other undefined factors designated by R. The double arrow lines indicated mutual association as measured by correlation coefficient. The single arrow represents direct influence as measured by path coefficient Pij.

Path coefficient was obtained by solving as set of simultaneous equation of the form.

$$r_{ny} = P_{ny} + r_n2P_{2y} + r_{n3}P_{3y} + \ldots\ldots\ldots\ldots\ldots$$

Where,

r_{ny} = represents the correlation between one component and resistant seed, P_{ny} represents path coefficient between that character and resistant seed and r_{n2} represents correlation between that character and each of the other components in turn.

$$\begin{pmatrix} r1_y \\ r2_y \\ r_{ny} \end{pmatrix} = \begin{pmatrix} r_{11} & r_{12} & r_{13} & \ldots\ldots & r1_n \\ r_{21} & r_{22} & r_{23} & \ldots\ldots & r2_n \\ r_n1 & r_n2 & r_n3 & \ldots\ldots & 1 \end{pmatrix}$$

Matrix-A Matrix-B

Where,

$r_{12} = r_{21}$ and so on and r_{1y}, correlation between between one component character and yield. The B matrix was inverted (B^{-1}) and path coefficient (Pij) were obtained as

$$(Pij) = A \times (B^{-1})$$

The indirect effects of a perticular character through other characters were obtained by multiplication of direct paths and perticular correlation between these characters seperatrely.

Indirect effects = rij x Pij

Where,

i = 1 to 9

j = 1 to 9

Pij = P_{1y} P_{2y} .... P_{ny}

Path coefficient (Pij) correlation coefficient (rij) and residual factors (R) were diagramatically presented. The residual factor i.e. variation in yield unacoounted by these associations was calculated with the following formula,

Residual factor (R) = $(1-R^2)$

Where,

$R^2 = P_{1y}\, r_{1y} + P_{2y}\, r_2y + \ldots\ldots\ldots\, P_{ny}\, r_{ny}$

Where,

P_{1y}, P_{2y} .... P_{ny} = Path values

r_{1y}, r_{2y}............ r_{ny} = Correlation coefficients

RESULTS

4. RESULTS

The present investigation on "Biochemical basis of resistance to stored grain pest (*Callosobruchus chinensis*) of different *Vigna* species" was carried out and results are presented under following heading.

4.1 Evaluation of levels of resistance to the bruchid

4.2 Biochemical parameters

4.3 Electrophoresis studies

4.4 Seed mycoflora study

4.1 Evaluation of level of resistance to bruchid

4.1.1 Mean performance of different parameters for evaluating resistance

The treatment mean values of different parameters for evaluating bruchid resistance in green gram, black gram and rice bean at different population of bruchid are presented in Table 2, 2a, 3, 3a, 4, 4a and Figure 1, 2.

4.1.1.1 Percent moisture content of seed (before the start of experiment)

The mean moisture content of the green gram genotypes studied ranged between 8.24 (T_2) to 8.45 (T_4) percent. The minimum seed moisture content was recorded in TARM-18 (3.10) in the treatment T_1 followed by PM-9339 (8.25) in the treatment T_2 and T_4, while the maximum seed moisture content was recorded by PM-2 (8.72) in the treatment T_1 and T_4 followed by AKM-8802 (8.67) in the treatment T_3.

The range of treatment mean seed moisture content among the black gram genotypes was 8.56 to 8.65 percent. The minimum seed moisture content was observed in PU-9503-8

(8.45 %) in the treatment T_2 followed by TAU-1 (8.47 %) in the treatment T_4. The maximum seed moisture content was observed in PU-9205-11-3 and TPU-4 (8.67 %) in the treatment T_5 followed by TAU-2, PU-9503-8 and TPU-4 (8.65 %) in the treatment T_1 and T_4.

The treatment mean seed moisture content among the rice bean genotypes ranged from 8.59 to 8.70 percent. The minimum seed moisture content was recorded in RBL-36 and RBL-50 (8.55) in the treatments T_2 and T_5 respectively, while the maximum seed moisture content was recorded for RBL-36 and RBL-99 (8.77) in the treatment T_3.

4.1.1.2 Initial germination (%)

The mean initial germination percent among the green gram genotypes ranged from 91.25 to 91.50 percent. The maximum initial germination was recorded for PM-2 (92.25) in the treatment T_3 and T_4 followed by PM-9339 (92.00) in the treatment T_2 while the minimum initial germination was recorded for PM-9339; AKM-8802 and Kopergaon (90.75) in the treatment T_1, T_4 and T_3 respectively.

The mean initial germination among the black gram genotypes ranged from 91.40 to 91.94 percent. The maximum initial germination was recorded by TAU-1 (92.75) in the treatment T_1 followed by PU-9205-11-3 (92.73) in the treatment T_5, while the minimum initial germination was recorded by TAU-2 (90.75) and PU-9205-11-3 (90.75) in the treatments T_2 and T_5 respectively.

The mean initial germination percent among the rice bean genotypes ranged between 92.40 to 93.65 percent. The highest initial germination was recorded by LRB-202 (94.75) in the treatment T_1, while the minimum initial germination was recorded by RBL-50 and RBL-99 (92.00) in the treatment T_1 and T_4 respectively.

4.1.1.3 Number of damaged seeds (%)

The variation for mean number of damaged seeds among the green gram genotypes studied ranged between 0 to 26.29 percent. All genotypes of green gram showed zero percent damage in the treatment T_1. The minimum number of damaged seeds was observed in case of TARM-18 (1.75) in the treatment T_2, while the maximum number of damaged seeds was observed in Kopergaon (39.25) in T_5 followed by AKM-8802 (28.25).

The mean number of damaged seeds among the black gram genotypes studied ranged from 0 to 25.80 percent. In treatment T1, number of damaged seeds was observed as zero percent. The minimum number of damaged seeds was observed in case of PU-9503-8 (3.00) in the treatment T_2 followed by TAU-1 (3.28) in the treatment T_2, while it was maximum in case of TAU-2 (34.50) in T_5 followed by TAU-1 (29.50).

There was no damage in five genotypes of rice bean in all the 5 treatments.

4.1.1.4 Loss in seed weight (%)

The mean loss in Seed weight among the green gram genotype studied, ranged from 0.00 to 21.95 percent. All five genotypes of green gram showed zero percent loss in seed weight in the treatment T_1. The minimum loss in seed weight was recorded in case of TARM-18 (0.30) in T_2, while it was maximum in case of Kopergaon (23.15) followed by AKM-8802 (25.10) in T_5.

In black gram genotypes, the mean loss in seed weight was ranged between 0 to 17.54 percent. The minimum loss in seed weight was observed in TAU-1 (2.03) followed by PU-9503-8 in T_2, while the maximum loss in seed weight was recorded in TAU-1 (27.00) followed by TAU-1 (19.20) in the treatment T_5.

There was no loss in seed weight in all the treatments of rice bean genotypes.

4.1.1.5 Germination after emergence of first generation of bruchid (%)

The mean germination after emergence of first generation of bruchid among the green gram genotypes, ranged between 59.60 to 90.60 percent. The maximum germination was recorded in case of Kopergaon (91.00) followed by AKM-8802 (90.76) in the treatment T_1, while it was minimum in case of Kopergaon (39.25) followed by AKM-8802 (51.50) in treatment T_5.

The variation in mean for germination after emergence of first generation of bruchid among the black gram genotypes ranged between 68.02 to 90.80 percent. It was observed maximum in case of TAU-1 (91.50) followed by TAU-2 (91.00) and PU-9205-11-3 (91.00) in the treatment T_1, while it was observed minimum in case of TAU-2 (58.75) followed by TAU-1 (66.25) in the treatment T_5.

Mean germination after emergence of first generation of bruchid among rice bean genotypes was ranged from 92.00 to 92.75 percent. The maximum germination after emergence of first generation of bruchid was recorded in case of LRB-202 (94.00) in the treatment T_1, while it was the lowest in case of RBL-36 and RBL-50 (91.00) in the treatment T_2. No reduction in germination was observed in rice bean.

4.1.1.6 Reduction in germination (%)

The mean reduction in germination among the green gram genotypes was ranged from 0.86 to 35.44 percent. The minimum reduction in germination was recorded in case of Kopergaon and PM-9339 (0.54) in T_1, while it was maximum in case of Kopergaon (56.88) followed by AKM-8802 (43.92) in T5.

The range of mean for reduction in germination among the black gram genotypes was between 1.02 to 25.53 percent. Reduction in germination was minimum in case of TPU-4, PU-

9503-8 and PU-9205-11-3 (0.81) in the treatment T_1, while it was maximum in case of TAU-2 (35.96) followed by TAU-1 (28.57) in T_5 treatment.

The reduction in germination of all the five genotypes of rice bean was ranged between 0.80 to 0.91 percent. The minimum reduction in germination was recorded in RBL-36 (0.53) followed by RBL-50 (0.20) in treatment T_1, while it was maximum in case of RBL-99 (1.34) in the treatment T_4.

4.1.1.7 Oviposition

The mean oviposition of green gram genotypes was ranged from 0 to 46.90. In the treatment T_1 there was no egg laid by bruchid. The minimum oviposition was recorded in TARM-18 (12) in T_2, while it was maximum in case of Kopergaon (56.50) followed by AKM-8802 (52.50) in T_5.

The range of mean oviposition among the black gram genotypes ranged from 0 to 52.75. In the treatment T_1 the eggs laying was not observed. The minimum oviposition was recorded by PU-9503-8 (12.00) in the treatment T_2, while it was maximum in case of TAU-2 (50.25) followed by TAU-1 (42.50) in the treatment T_5.

The mean oviposition of rice bean genotypes was ranged from 0 to 36.80. There were no eggs laid by bruchid in the treatment T_1. The minimum number of eggs laid was recorded in LRB-199 (10) in T_2 treatment, while it was maximum in case of LRB-202 (42) followed by RBL-36 (38) in the treatment (T_5).

4.1.1.8 Number of adult emerged

The mean number of adult emerged of green gram genotypes were ranged from 0 to 272.00. The minimum number of adult emerged in case of TARM-18 (22) in the treatment T_2, while

Table 2 : Mean performance of different parameters for evaluating resistance in green gram

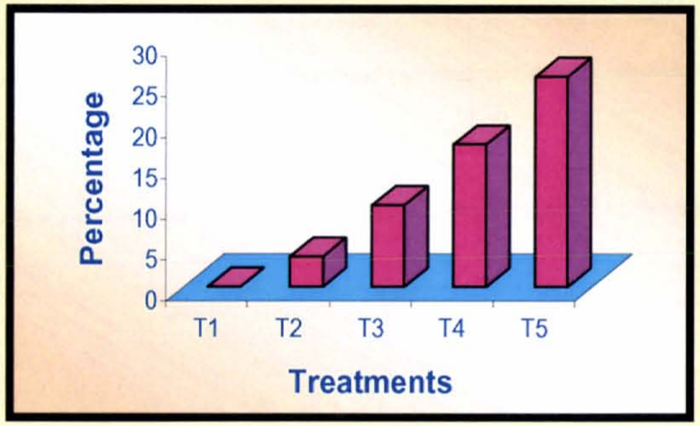
Treatment	Moisture content %					Initial Germination (%)					No. of Damaged seed (%)					Loss in Weight (%)				
	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon
T_1	8.70 (17.15)	8.35 (16.79)	8.55 (16.99)	8.10 (16.53)	8.32 (16.76)	91.50 (73.08)	90.75 (72.29)	91.75 (73.34)	91.25 (72.81)	91.75 (73.08)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4 05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
T_2	8.72 (17.18)	8.25 (16.69)	8.60 (17.05)	8.30 (16.74)	8.35 (16.79)	91.25 (17.81)	92.00 (73.58)	91.25 (72.81)	91.00 (72.55)	91.00 (72.55)	3.50 (10.64)	2.50 (9.29)	3.50 (10.64)	1.75 (7.39)	4.75 (12.44)	2.15 (8.38)	1.25 (6.40)	2.10 (8.31)	0.30 (4.05)	2.75 (9 48)
T_3	8.60 (17.05)	8.30 (16.74)	8.67 (17.12)	8.22 (16.66)	8 42 (16.87)	92.25 (73.89)	91.00 (72.55)	90.75 (72.31)	91.50 (73.30)	90.75 (72.31)	7.50 (15.78)	6.75 (14.97)	8.00 (16.35)	2.50 (8.84)	15.75 (23.33)	4.30 (13.28)	3.50 (10.74)	5.75 (13.79)	1.10 (5.95)	8.15 (16.57)
T_4	8.70 (17.15)	8.25 (16.56)	8.65 (17.10)	8.30 (16.74)	8.35 (16.79)	92.25 (73.87)	91.00 (72.55)	90.75 (72.50)	91.75 (73.37)	91.75 (73.37)	12.00 (20.18)	13.50 (21.51)	16.00 (23.54)	6.50 (15.24)	21.75 (27.75)	8.50 (16.52)	9.05 (18.55)	11.15 (16.94)	3.25 (10.36)	15.60 (23.23)
T_5	8.65 (17.10)	8.27 (16.71)	8.62 (17.07)	8.42 (16.87)	8.45 (16.89)	91.75 (73.37)	92.00 (73.58)	91.00 (72.58)	91.75 (72.81)	91.00 (72.55)	24.00 (29.65)	26.75 (31 12)	28.25 (32.08)	13.00 (21.04)	39.25 (38.78)	22.00 (27.97)	23.68 (29.06)	25.10 (30.07)	11.26 (19.64)	28.15 (32.01)
S.E. ±	0.09	0.09	0.11	0.12	0.17	0.72	0.39	0.58	0.67	0.53	0.91	0.83	0.84	1.02	0.89	0.85	0.46	0.58	0.47	0.64
CD at 5 %	0.27	0.28	0.33	0.36	0.52	2.19	1.18	1.69	2.04	1.62	2.75	2.51	2.53	3.09	2.69	2.56	1.40	1.75	1.43	1.93

Treatment	Germination after emergence of first generation of bruchid (%)					Reduction in Germination (%)					No. of eggs laid by female per 50 seeds					No of adult emerged				
	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon
T_1	90.50 (72.06)	90.75 (72.31)	90.76 (72.31)	90.25 (71.80)	91.00 (72.56)	1.08 (6.11)	0.54 (5.02)	1.06 (6.10)	1.08 (6.12)	0.54 (5.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2	80.75 (64.02)	82.00 (64.94)	83.50 (66.10)	86.50 (68.48)	77.50 (61.73)	11.51 (19.73)	10.85 (19.07)	8.50 (16 67)	4.94 (12.62)	14.82 (22.51)	16.50	16.75	16.75	12 00	19.50	50.00	41.50	77.5	22.00	90 50
T_3	74.00 (56.37)	76.50 (61.03)	74.23 (59.52)	84.25 (69.28)	66.75 (54.79)	18.94 (25.73)	15.91 (23.38)	18.49 (25.44)	4.92 (12.24)	26.44 (30.92)	20.25	14.50	27.75	20.50	29.75	109.75	106.50	137.75	58.00	244 50
T_4	68.50 (55 88)	66.50 (54.67)	69.50 (56.52)	87.25 (68.51)	55.50 (48 30)	25.91 (30.57)	27 09 (31.23)	23.63 (29.00)	5 71 (13.60)	39.08 (38.68)	35.50	26.50	36.00	25.50	37.00	141.50	181.50	197.50	75.00	304.50
T_5	65.00 (53.74)	55 75 (48.30)	51 50 (45.86)	86.50 (67.22)	39.25 (38.77)	29.15 (32.65)	39 36 (38.83)	43.92 (41.48)	7 92 (16.26)	56.88 (48.96)	40.75	30.50	52.75	37.50	56.50	261.75	281.75	300.00	87.00	429.50
S.E. ±	0 96	1 09	1.12	0.07	0 96	1.07	1 38	1 46	1.49	1.06	2.05	1.81	1.87	1.69	2.23	2.32	2.40	9.07	4.40	2.54
CD at 5 %	2.92	3.28	3.39	3.24	2.92	3.25	4.16	4.41	4.52	3 20	6.18	5.47	5.65	5.10	6.73	7.01	7.23	27 36	13 28	7.68

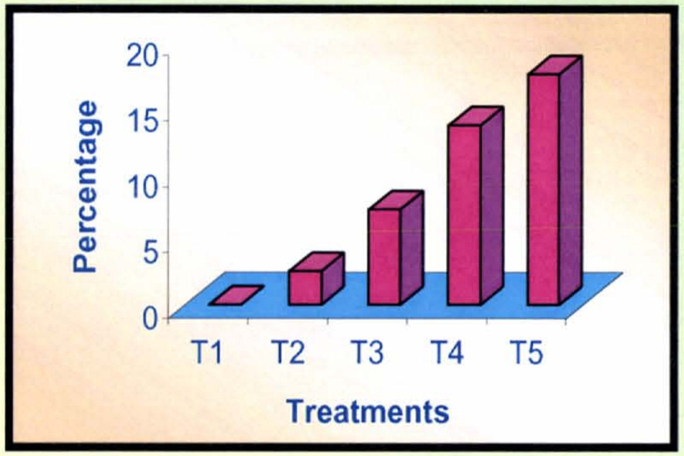
* Figures in parenthesis are aresin values

Table 2a: Average performance of treatments in green gram

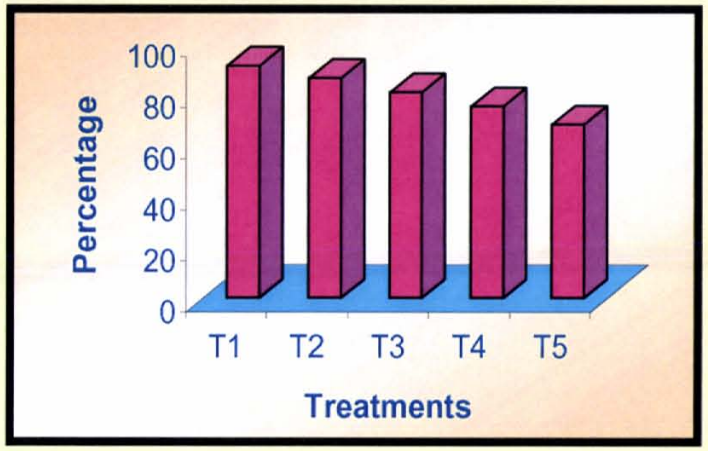
Treatments	Moisture content (%)	Initial germination (%)	No. of damaged seeds (%)	Loss in seed weight (%)	Germination after emergence of first generation of bruchid	Reduction in germination (%)	Oviposition	No. of adult emerged
T_1	8.40	91.40	0.00	0.00	90.60	0.86	0.00	0.00
T_2	8.24	91.30	3.20	1.71	82.05	10.12	15.85	56.30
T_3	8.44	91.25	8.10	4.56	75.14	16.94	20.85	131.30
T_4	8.45	91.50	13.95	9.20	69.45	24.28	32.90	180.00
T_5	8.40	91.50	26.29	21.93	59.60	35.44	46.90	272.00



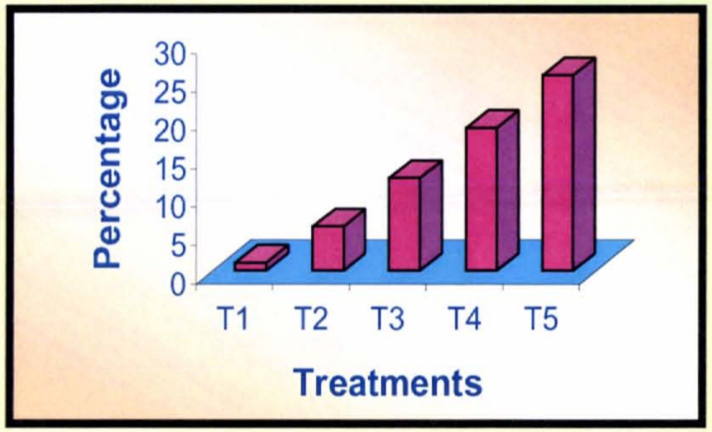
Number of damaged seeds



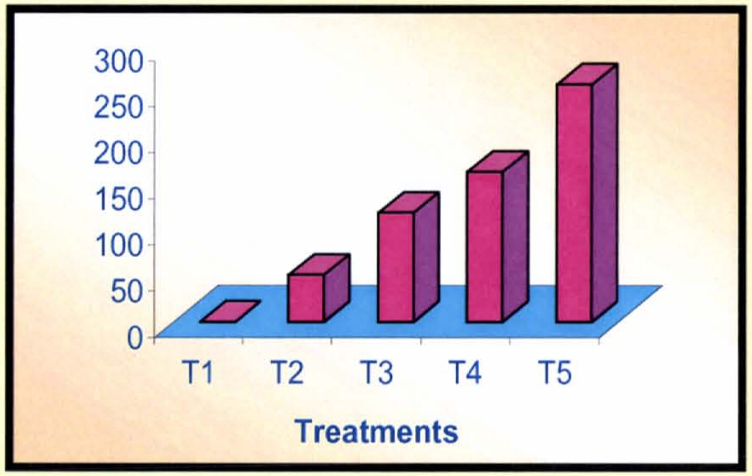
Loss in seed weight



Germination after emergence of first generation of bruchid



Reduction in germination



Number of adult emerged

Fig. 1 : Parameters of evaluating resistance to *Callosobruchus chinensis* in green gram genotypes

Table 3 : Mean performance different parameters for evaluating resistance in black gram

Treatment	Moisture content (%)					Initial Germination (%)					No. of Damaged Seed (%)					Loss in Weight (%)				
	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3
T_1	8.62 (17.07)	8.65 (17.10)	8.72 (17.18)	8.50 (16 94)	8.65 (17.10)	92.75 (74.46)	92.25 (73.87)	91.00 (72.55)	91.00 (74.49)	92.73 (73.58)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
T_2	8.57 (17.02)	8.60 (17.050	8.57 (17.02)	8.45 (16.89)	8.62 (17.07)	92.75 (74.59)	90.75 (72.31)	91.00 (72.55)	91.50 (72.29)	91.25 (72.81)	3.25 (10.29)	4.25 (11.80)	4.00 (12.50)	3.00 (9.58)	4.10 (11.30)	2.03 (8.20)	3.12 (8.30)	3.15 (10.20)	2.11 (10.17)	2.46 (9.00)
T_3	8.55 (17.00)	8.55 (17.00)	8.67 (17.12)	8.65 (17.10)	8.57 (17.02)	91.25 (72.81)	91.75 (73.37	91.75 (73.37)	91.50 (73.08)	90.75 (72.31)	11.75 (20.01)	16.25 (23.72)	7.00 (15.24)	8.25 (16.58)	7.00 (15.24)	10.00 (18.38)	10.17 (20.45)	6.68 (14.97)	8.30 (14.99)	4.10 (11.68)
T_4	8.47 (16.92)	8.67 (17.12)	8.65 (17.10)	8.65 (17.07)	8.60 (17.05)	92.75 (74.46)	92.50 (74.19)	91.00 (72.55)	92.00 (73.61)	91.25 (72.83)	18.00 (25.08)	25.5 (30.25)	19.00 (25.74)	13.00 (21.08)	12.00 (20.22)	16.07 (23.60)	21.08 (27.30)	14.25 (22.14)	9.50 (17.95)	7.46 (15.81)
T_5	8.60 (17.05)	8.57 (17.02)	8.67 (17.12)	8.65 (17.10)	8.67 (17.12)	92.75 (74.43)	91.75 (73.37)	91.25 (72.87)	91.75 (73.37)	90.75 (92.31)	29.50 (32.90)	34.50 (35.97)	25.00 (30.33)	21.00 (27.27)	19.00 (25.87)	19.20 (25.99)	27.00 (31.31)	18.20 (25.25)	12.10 (20.36)	11.20 (19.55)
S.E. ±	0.11	0.80	0.07	0.15	0.10	1.03	0.81	0.68	0.80	0.54	0.70	0.82	1.27	1.23	0.84	0.63	0.78	0.50	0.52	0.47
CD at 5 %	0.35	0.24	0.23	0.45	0.31	3.12	2.47	2.06	2.43	1.63	2.13	2.48	3.84	3.71	2.54	1.89	2.37	1.53	1.59	1.42

Treatment	Germination after emergence of first generation of bruchid (%)					Reduction in Germination (%)					No. of egg laid by female per 50 seeds					No of adult emerged				
	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3
T_1	91.50 (73.08)	91.00 (72.58)	90.25 (71.80)	90.25 (71.80)	91.00 (72.58)	1.33 (6.69)	1.35 (6.58)	0.81 (5.50)	0.81 (5.50)	0.81 (5 50)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2	84.25 (66.63)	83.25 (65.91)	87.25 (69.11)	87.25 (69.08)	88.00 (69.75)	9.07 (17.23)	8.25 (16.33)	4.11 (11.56)	3.84 (11.16)	3.54 (10.56)	26.25	22.75	17.00	12.00	13.25	7.25	89.25	59.50	49.75	47.75
T_3	81.75 (64.72)	73.00 (58.72)	80.00 (63.64)	83.75 (66.23)	83.50 (66.04)	10.40 (18.76)	20.39 (26.76)	12.91 (21.00)	8.95 (17.34)	7.98 (16.40)	32.75	31.25	24.50	24.00	21.25	131.25	184.75	100 75	84.75	64.5.0
T_4	74.25 (59 53)	67.00 (54.95)	77.50 (61.70)	77.00 (61.34)	79.50 (63.08)	21.84 (27.85)	27.57 (31.65)	14.83 (22.61)	16.30 (23.81)	12.85 (20.95)	37 25	47.75	34.00	30.50	34.25	185.00	251.50	164 50	112.75	99 00
T_5	66.25 (54.51)	58 75 (50.04)	67.00 (54.95)	73.50 (59.05)	75.50 (60.33)	28.57 (32 25)	35.96 (36 83)	26.52 (30.94)	19.84 (26.38)	16.78 (24.16)	42 50	50.25	41.75	40.00	38.00	304.75	395.00	239 75	194.50	154.25
S.E. ±	0.89	0.99	0 65	0.65	0.55	1.26	1.30	0.94	0.86	0.88	1.52	1.21	1.50	0.90	1.55	3.09	5.17	4.43	3.65	2.23
CD at 5 %	2.70	2.98	1.89	1.98	1.67	3.81	3 94	2.84	2.59	2.66	4.59	3.65	4.53	2.80	4.68	9.09	15.5	13.35	11.02	6.74

* Figures in parenthesis are aresin values

Table 3a : Average performance of treatments in black gram

Treatments	Moisture content (%)	Initial germination (%)	No. of damaged seeds (%)	Loss in seed weight (%)	Germination after emergence of first generation of bruchid	Reduction in germination (%)	Oviposition	No. of adult emerged
T_1	8.62	91.94	0.00	0.00	90.80	1.02	0.00	0.00
T_2	8.56	91.45	3.72	2.57	86.00	5.76	18.25	50.70
T_3	8.56	91.40	10.05	7.26	80.40	12.12	26.75	118.2
T_4	8.60	91.90	17.50	13.67	75.05	18.67	36.75	162.55
T_5	8.65	91.65	25.80	17.54	68.02	25.53	52.75	257.65

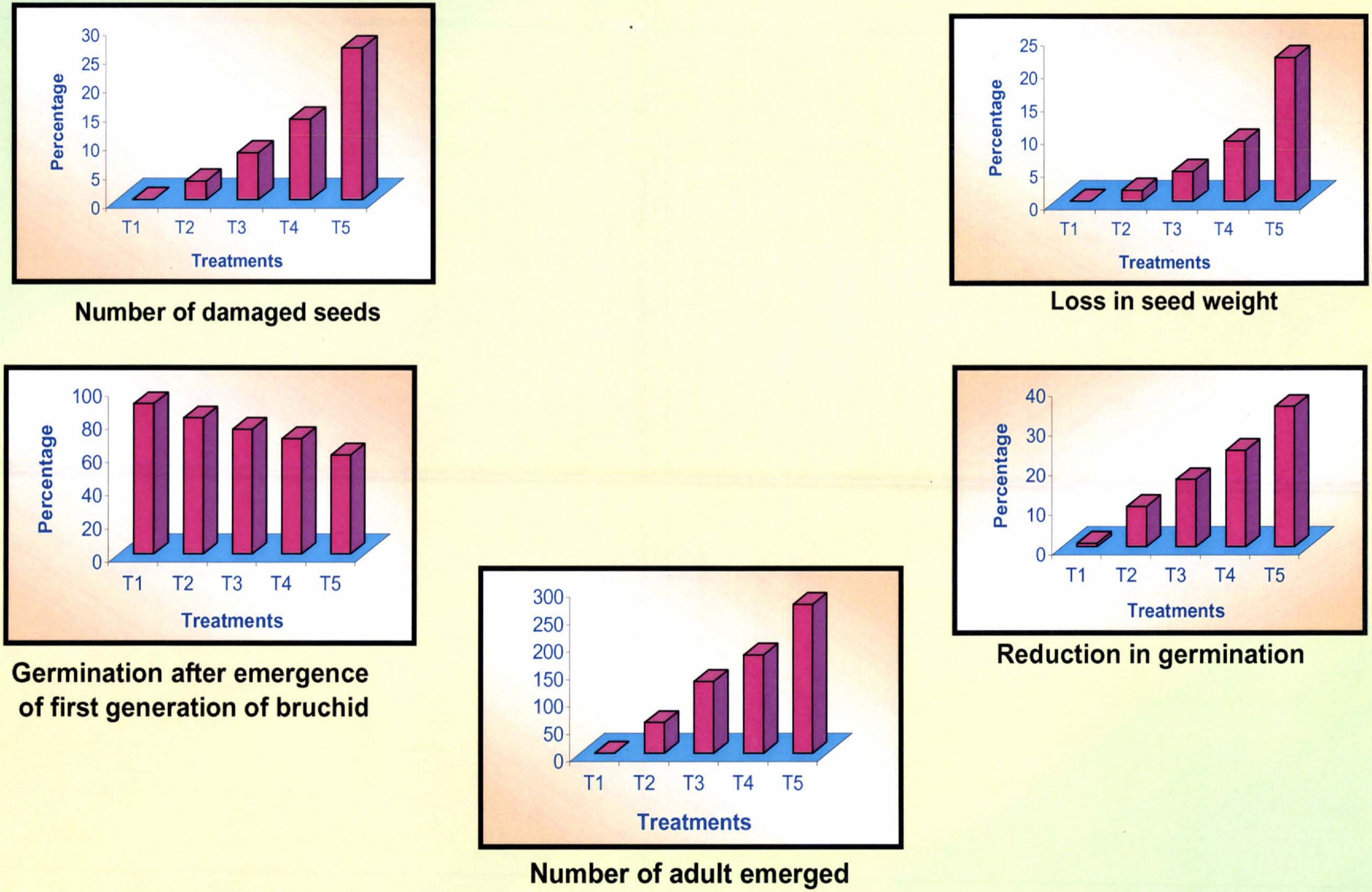


Fig. 2 : Parameters of evaluating resistance to *Callosobruchus chinensis* in black gram genotypes

Table 4 : Mean performance of different parameters for evaluating resistance in rice bean.

Treatment	Moisture content (%)					Initial Germination (%)					No. of Damaged seed (%)					Loss in Weight (%)				
	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202
T_1	8.62 (17.07)	8.50 (16.95)	8.70 (17.15)	8.57 (17.02)	8.67 (17.12)	93.00 (74.73)	92.00 (73.64)	93.00 (74.75)	93.25 (74.96)	94.75 (76.89)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2	8.55 (16.99)	8.65 (17.10)	8.67 (17.12)	8.60 (17.05)	8.52 (16.97)	92.00 (73.64)	92.00 (73.64)	93.00 (74.78)	93.00 (74.71)	93 25 (74.99)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_3	8.77 (17.23)	8.67 (17.12)	8.77 (17.23)	8.65 (17.10)	8.67 (17.12)	92.00(73.61)	93.00 (74.74)	92.00 (73.64)	93.00 (74.67)	93.50 (75.28)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_4	8.50 (16.94)	8.67 (17.12)	8.60 (17.05)	8.67 (17.12)	8.70 (17.15)	93.00 (75.02)	92.50 (73.64)	92.00 (73.64)	92.00 (73.64)	92.50 (74.19)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_5	8.72 (17.17)	8.55 (17.00)	8.70 (17.15)	8.72 (17.80)	8.70 (17.15)	93 00 (75.28)	93.00 (74.75)	93.00 (74.75)	92.50 (74.11)	94.00 (75.94)	0.00	0.00	0.00	0.00	0 00	0.00	0.00	0.00	0.00	0.00
S.E. ±	0.13	0.07	0.06	0.07	0.08	0.88	0.97	1.04	0.63	0.96										
CD at 5 %	0 41	0.22	0.20	0.21	0.26	2.66	2.93	3.13	1.99	2.91										

Treatment	Germination after emergence of first generation of bruchid (%)					)Reduction in Germination (%)					No. of egg laid by female per 50 seeds					No of adult emerged				
	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202
T_1	92.50 (74 17)	91.50 (72.83)	92.75 (73.64)	93.00 (73.64)	94.00 (73.33)	0.53 (4.99)	0.80 (5.47)	1.07 (5.93)	0.53 (5.93)	1.31 (6.50)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2	91.00 (72.58)	91.00 (72.81)	92.00 (73.63)	92.50 (74.14)	93.50 (73.87)	1.07 (6.09)	0.79 (5.60)	1.06 (6.06)	0.55 (6.10)	1.08 (6.07)	11	13	15	10	12	0.00	0 00	0.00	0.00	0.00
T_3	91.50 (73.13)	92.10 (73.61)	91.50 (73.11)	92.50 (73.321)	93.00 (74.40)	054 (5.02)	1.06 (6.07)	0.53 (5.00)	1.34 (5.00)	0.79 (5.44)	19	22	25	18	26	0.00	0.00	0.00	0.00	0 00
T_4	92.00 (74.19)	91.95 (73.08)	92.00 (72.31)	92.00 (72 82)	92.75 (73.36)	0.80 (5.45)	0.53 (4.98)	1.34 (6.71)	0.80 (6.75)	0.80 (5.46)	30	30	28	25	31	0 00	0.00	0.00	0.00	0.00
T_5	92.25 (74.40)	92 50 (74.19)	92.50 (73.37)	91.75 (73.31)	93.10 (74.75)	079 (5.59)	0.53 (4.99)	1.33 (6.70)	0.82 (6.71)	1.06 (6.05)	38	35	37	32	42	0.00	0 00	0 00	0.00	0 00
S, E. ±	0.86	0.76	0.84	0.62	0.86	0.72	0.73	0.96	0.67	0.69	1.36	2.16	2.46	1.80	1.95					
CD at 5 %	2.603	2.300	2.54	1.87	2.594	2.20	2.20	2.90	2.02	2.10	4.11	6.53	7.42	5 44	5.90					

* Figures in parenthesis are aresin values

Table 4a : Average performance of treatments in rice bean.

Treatments	Moisture content (%)	Initial germination (%)	No. of damaged seeds (%)	Loss in seed weight (%)	Germination after emergence of first generation of bruchid	Reduction in germination (%)	Oviposition	No. of adult emerged
T_1	8.61	93.20	0.00	0.00	92.75	0.80	0.00	0.00
T_2	8.59	93.65	0.00	0.00	92.00	0.91	12.20	0.00
T_3	8.70	92.70	0.00	0.00	92.12	0.85	22.00	0.00
T_4	8.62	92.40	0.00	0.00	92.14	0.85	28.80	0.00
T_5	8.67	93.10	0.00	0.00	92.42	0.90	36.80	0.00

maximum in case of Kopergaon (429.50) followed by AKM-8802 (300) in the treatment T_5.

The range of mean number of adult emerged in black gram genotypes were ranged from 0 to 257.65. The minimum number of adult emerged was recorded in case of PU-9502-11-3 (47.75) in the treatment T_2, while it was maximum in case of TAU-2 (395.00) followed by TAU-1 (304.75) in the treatment T_5.

In rice bean genotype there was no adult emerged in all the five treatments.

The present investigation was undertaken to study the effect of different levels of *Callosobruchus chinensis* infestation on deterioration and evaluation of resistance of green gram, black gram and rice bean seed under ambient condition. The deterioration and damage of seed was increasing as the pair of *Callosobruchus chinensis* increased. Therefore the evaluation of resistance was done on the basis of damaged seed (%) on release of maximum pair of bruchid i.e. 8 pair (T5). The resistance to bruchid was evaluated by recording the observation viz. oviposition, number of adult emerged, loss in seed weight, seed hardness, 100 seed weight, reduction in germination and number of resistant seed from the seeds infected by releasing maximum pair of bruchid (8 pair). The data collected was statistically analyzed and results are presented below.

4.1.2 Mean performance of different parameters for resistance at maximum population of bruchid

The mean values of different parameters studied for evaluating resistance in green gram, black gram and rice bean are presented in Table 5, 6, 7 and Figure 3, 4 respectively.

4.1.2.1 Oviposition

In green gram oviposition were minimum in case of TARM-18 (37.50) followed by PM-2 (40.75), PM-9339 (47.00) and AKM-8802 (52.75), while maximum in case of Kopergaon (56.50). The genotypes, Kopergaon, AKM-8802 and PM-9339 recorded more oviposition.

The mean oviposition among the black gram genotypes was ranged from 42.50 to 50.25 with mean of 42.50. The minimum oviposition was recorded by PU-9205-11-3 (38.00) followed by PU-9503-8 (40.00), TPU-4 (41.75) and TAU-1 (42.50), while the maximum oviposition was recorded in TAU-2 (50.25).

The mean oviposition in rice bean genotypes was ranged from 32 to 42. The lowest oviposition was observed in the case of LRB-199 (32) followed by RBL-50 (35), RBL-99 (37) and RBL-36 (38) in ascending order. The maximum oviposition was noticed in LRB-202 (42).

On the basis of mean performance oviposition were maximum in case of green gram (46.90) followed by black gram (42.50) and rice bean (36.80) Fig. 5.

4.1.2.2 Number of adult emerged

Number of adult emerged were minimum in case of TARM-18 (87.00) followed by PM-2 (261.75), PM-9339 (281.75) and AKM-8802 (300) in ascending order. While maximum number of adult emerged were observed in case of Kopergaon (429.50).

Mean number of adult emerged among black gram genotypes, ranged from 154.25 to 395 with an average of 257.65. The minimum number of adult emerged were recorded in PU-9205-11-3 (154.25) followed by in ascending order PU-9503-8 (194.50), TPU-4 (239.75) and TAU-1 (304.75), while maximum number of adults were emerged in TAU-2 (395).

In rice bean, there was no emergence of adult in all five genotypes.

On the basis of average number of adult emerged it was found that there was no emergence of adult in rice bean and number of adult emerged was highest in green gram (275) followed by black gram (257.65).

4.1.2.3 Loss in seed weight (%)

Mean loss in seed weight among the green gram genotypes, ranged from 11.26 to 28.15 percent with an average of 22.03 percent. The minimum loss in seed weight was recorded in TARM-18 (11.28) followed in ascending order by PM-2 (22.00), PM-9339 (23.68) and AKM-8802 (25.10), while the maximum loss in seed weight was observed in Kopergaon (28.15).

In black gram, loss in seed weight was minimum in PU-9205-11-3 (11.20) followed in ascending order by PU-9503-8 (12.10), TPU-4 (18.20) and TAU-1 (19.20), while the maximum loss in seed weight was found in TAU-2 (27). Three genotypes TAU-2, TAU-1 and TPU-4 had with maximum loss in seed weight.

There was no loss in seed weight in all five rice bean genotypes.

Overall average performance showed that the green gram had maximum loss in seed weight (22.03) followed by black gram (17.54) and no loss in seed weight in rice bean.

4.1.2.4 Seed hardness (kg. cm^{-2})

The mean seed hardness among green gram genotypes ranged between 3.40 to 4.80 kg/cm^2 with an average of 4.11 kg/cm^2. The maximum seed hardness was recorded by TARM-18 (4.80) followed by PM-9339 (4.30), PM-2 (4.22) and AKM-8802 (3.82), while the minimum seed hardness was recorded in Kopergaon (3.40).

Seed hardness of five black gram genotypes studied, ranged between 5.02 to 5.78 kg/cm^2, with an average of 5.35 kg/cm^2. The genotype PU-9205-11-3 showed the maximum seed hardness (5.72) followed by PU-9503-8 (5.62), TPU-4 (5.32), TAU-1 (5.10), while TAU-2 showed minimum seed hardness (5.02). The two genotypes, PU-9205-11-3 and PU-9503-8 had maximum seed hardness.

The range of mean seed hardness among the rice bean genotypes was between 6.12 to 6.50 kg/cm^2, with an average of 6.32 kg/cm^2. The maximum seed hardness was observed in RBL-50 (6.50) followed by LRB-202 (6.47), RBL-36 (6.30) and RBL-99 (6.25), while the minimum seed hardness was observed in LRB-199 (6.12).

According to overall average mean performance, the seed hardness was maximum in rice bean (6.32) followed by black gram (5.32) and green gram (4.11).

4.1.2.5 100 seed weight (g)

The mean 100 seed weight among the green gram genotypes ranged between 3.19 to 4.66 g with an average of 3.79 g. The minimum 100 seed weight was recorded by TARM-18 (3.19) followed in ascending order by PM-9339 (3.30), PM-2 (3.66) and AKM-8802 (4.15), while maximum 100 seed weight was recorded in Kopergaon (4.66).

The variation for 100 seed weight among the black gram genotypes ranged between 4.56 to 5.76 g. The genotype PU-9205-11-3 showed a minimum 100 seed weight (4.56) followed in ascending order by PU-9503-8 (4.72), TAU-1 (5.13) and TPU-4 (5.50), where as TAU-2 showed the maximum 100 seed weight (5.76).

In rice bean genotypes, 100 seed weight was observed minimum in case of LRB-202 (5.07) followed in ascending order by

RBL-50 (5.10), RBL-36 (5.16) and LRB-199 (5.27), while maximum 100 seed weight was in RBL-99 (6.18).

On the basis of overall average rice bean had a maximum 100 seed weight (5.35) followed by black gram (5.13) and green gram (3.79).

4.1.2.6 Number of resistant seed (per cent)

The mean percentage of resistant seed among the green gram genotypes was ranged between 60.75 to 87.00 percent with an average of 73.65. The maximum percentage of resistant seed was recorded in TARM-18 (87.00) followed by PM-2 (76.50), PM-9339 (73.25) and AKM-8802 (71.25), while the minimum percentage of resistant seeds was found in Kopergaon (60.75).

The overall percentage of resistant seed among the black gram genotypes ranged between 65.50 to 81.00 percent, with the maximum percentage of resistant seed in case of PU-9205-11-3 (81.00) followed by PU-9503-8 (79.00), TPU-4 (75.00) and TAU-1 (70.50). The three genotypes, PU-9205-11-3, PU-9503-8 and TPU-4 had more percentage of resistant seed.

All five genotypes of rice bean showed 100 per cent resistance to bruchid.

On the basis of overall percentage of resistance seed, rice bean showed 100 percent resistance to bruchid, and was followed by black gram (74.20) and green gram (73.65).

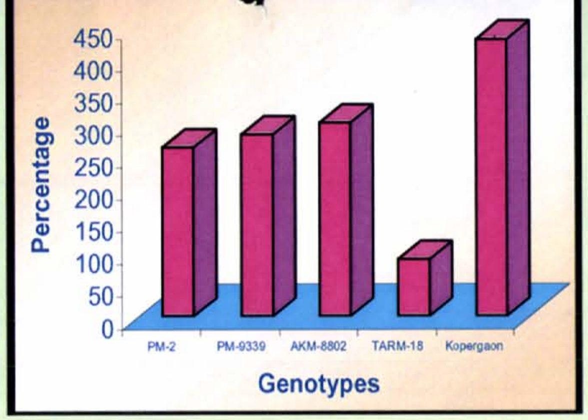
4.1.2.7 Reduction in germination (%)

The variation for reduction in germination percent among the green gram genotypes was ranged between 7.92 to 56.88 percent with an average of 35.45 percent. The minimum reduction in germination percent was recorded by TARM-18 (7.92) followed in ascending order by PM-2 (29.15), PM-9339 (39.36) and AKM-8802 (43.92), while the maximum reduction in germination percent was recorded in Kopergaon (56.88).

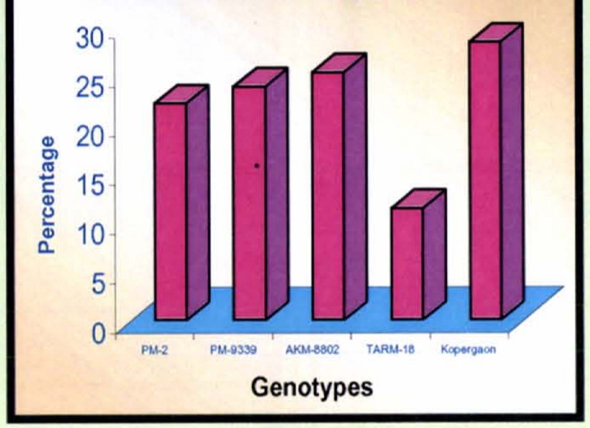
Table 5 : Mean performance of different parameters for evaluating resistance in green gram genotypes at the maximum population of bruchid

Sr. No.	Genotypes	Oviposition	No. of adult emerged	loss in seed weight (%)	Seed hardness (Kg/cm2)	100 seed weight (gm)	Reduction in germination (%)	No. of resistant seed (%)
1	PM-2	40.75	261.75	22.00	4.22	3.30	29.15 (31.83)	76.50 (61.03)
2	PM-9339	47.00	281.75	23.68	4.30	3.66	39.36 (38.83)	73.25 (58.87)
3	AKM-8802	52.75	300.00	25.10	3.82	4.15	43.92 (41.48)	71.25 (57.91)
4	TARM-18	37.50	87.00	11.26	4.80	3.19	7.92 (16.26)	87.00 (68.95)
5	Kopergaon	56.50	429.50	28.15	3.40	4.66	56.88 (48.96)	60.75 (51.21)
	S.E. ± CD at 5 %	2.18 6.58	3.27 9.86	0.90 2.71	0.15 0.45	0.03 0.11	1.37 4.15	0.90 2.71
	Mean	46.90	272.0	22.03	4.11	3.79	35.45	73.65

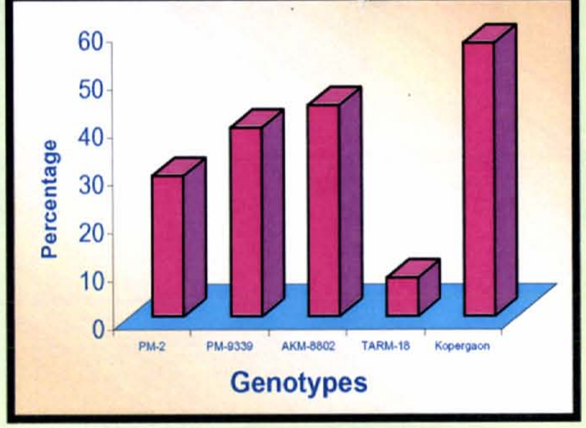
* Figures in parenthesis are arcsin value



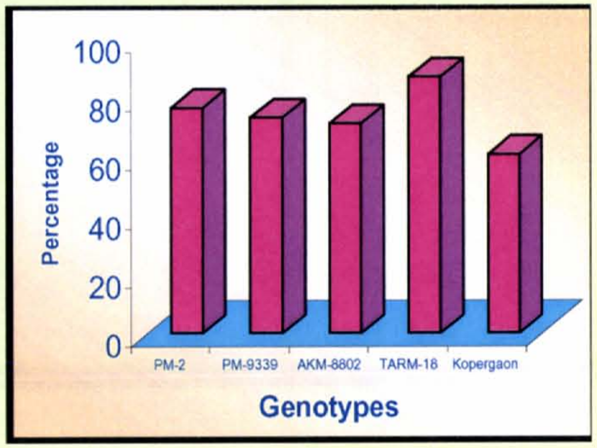
Number of adult emerged



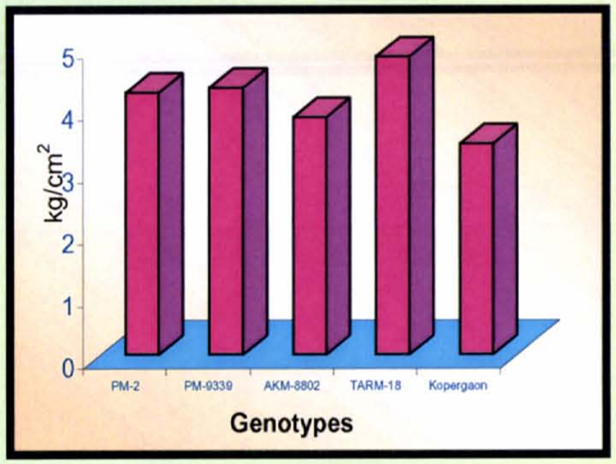
Loss in weight of seed



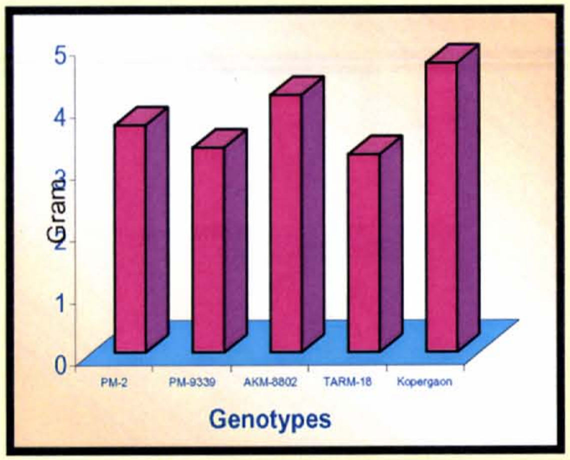
Number of resistant seed



Reduction in germination



Seed hardness



100 seed weight

Fig. 3 : Parameters of evaluating resistance to *Callosobruchus chinensis* in green gram genotypes at maximum population of bruchid

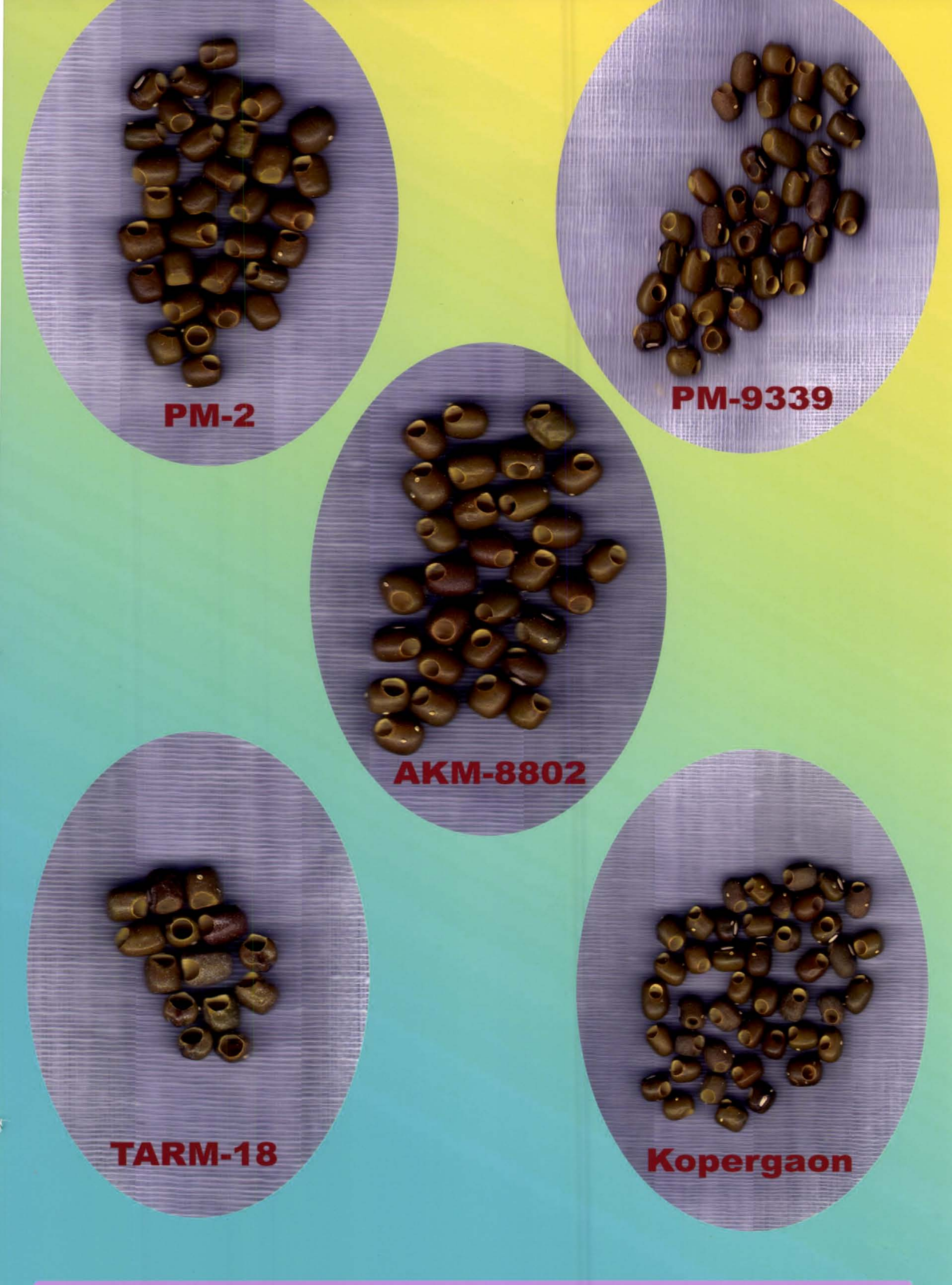


PLATE 3: Damaged seeds of green gram variety

Table 6 : Mean performance of different parameters for evaluating resistance in black gram genotypes at the maximum population of bruchid

Sr. No	Genotypes	Oviposition	No. of adult emerged	loss in seed weight (%)	Seed hardness (Kg/cm^2)	100 seed weight (gm)	Reduction in germination (%)	No. of resistant seed (%)
1	TAU-1	42.50	304.75	19.20	5.10	5.13	28.57 (32.25)	70.50 (57.10)
2	TAU-2	50.25	395.00	27.00	5.02	5.76	35.96 (35.92)	65.50 (54.03)
3	TPU-4	41.75	239.75	18.20	5.32	5.50	26.52 (30.94)	75.00 (60.00)
4	PU-9503-8	40.00	194.50	12.10	5.62	4.72	19.84 (26.38)	79.00 (62.73)
5	PU-9205-11-3	38.00	154.25	11.20	5.72	4.56	16.78 (24.16)	81.00 (64.16)
	S.E. ±	1.44	5.10	0.69	0.11	0.07	1.19	1.34
	CD at 5 %	4.35	15.38	2.10	0.35	0.22	3.60	4.04
	Mean	42.50	257.65	17.54	5.35	5.13	25.53	74.30

* Figures in parenthesis are arcsin value

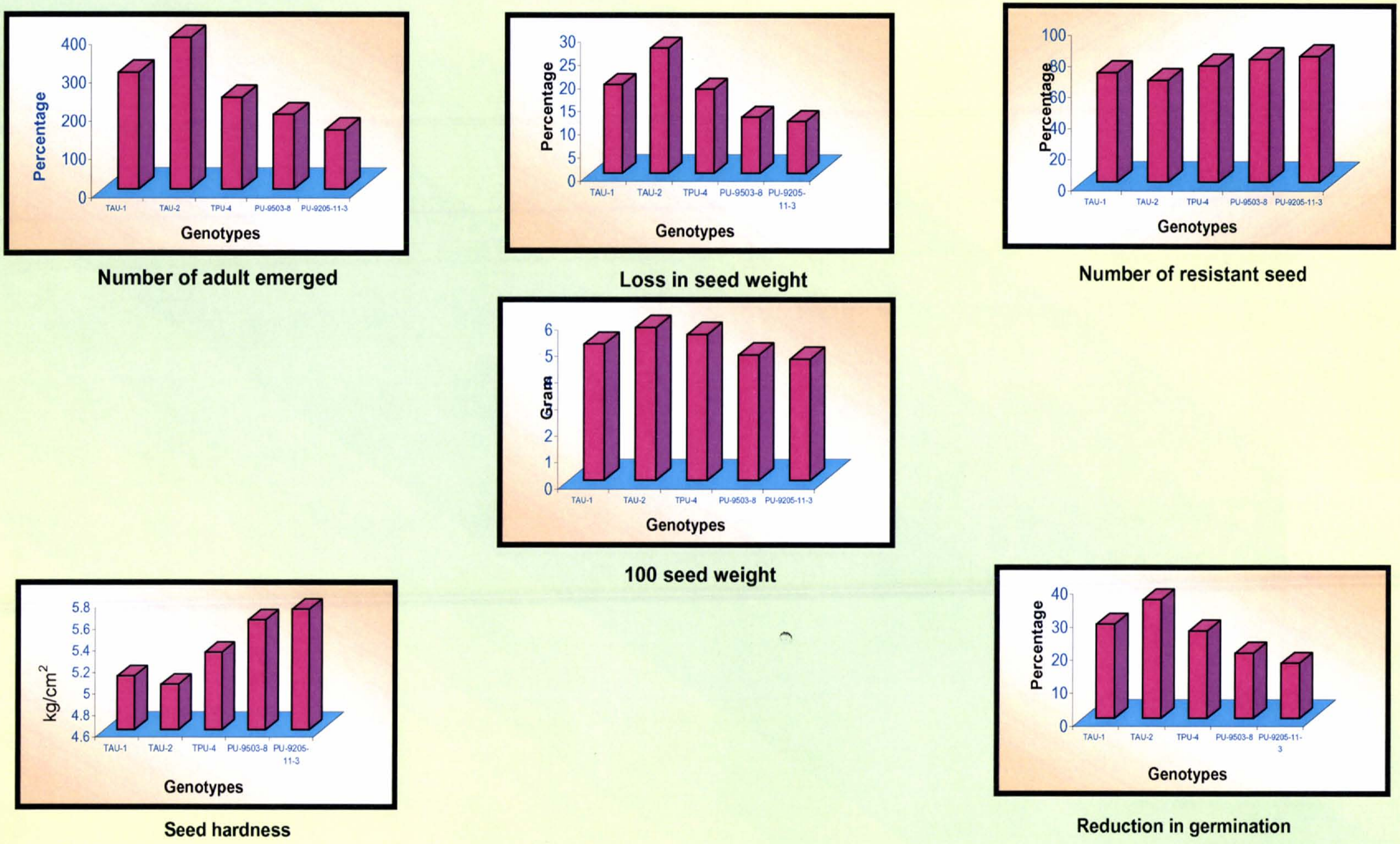


Fig. 4 : Parameters of evaluating resistance to *Callosobruchus chinensis* in black gram genotypes at maximum population of bruchid

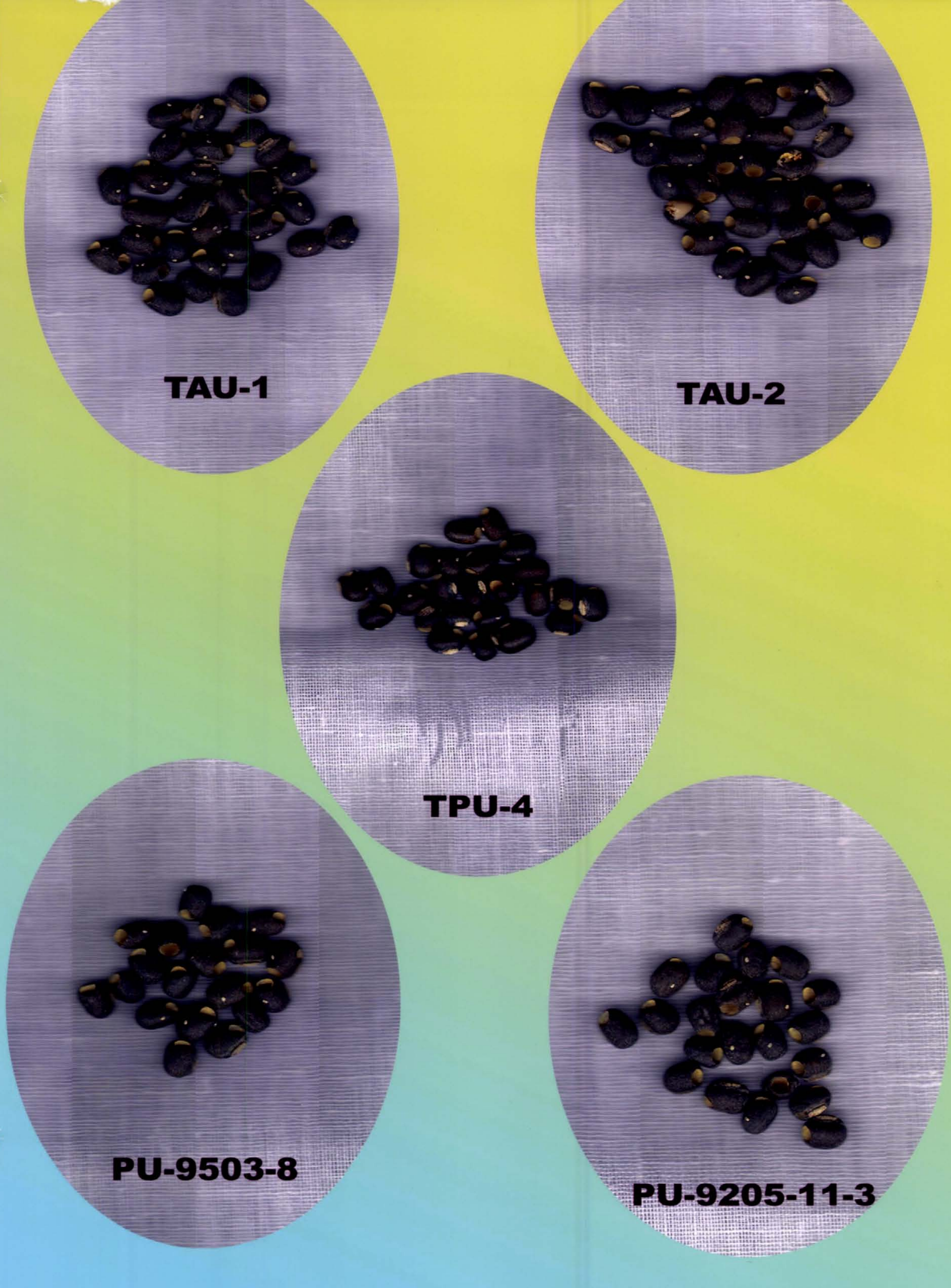
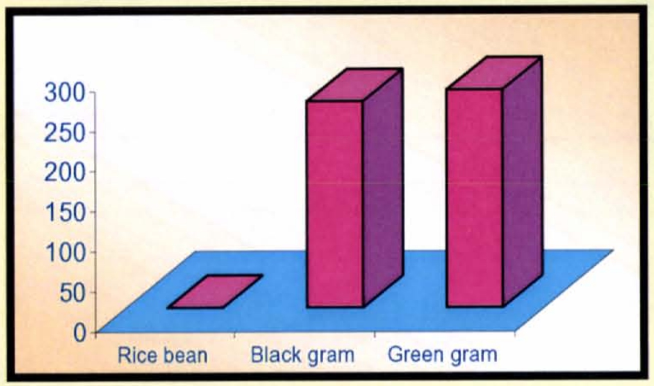


PLATE 4: Damaged seeds of black gram varieties

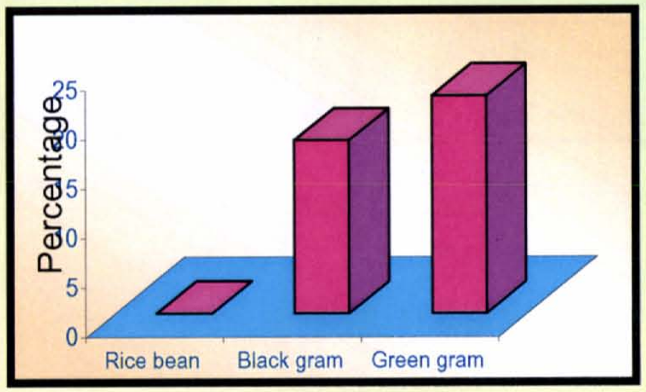
Table 7 : Treatment mean performance different parameters for evaluating resistance in rice bean at the maximum population of bruchid

Sr No.	Genotypes	Oviposition	No. of adult emerged	loss in seed weight (%)	Seed hardness (Kg/cm^2)	100 seed weight (gm)	Reduction in germination (%)	No. of resistant seed (%)
1	RBL-36	38	-	-	6.30	5.16	1.33 (6.70)	100
2	RBL-50	35	-	-	6.50	5.10	0.80 (5.47)	100
3	RBL-99	37	-	-	6.25	6.18	0.79 (5.59)	100
4	LRB-199	32	-	-	6.12	5.27	0.53 (4.99)	100
5	LRB-202	42	-	-	6.47	5.07	1.06 (6.05)	100
	S.E. ±	-	-	-	0.12	0.05	0.98	-
	CD at 5 %				0.36	0.16	2.95	
	Mean	36.80	0.00	0.00	6.32	5.35	0.90	100

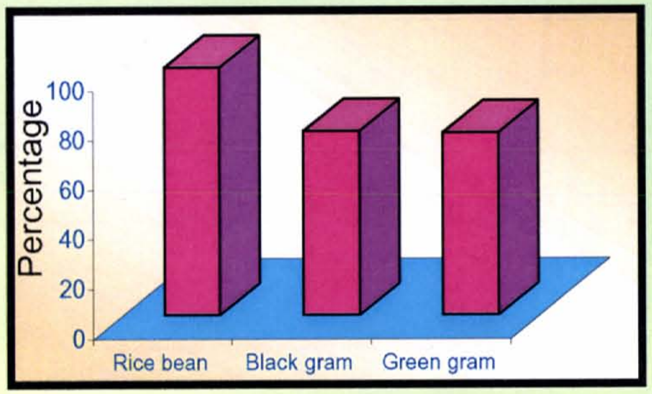
- Figures in parenthesis are arcsin value



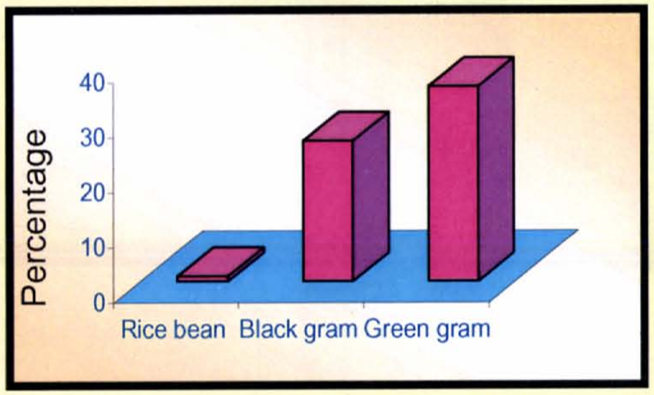
Number of adult emerged



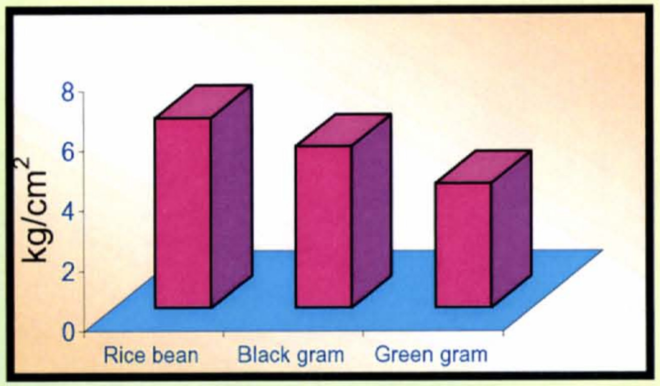
Loss in seed weight



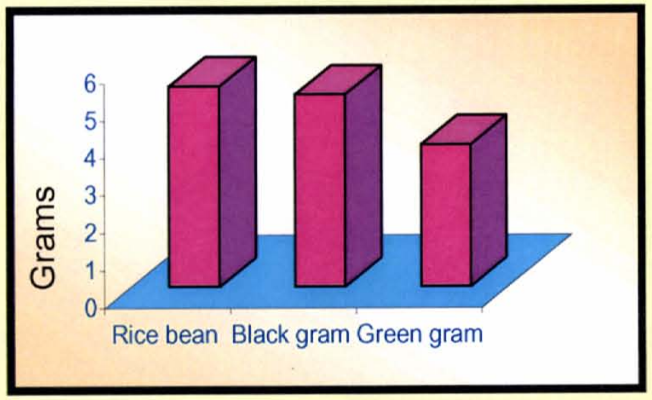
Number of resistant seed



Reduction in germination



Seed hardness



100 seed weight

Fig. 5 : Parameters of evaluating resistance to *Callosobruchus chinensis* **in *vigna* species at maximum population of bruchid**

Mean reduction in germination percent among the black gram genotypes was ranged between 16.78 to 35.96 percent. The lowest reduction in germination percent was observed in PU-9205-11-3 (16.78) followed by PU-9503-8 (19.84), TPU-4 (26.52) and TAU-1 (28.57), while the highest reduction in germination percent was recorded in TAU-2 (35.96).

The range of reduction in germination percent among rice bean genotypes was between 0.53 to 1.33 percent with the lowest reduction in germination percent in the case of LRB-199 (0.53) followed in ascending order by RBL-99 (0.79), RBL-50 (0.80) and LRB-202 (1.06) and maximum reduction in germination percent was noticed in RBL-36 (1.33).

On the basis of overall average, rice bean showed minimum reduction in germination percent (0.90) followed in ascending order by black gram (25.53) and green gram (35.45).

4.1.2.8 Correlation coefficient between various parameters of resistance to bruchid in green gram

The correlations for seven characters studied are presented in Table 8. Only significant correlation either in positive or negative directions are described in this chapter.

Oviposition

Oviposition exhibited significant negative correlation with resistant seed (r= -0.486) and seed hardness (r = -0.552).

Number of adult emerged

Number of adult emerged showed highly positive and significant association with loss in seed weight (r = 0.902) and 100 seed weight (r = 0.652), while it was highly significant and negatively correlated with resistant seed (r =-0.953) and seed hardness (r = -0.744).

Seed hardness

Seed hardness had positive and significant correlation with resistant seed (r=0.757), while seed hardness was significant negatively associated with loss in seed weight (r = -0.656).

Loss in seed weight

Loss in seed weight was significant negatively correlated with resistant seed (r =-0.874), while highly significant and positively correlated with 100 seed weight (r = 0.507).

100 seed weight

100 seed weight had highly significant and negative correlation with resistant seed (r = -0.632).

Path Coefficient Analysis

To find out direct and indirect contribution from each of the characters towards resistance to bruchid, path coefficient analysis was carried out. The correlation coefficient being more important were only partitioned into direct and indirect effects, which are presented in Table 9 and Fig. 6.

4.1.2.9 Direct and Indirect effect of various parameters of resistance to bruchid in green gram

Resistant seed vs. oviposition

Oviposition showed significant negative correlation with resistant seed (-0.486), which may be due to negative direct effect of oviposition (-0.132) on resistant seed. Number of adult emerged (-0.220) had highest negative indirect effect followed by seed hardness (-0.079) and loss in seed weight (-0.068).

Resistant seed vs. number of adult emerged

Number of adult emerged showed high negative direct effect (-0.516) on resistant seed. The indirect effects through loss

Table 8 : Correlation coefficient between various parameters and resistant seed to bruchid in green gram

.30 Sr. No.	Characters	No. of adult emerged	Seed hardness	Loss in seed weight	100 seed weight	Resistant seed
1	Oviposition	0.427	-0.552*	0.384	0.014	-0.486*
2	No. of adult emerged		-0.774**	0.902**	0.652**	-0.953**
3	Seed hardness			-0.656**	-0.242	0.757**
4	Loss in seed weight				0.507**	-0.874**
5	100 seed weight					-0.632**

** Significant at 1% level
* Significant at 5% level

in seed weight (-0.159), 100 seed weight (-0.112), seed hardness (-0.111) and oviposition (-0.056) were negative. The total correlation with resistant seed was negative and highly significant (-0.953).

Resistant seed vs. seed hardness

Seed hardness exhibited positive direct effect (0.143) on resistant seed. Its indirect effect through number of adult emerged (0.399) was positive and highest followed by loss in weight (0.116), number of eggs laid (0.072) and 100 grain weight (0.041). The total correlation with resistant seed was highly significant and positive (0.757) mostly due to strong indirect effect via. number of adult emerged.

Resistant seed vs. loss in seed weight

Loss in seed weight (-0.177) evinced negative direct effect on resistant seed. It showed high negative indirect effect through number of adult emerged (-0.465) followed by seed hardness (-0.094), 100 seed weight (-0.087) and oviposition (-0.050). The total correlation with resistant seed was negative and highly significant (-0.874) mostly due to strong indirect effect via. number of adult emerged.

Resistant seed vs. 100 seed weight

100 seed weight showed negative direct effect (-0.171) on resistant seed. It rendered highly negative indirect effect through number of adult emerged (-0.336) followed by loss in seed weight (-0.089) and seed hardness (-0.034).

Table 9 : Direct and Indirect effect of various parameters and resistant seed to bruchid in green gram.

Sr. No.	Characters	Oviposition	No. of adult emerged	Seed hardness	Loss in seed weight	100 seed weight	Correlation Coefficient (r)
1	Oviposition	<u>-0.132</u>	-0.220	-0.079	-0.068	-0.002	-0.486*
2	No. of adult emerged	-0.056	<u>-0.516</u>	-0.111	-0.159	-0.112	-0.953**
3	Seed hardness	0.072	0.399	<u>0.143</u>	0.116	0.041	0.757**
4	Loss in seed weight	-0.050	-0.465	-0.094	<u>-0.177</u>	-0.087	-0.874**
5	100 seed weight	-0.001	-0.336	-0.034	-0.089	<u>-0.171</u>	-0.632**

Under line figure indicate direct effect
Residual effect = 0.2717

** Significant at 1% level
* Significant at 5% level

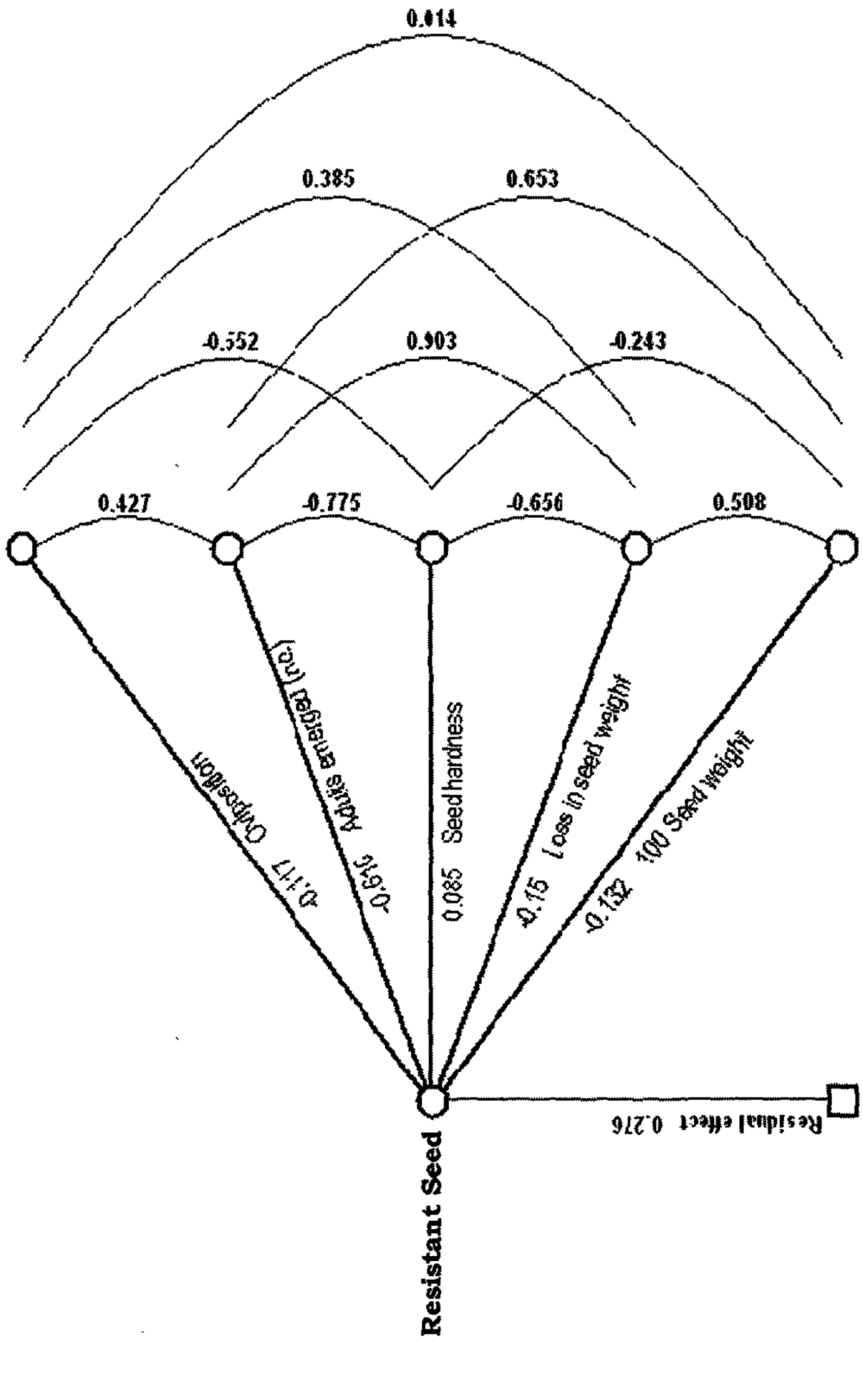


Fig. 6 : Path Diagram (GG)

4.1.2.10 Correlation coefficient between various parameters of resistance to bruchid in black gram

The correlations for seven characters studied for resistance to bruchid are presented in Table 10. Only significant correlation either in positive or negative directions are described in this chapter.

Number of adult emerged

Number of adult emerged exhibited highly positive and significant correlation with loss in seed weight (r=0.922) and positive significant correlation with 100 seed weight (r=0.524); while highly negative and significant association with resistant seed (r=-0.742).

Seed hardness

Seed hardness was significant positively correlated with resistant seed (r=0.538).

Loss in seed weight

Loss in seed weight showed highly significant and positive correlation with 100 seed weight (r=0.566), while it was negatively correlated with resistant seed (r=-0.781).

100 seed weight

100 seed weight had significant negative correlation with resistant seeds (r=-0.484).

Table 10 : Correlation coefficient between various parameters and resistant seed to bruchid in black gram

Sr. No.	Characters	No. of adult emerged	Seed hardness	Loss in seed weight	100 seed weight	Resistant seed
1	Oviposition	0.171	-0.187	0.304	0.270	-0.023
2	No. of adult emerged		0.173	0.922**	0.524*	-0.742**
3	Seed hardness			-0.297	0.222	0.538*
4	Loss in seed weight				0.566**	-0.781**
5	100 seed weight					-0.484*

** Significant at 1% level
* Significant at 5% level

4.1.2.11 Direct and indirect effects of various parameters of resistance to bruchid in black gram

To find out direct and indirect contribution of each character, path coefficient analysis was carried out, which are presented in Table 11and Fig. 7.

Resistant seed vs. oviposition

Oviposition showed positive direct effect (0.320) and it had negative indirect effect through almost all the characters and thus leading to non-significant negative correlation with resistant seed (-0.023).

Resistant seed vs. number of adult emerged

Number of adult emerged exhibited negative direct effect (-0.306). The indirect effect through 100 seed weight (-0.221), loss in seed weight (-0.188) and seed hardness (-0.100) was negative, where as oviposition (0.055) showed positive indirect effect.

Resistant seed vs. seed hardness

Seed hardness showed high positive direct effect (0.579) on resistant seed. Its indirect effects through 100 seed weight (-0.094) and oviposition (-0.060) were negative, as against loss in seed weight (0.060) and number of adult emerged (0.053) showed positive indirect effect. The total correlation with resistant seed was positive and significant (r=0.558).

Resistant seed vs. loss in seed weight

Loss in seed weight showed highly negative and significant correlation with resistant seed (r=0.781). Similarly it had negative direct effect (-0.204) on resistant seed. It's indirect

Table 11 : Direct and Indirect effect of various parameters on resistant seed to bruchid in black gram.

Sr. No.	Characters	Oviposition	No. of adult emerged	Seed hardness	Loss in seed weight	100 seed weight	Correlation Coefficient (r)
1	Oviposition	0.320	-0.052	-0.108	-0.062	-0.114	-0.023
2	No. of adult emerged	0.005	-0.306	-0.100	-0.188	-0.221	-0.742**
3	Seed hardness	-0.060	0.053	0.579	0.060	-0.094	0.538*
4	Loss in seed weight	0.097	-0.283	-0.172	-0.204	-0.238	-0.781**
5	100 seed weight	0.086	-0.160	0.129	-0.115	-0.422	-0.484*

Under line figure indicate direct effect
Residual effect = 0.3225
** Significant at 1% level
* Significant at 5% level

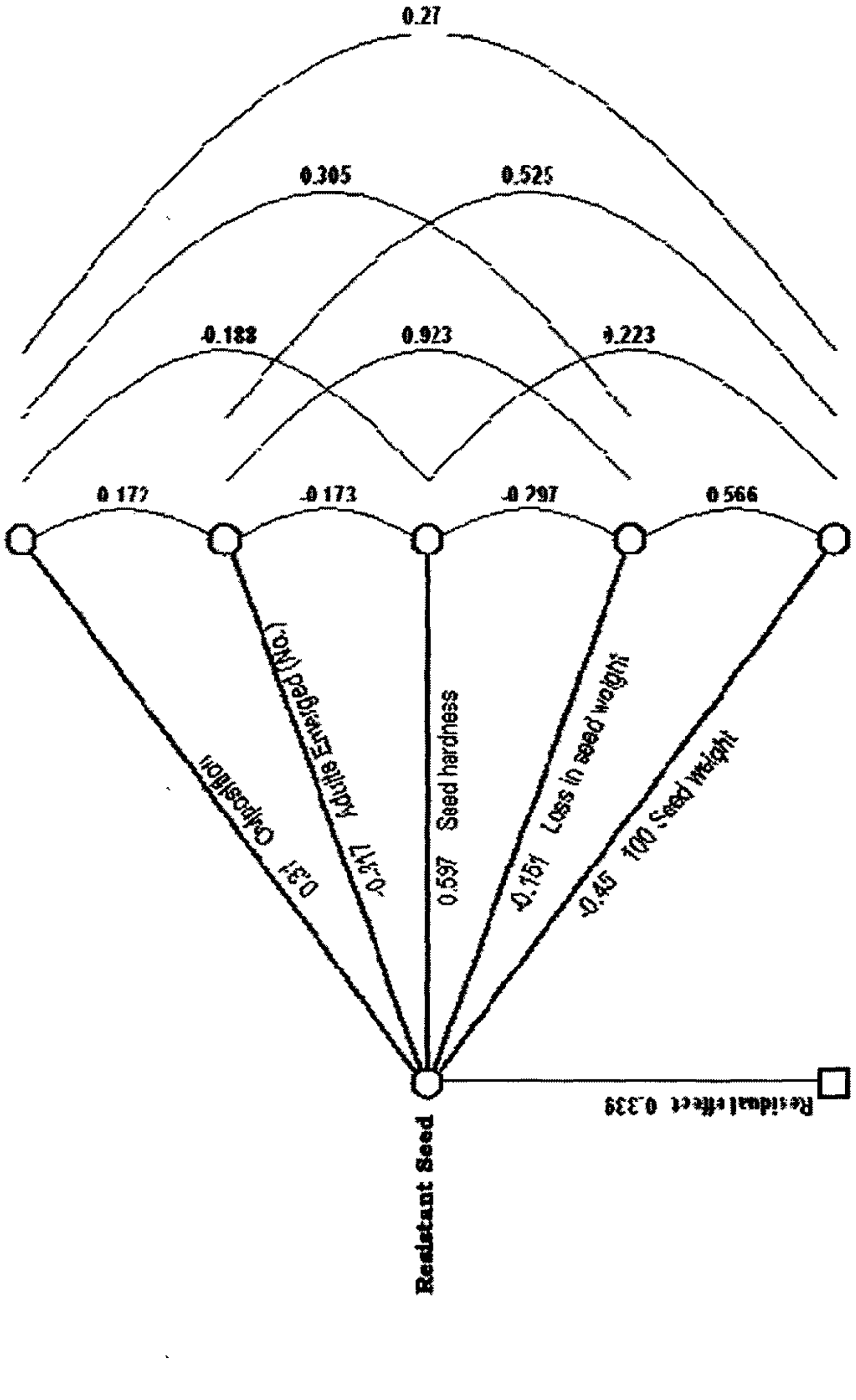


Fig. 7 : Path diagram (BG)

effect via. number of adult emerged (-0.283), 100 seed weight (-0.238) and seed hardness (-0.172) were negative and through remaining characters were positive.

Resistant seed vs. 100 seed weight

100 seed weight showed negative and high direct effect on resistant seed. It showed positive indirect effect through seed hardness (0.129) and oviposition (0.086). Its indirect effect through number of adult emerged (-0.160), loss in seed weight (-0.115).

4.2 Biochemical parameters

The mean values of biochemical parameters studied for evaluating resistance in green gram, black gram and rice bean are presented in Table 12, 13, 14 and Fig. 8, 9, 10, and 11 respectively.

4.2.1 ∝-amylase inhibitor (µ moles of maltose formed/10 min)

Quantification of five green gram genotypes for ∝-amylase inhibitor potential (measure in terms of percent inhibitor of ∝-amylase potential revealed that the alpha-amylase inhibitor potential varied from 12.67 to 16.00 with average of 14.11. A maximum ∝-amylase inhibitor potential 16.00 was recorded in genotype TARM-18 followed by PM-2 (15.30), PM-9339 (13.45) and AKM-8802 (13.15) as against a minimum in Kopergaon(12.64). The genotypes TARM-18 (16.00) and PM-2 (15.30) recorded more than the average ∝-amylase inhibitor potential.

∝-Amylase inhibitor potential (measured in terms of percentage inhibition of ∝-amylase activity) of five black gram genotypes was ranged from 22.60 to 27.17 with a mean ∝-amylase inhibitor potential of 25.18. Genotype PU-9503-8 recorded a maximum ∝-amylase inhibitor potential i.e. 27.17, followed by PU-

9205-11-3 (26.00), TPU-4 (25.62) and TAU-1 (24.52) and it was minimum in TAU-2 (22.60). Genotypes, PU-9503-8, PU-9205-11-3 and TPU-4 were recorded more alpha amylase inhibitor potential than the average.

The ∝-amylase inhibitor potential of rice bean was found to vary from 24.51 to 28.37 with an average inhibitor potential of 25.59. ∝-Amylase inhibitor potential was observed maximum in case of RBL-36 (28.37) followed by RBL-99 (25.05), LRB-199 (25.02) and RBL-50 (25.00), while the minimum in case of LRB-202 (24.51). Only one genotype viz. RBL-36 recorded as more alpha amylase inhibitor potential than the average.

There was no much difference in the average of alpha amylase potential between rice bean and black gram, whereas the green gram exhibited low ∝-amylase inhibitor potential.

4.2.2 Trypsin inhibitor (units/ml/10 min)

As evident form Table 12 the trypsin inhibitor potential was found to vary from 53.00 to 93.20 with an average of 69.16 in green gram genotypes. The trypsin inhibitor potential was observed maximum in case of TARM-18 (93.20) followed by PM-2 (74.50), PM-9339 (72.00) and AKM-8802 (56.50), while it was minimum in case of Kopergaon (53.00). The genotypes TARM-18, PM-2 and PM-9339 were exhibited with more trypsin inhibitor potential than the average.

The variation for trypsin inhibitor potential in black gram genotypes was ranged from 59.50 to 70.25. The maximum trypsin inhibitor potential was observed in genotypes PU-9205-11-3 (70.25 followed by PU-9503-8 (68.7), AKM-8802 (67.25) and TAU-1 (64.50), where as TAU-2 showed the minimum trypsin inhibitor potential (59.50). The average mean performance of five genotype of black gram was 66.10 units/ml/10 min. The three genotypes, PU-9205-11-3 (70.25), PU-9503-8 (68.75) and TPU-4

(67.25) were observed with more tyrpsin inhibitor potential than the average.

The mean trypsin inhibitor potential among the rice bean genotypes studied ranged from 71.50 to 82.50 units/ml/10 min with an average of 78.18. A maximum trypsin inhibitor potential was observed in LRB-199 (82.50) followed by LRB-202 (79.75), RBL-99 (87.50) and RBL-50 (78.25) as against a minimum (71.50) was recorded in RBL-36. Four genotypes, LRB-202, LRB-199, RBL-99 and RBL-50 were recorded with the maximum trypsin inhibitor potential than the average.

On the basis of average over the crop it was observed that rice bean had the highest trypsin inhibitor potential than black gram and green gram.

4.2.3 Protein content (%)

The mean protein content in grain among green gram genotypes studied ranged from 19.79 to 28.54 per cent. The minimum protein content was recorded by TARM-18 (19.79) and PM-9339 (20.77), while maximum protein content was recorded in Kopergaon (28.54). The genotypes Kopergaon and AKM-8802 showed more protein percent than the average (23.31).

In black gram, the minimum protein content was recorded in PU-9205-11-3 (16.62) followed by PU-9503-8 (17.38), while the maximum protein content was recorded in TAU-2 (24.93). The variation for protein content was ranged from 17.38 to 24.93 per cent. Two genotypes viz. TAU-2 and TAU-1 showed more protein content than the mean performance.

In case of rice bean, the variation for protein content was ranged between 20.34 to 23.07 per cent. The minimum protein content was recorded in RBL-99 (20.35) followed by RBL-36 (21.43), while maximum protein was observed in LRB-202 (23.07). The genotypes RBL-36 (21.43), RBL-50 (22.09), LRB-199

(21.65) and LRB-202 (23.07) showed more protein percent than the average.

The average mean performance of protein content over the crop showed that the green gram had maximum protein content (23.31) followed by rice bean (21.71) and black gram (20.05).

4.2.4 Total phenol (mg/100 g)

Total phenol content ranged from 22.5 to 57.5 among the green gram genotypes. The maximum total phenol content was observed in TARM-18 (57.5), followed by PM-9339 (37.5) and PM-2 (32.5), while the minimum total phenol content was recorded in Kopergaon (22.5). The genotypes TARM-18 and PM-2 showed more total phenol content than the mean performance (34.97).

The mean total phenol content in the black gram genotypes studied ranged from 32.5 to 55.0. The genotype PU-9205-11-3 showed the maximum total phenol content (55.0) followed by PU-9503-8 (42.5) and TPU-4 (42.5) while the minimum total phenol content was recorded in TAU-2 (32.5). Two genotypes viz. PU-9503-8 (47.5) and PU-9205-11-3 (55.0) were recorded as more total phenol content than the average (42.5).

The total phenol exhibited range of 50.0 to 65.0 mg/100 mg among the genotypes rice bean. The maximum total phenol content was recorded in RBL-50 (65.0) followed by RBL-99 (60), RBL-36 (57.5) and LRB-199 (52.5), while the minimum was recorded in LRB-202 (50). RBL-36, RBL-99 and RBL-50 were recorded as more total phenol content than the average (57.0).

According to average over the crop, rice bean crop exhibited maximum total phenol content (57.0) followed by black gram (42.5). The minimum total phenol content was recorded in green gram (34.97).

4.2.5 Tannic acid (%)

The tannic acid content of the five different genotypes of green gram varied from 0.74 to 1.28 per cent. Genotype TARM-18 recorded maximum tannic acid content i.e. 1.28 followed by PM-2 (1.10), AKM-8802 (0.96) and PM-9339 (1.06) as against genotype Kopergaon that recorded the minimum tannic acid of 0.74 percent. The genotypes viz. PM-2 (1.10), PM-9339 (1.06) and TARM-18 (1.28) were more tannic acid content than the average (1.02).

The variation for tannic acid content was ranged from 1.02 to 1.45 in black gram genotypes. It was recorded maximum in PU-9503-8 (1.45) followed by PU-9205-11-3 (1.30), TAU-1 (1.21) and TPU-4 (1.11). The genotype TAU-2 showed the minimum tannic acid content i.e. 1.02. The genotypes, PU-9503-8 and PU-9205-11-3 were showed more tannic acid content than the average (1.21).

The maximum tannic acid content of rice bean genotypes was recorded in case of RBL-99 (1.98), while the minimum in case of RBL-36 (1.70). Three genotypes RBL-99, LRB-190 and LRB-202 was recorded more tannic acid content than the average (85).

On the basis of average over the crop, rice bean recorded high tannic acid content i.e. 1.85 followed by black gram (1.21) and green gram (1.02).

4.2.6 Polyphenol oxidase activity (ΔA min^{-1} mg^{-1} soluble proteins)

The variation for polyphenol oxidase activity among green gram genotypes ranged between 2.41 to 6.48. The maximum polyphenol oxidase activity of 6.48 was recorded in case of Kopergaon followed by TARM-18 (5.97), PM-9339 (3.07) and PM-2 (2.48), while the minimum in AKM-8802 (2.41). The genotypes

Kopergaon and TARM-18 were found to have more polyphenol oxidase activity than the average (4.08).

The mean polyphenol oxidase activity among the black gram genotypes studied ranged from 3.45 to 5.89 with an average of 4.73. Genotype TAU-2 had maximum polyphenol oxidase activity i.e. 5.89 of soluble protein followed by TPU (5.43), PU-9205-11-3 (5.31) and PU-9503-8 (3.57), while the minimum polyphenol oxidase activity was recorded in TAU-1 (3.45). The genotypes TAU-2, TPU-4 and PU-9205-11-3 exhibited more polyphenol oxidase activity than average means performance (4.73).

Polyphenol oxidase activity in rice bean genotypes was observed to be maximum in case of LRB-202 (4.15) followed by RBL-36 (3.95), RBL-50 (3.50) and LRB-199 (3.25), while minimum in case of RBL-99 (2.91). The genotype LRB-202 and RBL-50 showed maximum polyphenol oxidase activity than average (3.50).

According to average over the crop it was observed that black gram had maximum polyphenol oxidase activity (4.73), while rice bean showed minimum polyphenol oxidase activity (3.50).

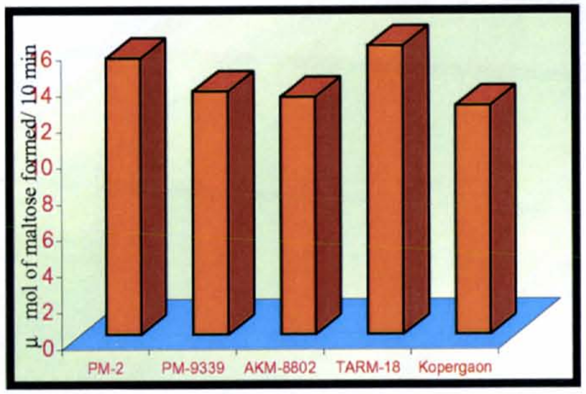
4.2.7 Peroxidase activity (ΔA $min^{-1}mg^{-1}$ soluble protein)

The variation for peroxidase activity among the green gram genotypes was ranged between 2.15 to 5.12 of protein with an average performance of 3.13. Kopergaon had maximum peroxidase activity i.e. 5.12, while TARM-18 had minimum peroxidase activity (2.15). Only Kopergaon was recorded with maximum peroxidase activity than the mean performance.

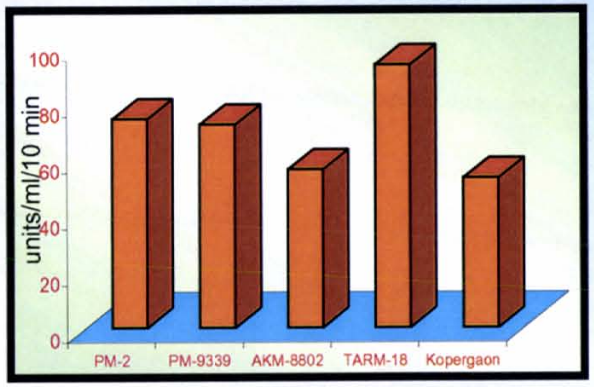
The mean peroxidase activity among the black gram genotypes studied was ranged between 2.66 to 5.02. The maximum peroxidase activity was recorded by TAU-1 (5.02) followed by PU-9205-11-3 (3.50), TAU-2 (3.36) and PU-9503-8

Table 12 : Biochemical parameters recorded on genotypes of green gram

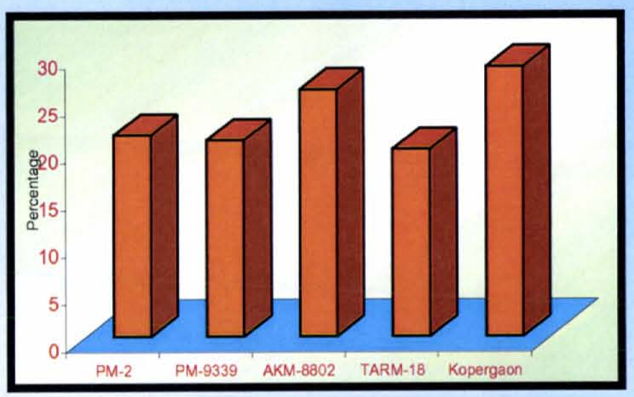
Sr. No.	Genotypes	∝-amylase inhibitor activity (μ mol of maltose formed /10 min)	Trypsin inhibitor activity (Unities /ml/10 min)	Protein (%)	Total phenol (mg/100 gm)	Tannic acid (%)	Polyphenol oxidase activity (A/min/mg of soluble protein)	Peroxidase activity (A/min/ mg of soluble protein)
1	PM-2	15.30	74.00	20.77	32.50	1.10	2.48	3.00
2	PM- 9339	13.45	72.00	21.32	37.35	1.06	3.07	2.92
3	AKM - 8802	13.15	56.50	26.13	25.00	0.96	2.41	2.48
4	TARM -18	16.00	93.20	19.79	57.50	1.28	5.97	2.15
5	Kopergaon	12.67	53.00	28.54	22.50	0.74	6.48	5.12
	S.E. ±	0.52	0.78	0.42	1.39	0.06	0.25	0.20
	CD at 5 %	1.58	2.36	1.29	4.19	0.20	0.75	0.61
	Mean	14.11	69.74	23.31	34.97	1.02	4.08	3.13



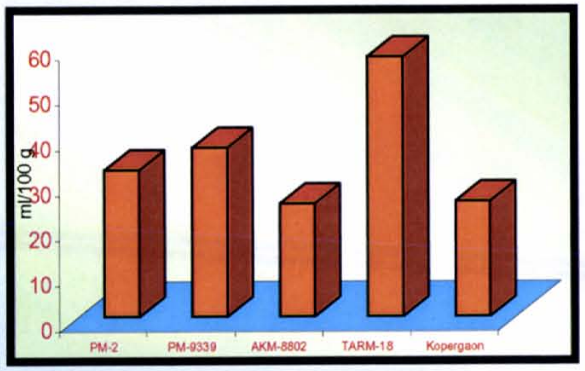
Alpha-amylase inhibitor activity



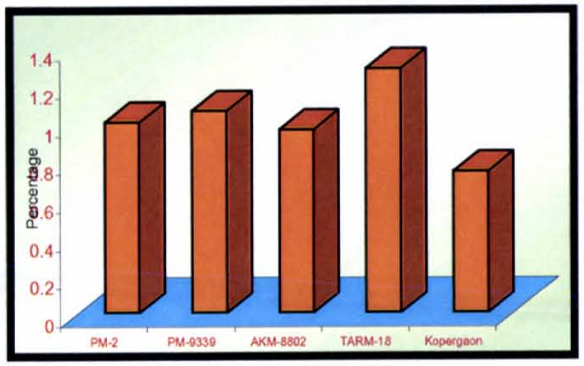
Trypsin inhibitor activity



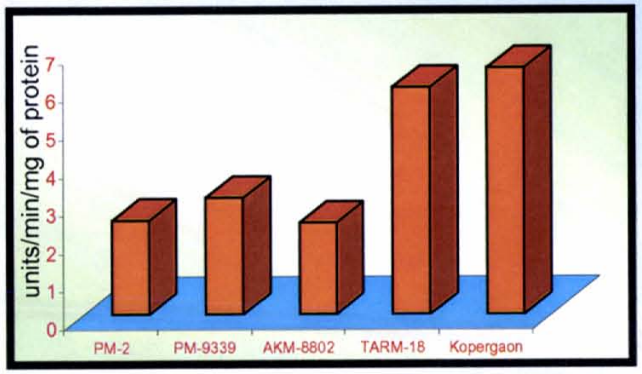
Protein percent



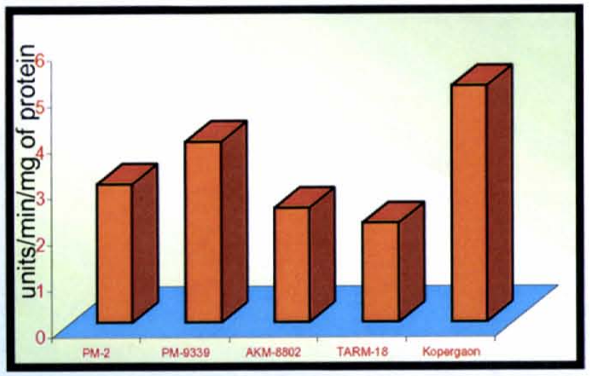
Total phenol



Tannic acid



Polyphenol oxidase activity



Peroxidase activity

Fig. 8 : Biochemical parameters of evaluating resistance to *Callosobruchus chinensis* n green gram genotypes

Table 13 : Biochemical parameters recorded on genotypes of black gram

Sr. No.	Genotypes	∝-amylase inhibitor activity (µ mol of maltose formed /10 min)	Trypsin inhibitor activity (Units/ml/10 min)	Protein (%)	Total phenol (mg/100 gm)	Tannic acid (%)	Polyphenol oxidase activity (A/min/mg of soluble protein)	Peroxidase activity (A/min/ mg of soluble protein)
1	TAU-1	24.52	64.50	21.54	35.00	1.21	3.45	5.02
2	TAU-2	22.60	59.50	24.93	32.50	1.02	5.89	3.36
3	TPU-4	25.62	67.25	19.79	42.50	1.11	5.43	2.66
4	PU-9503-8	27.17	68.75	17.38	47.50	1.45	3.57	3.14
5	PU-9205-11-3	26.00	70.25	16.62	55.00	1.30	5.31	3.50
	S.E. ±	0.64	1.00	0.25	0.87	0.05	0.26	0.15
	CD at 5 %	1.93	3.02	0.76	2.65	0.15	0.80	0.46
	Mean	25.18	66.10	20.05	42.50	1.21	4.73	3.53

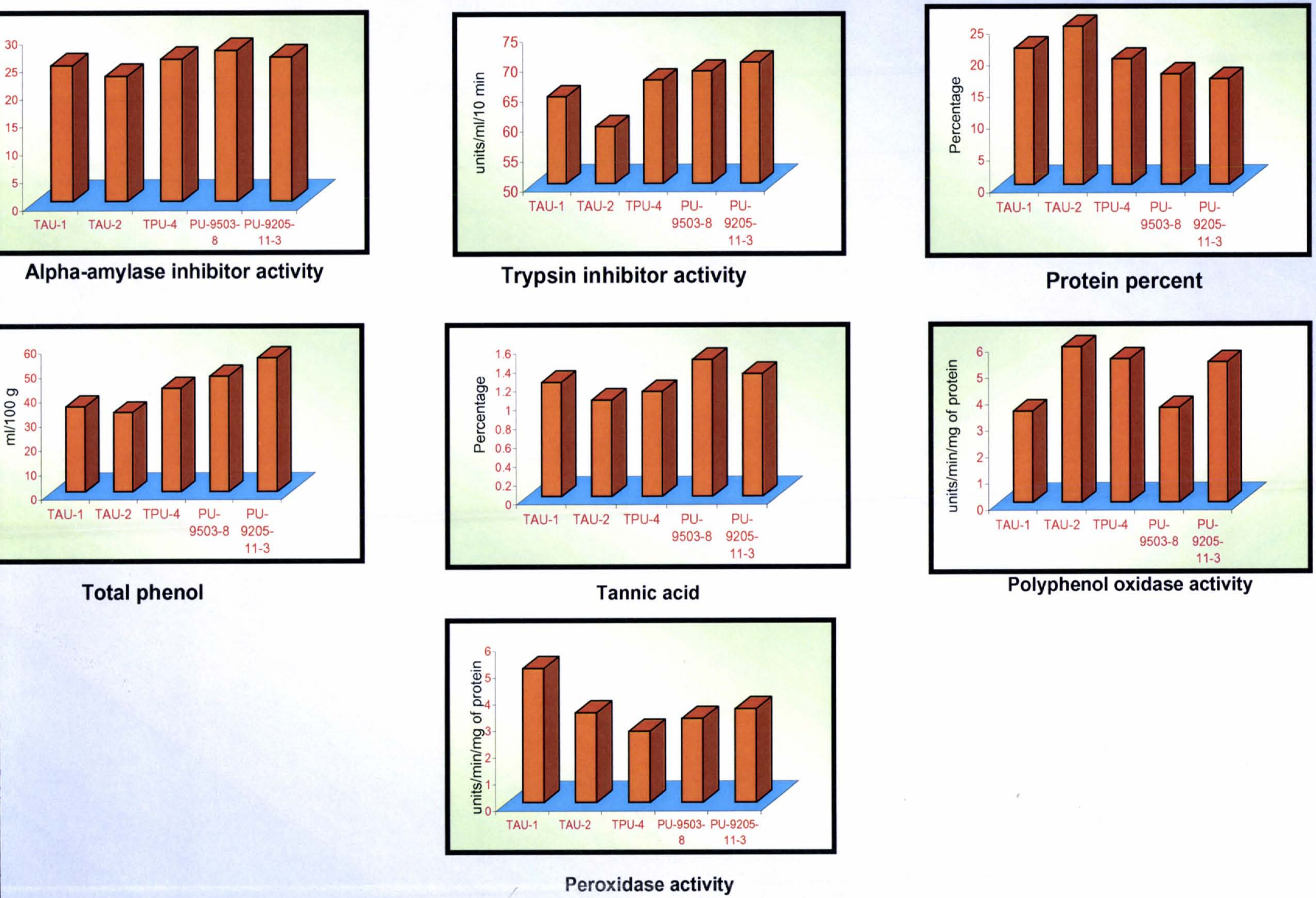
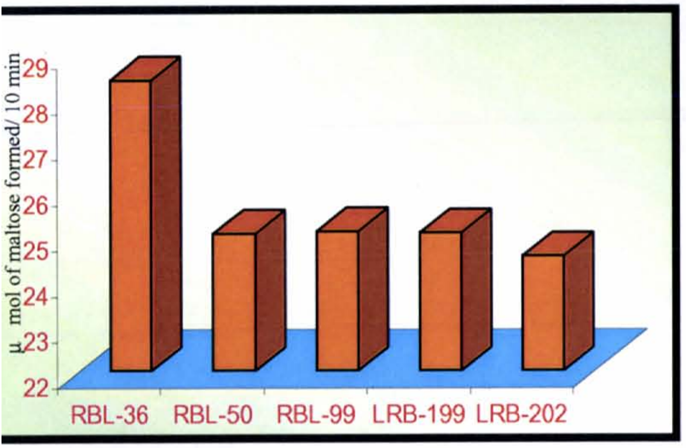


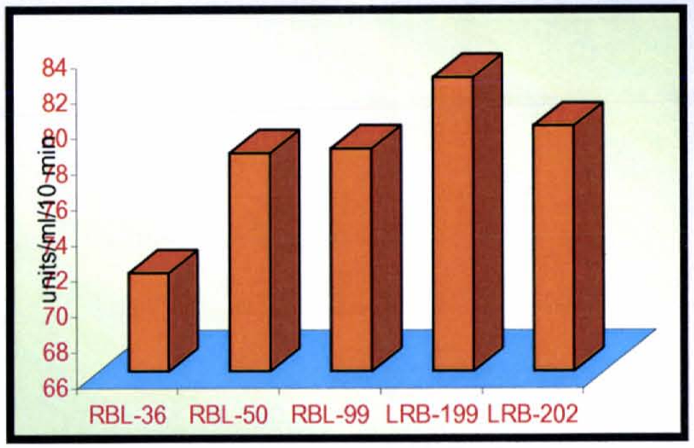
Fig. 9 : Biochemical parameters of evaluating resistance to *Callosobruchus chinensis* in black gram genotypes

Table 14 : Biochemical parameters recorded on genotypes of rice bean

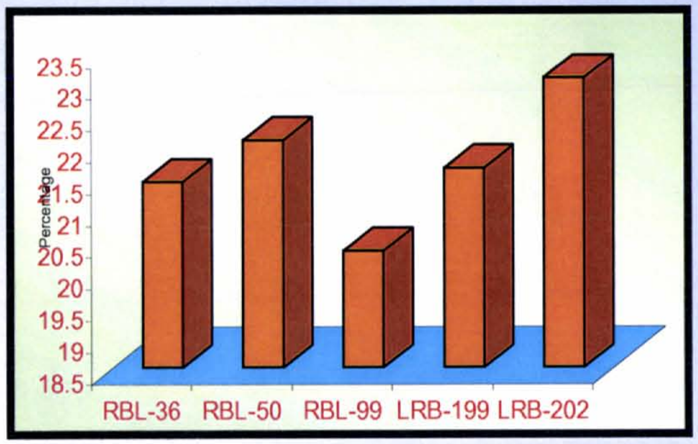
Sr. No.	Genotypes	∝-amylase inhibitor activity (μ mol of maltose formed /10 min)	Trypsin inhibitor activity (Units/ml/10 min)	Protein (%)	Total phenol (mg/100 gm)	Tannic acid (%)	Polyphenol oxidase activity (A/min/mg of soluble protein)	Peroxidase activity(A /min/mg of soluble protein)
1	RBL-36	28.37	71.50	21.43	57.50	1.70	3.95	7.26
2	RBL-50	25.00	78.25	22.09	65.00	1.75	3.50	6.56
3	RBL-99	25.05	78.50	20.34	60.00	1.98	2.91	5.57
4	LRB-199	25.02	82.50	21.65	52.50	1.90	3.25	7.01
5	LRB-202	24.51	79.75	23.07	50.00	1.95	4.15	7.15
	S.E. ±	0.72	0.77	0.29	0.90	0.14	0.15	0.20
	CD at 5 %	2.19	2.33	0.89	2.73	0.42	0.48	0.62
	Mean	25.59	78.10	21.71	57.00	1.85	3.50	6.71



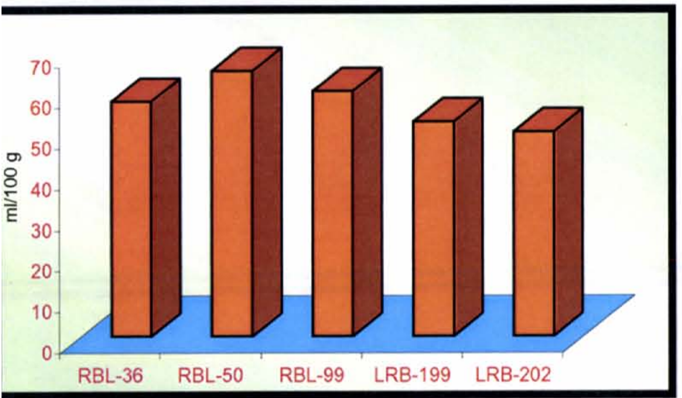
Alpha-amylase inhibitor activity



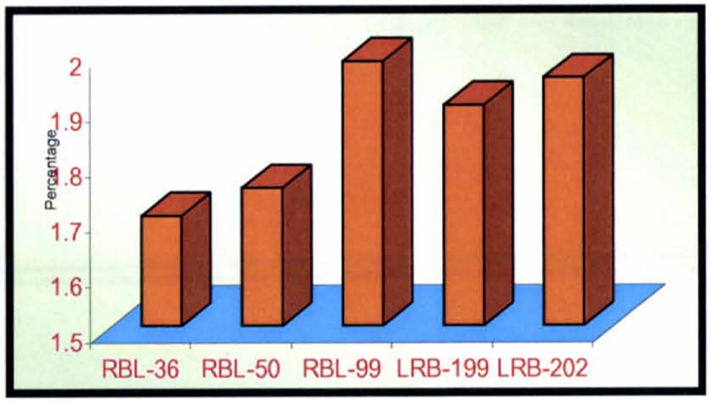
Trypsin inhibitor activity



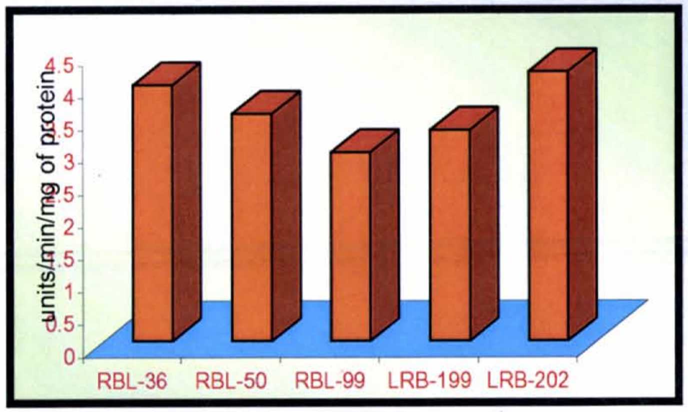
Protein percent



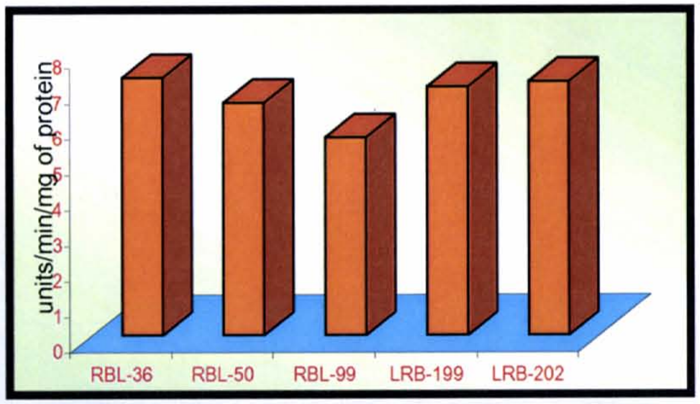
Total phenol



Tannic acid



Polyphenol oxidase activity



Peroxidase activity

Fig. 10 : Biochemical parameters of evaluating resistance to *Callosobruchus chinensis* in rice bean

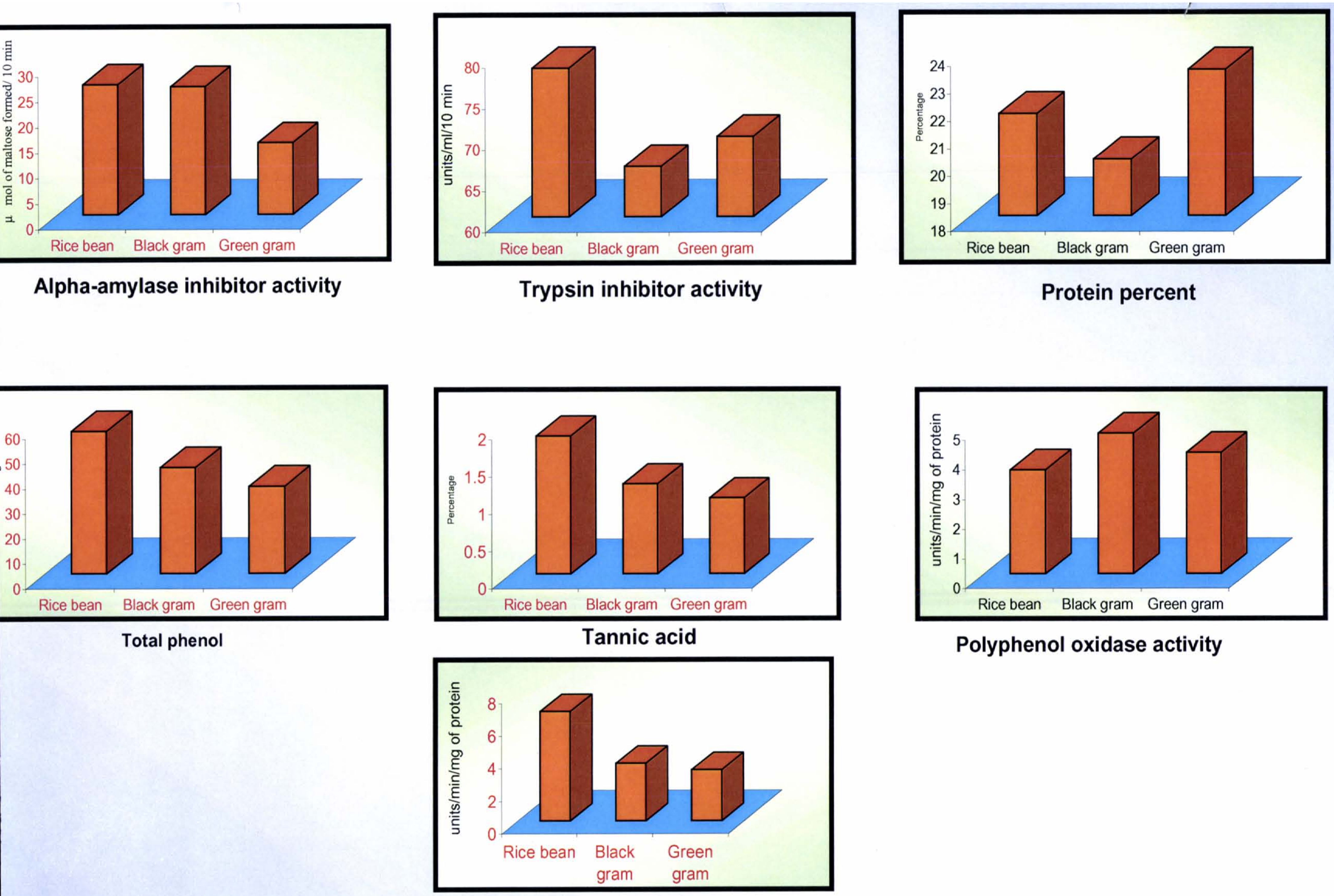


Fig. 11 : Biochemical parameters of evaluating resistance to *Callosobruchus chinensis* in *vigna* species

(3.14), while the minimum peroxidase activity was recorded by TPU-4 (2.66). The genotype TAU-1 (5.02) was recorded as more peroxidase activity than the average performance (3.53).

Peroxidase activity of five rice bean genotypes was ranged from 5.57 to 7.26 with a mean performance of 6.71. The genotype RBL-36 showed the maximum peroxidase activity (7.26) followed by LRB-202 (7.15), LRB-199 (7.01) and RBL-50 (6.56), where as RBL-99 was showed minimum peroxidase activity (5.57).

On the basis of average over the crop, it was observed that rice bean showed maximum peroxidase activity (6.71) followed by black gram (3.53) and green gram (3.13).

4.2.8 Correlation coefficient between biochemical parameters and resistant seed to bruchid in green gram

The correlation for biochemical parameter studies are presented in Table 15.

∝- amylase inhibitor

∝-Amylase inhibitor activity showed positive and highly significant correlation with total phenol (r=0.768), trypsin inhibitor activity (r=0.659) and resistant seed (0.566) and it also showed positive significant correlation with tannic acid (r=0.498). ∝-Amylase inhibitor activity was negative and highly significant correlation with protein content (r= -0.635).

Trypsin inhibition

The association between trypsin inhibitor potential and resistant seed (r=0.866) was positive and highly significant. Further it showed highly significant and positive correlation with total phenol (r=0.954) and tannic acid (r=0.789) while it was highly significant and negatively correlated with peroxidase activity

Table 15 : Correlation coefficient between biochemical parameters and resistant seed to bruchid in green gram.

Sr. No.	Characters	Trypsin inhibitor	Protein (%)	Polyphenol oxidase	Tannic acid (%)	Total phenol (mg/100 gm)	Peroxidase	Correlation Coefficient (r)
1	Alpha amylase inhibitor	0.659**	-0.635**	-0.026	0.498*	0.768**	-0.618**	0.566**
2	Trypsin inhibitor		-0.520*	0.043	0.789**	0.954**	-0.694**	0.866**
3	Protein (%)			0.236	-0.602**	-0.592**	0.773**	-0.687**
4	Polyphenol oxidase				-0.132	0.172	0.420	0.025
5	Tannic acid (%)					0.720**	-0.683**	0.777**
6	Total phenol (mg/100 gm)						-0.664**	0.848**
7	Peroxidase							-0.803**

** Significant at 1% level
* Significant at 5% level

(r= -0.694) and significant negative with protein content (r= -0.520).

Protein content

Protein content exhibited highly significant and positive correlation with peroxidase activity (r=0.773) while it was negatively and highly significant correlation with resistant seed (r= -0.687), tannic acid (r=-0.602) and total phenol (r=-0.592).

Tannic acid

Tannic acid was highly significant and positively correlated with resistant seed (r=0.777) and total phenol (r=0.720) but there was negative and highly significant correlation with peroxidase activity (r=-0.682).

Total phenol

Total phenol showed positive and highly significant correlation with resistant seed (r=0.848) while its association was highly significant and negative with peroxidase activity (r=-0.664).

Peroxidase activity

Peroxidase activity exhibited negative and highly significant correlation with resistant seed (r=-0.803).

4.2.9 Direct and indirect effect of biochemical parameters on resistant seed to bruchid in green gram

Resistant seed vs. alpha- amylase inhibitor

α-amylase showed negative direct effect (-0.231) on resistant seed while it's negligible negative indirect effect through polyphenol oxidase (-0.007) and total phenol (-0.005). It had positive and highest indirect effect through trypsin inhibitor activity (0.326) followed by peroxidase activity (0.322), protein

Table 16 : Direct and Indirect effect of biochemical parameters and resistant seed to bruchid in green gram.

Sr. No.	Characters	Alpha amylase inhibitor	Trypsin inhibitor	Protein (%)	Polyphenol oxidase	Tannic acid (%)	Total phenol (mg/100 gm)	Peroxidase	Correlation Coefficient (r)
1	Alpha amylase inhibitor	<u>-0.231</u>	0.326	0.129	-0.007	0.032	-0.005	0.322	0.566**
2	Trypsin inhibitor	-0.152	<u>0.494</u>	0.106	0.012	0.051	-0.006	0.361	0.866**
3	Protein (%)	0.147	-0.257	<u>-0.204</u>	0.605	-0.039	0.004	-0.402	-0.687**
4	Polyphenol oxidase	0.006	0.021	-0.048	<u>0.274</u>	-0.008	-0.001	-0.218	0.025
5	Tannic acid (%)	-0.115	0.390	0.123	-0.036	<u>0.065</u>	-0.005	0.355	0.777**
6	Total phenol (mg/100 gm)	-0.177	0.472	0.121	0.047	0.047	<u>-0.007</u>	0.345	0.848**
7	Peroxidase	0.143	-0.034	-0.158	0.115	-0.044	0.004	<u>-0.520</u>	-0.803

Under line figure indicate direct effect
Residual effect = 0.3035
** Significant at 1% level
* Significant at 5% level

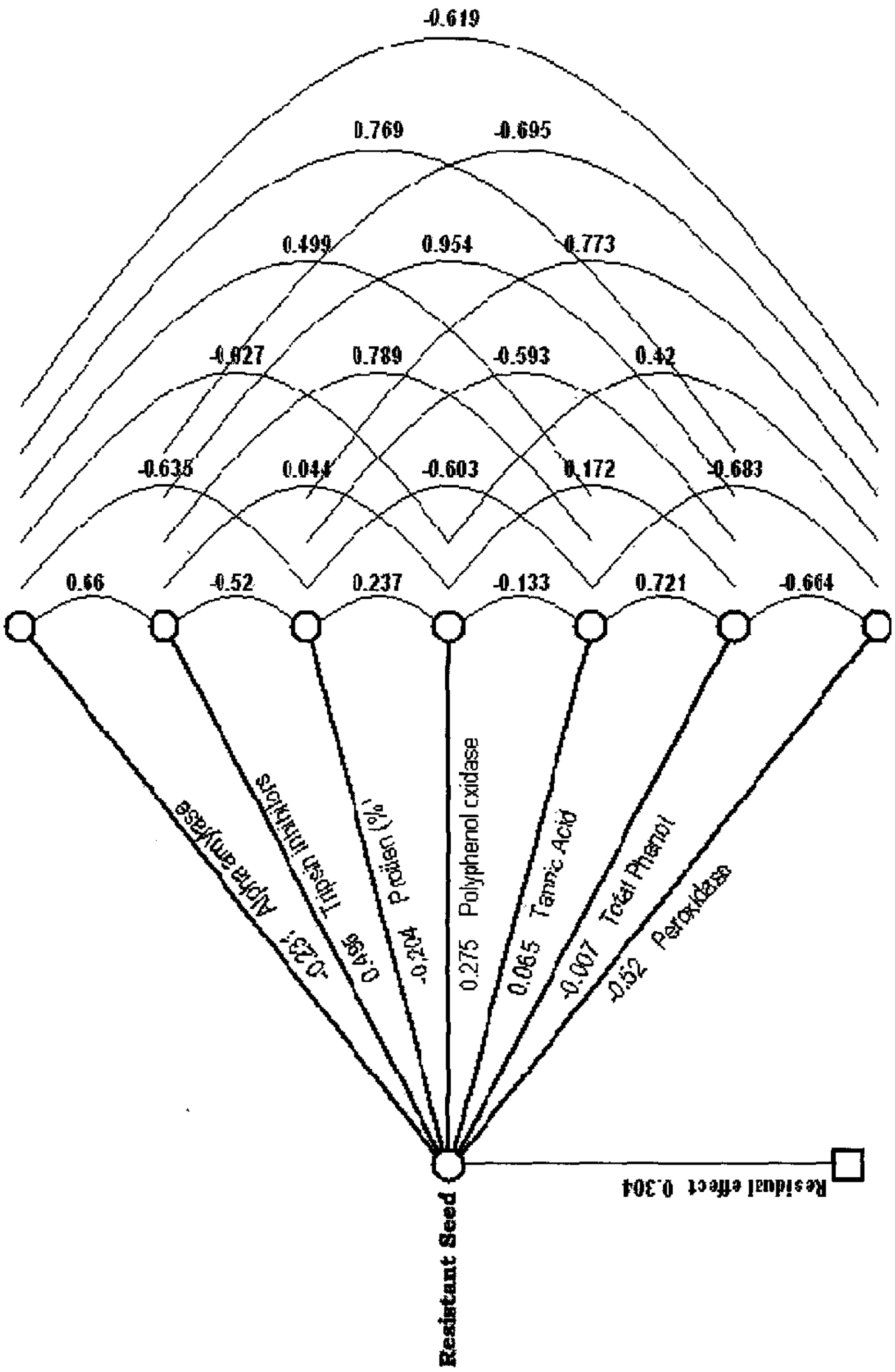


Fig. 12 : Path Diagram (GG)

content (0.129) and tannic acid (0.032). The total correlation with resistant seed was positive and highly significant (0.566) presented in table 16 and Fig. 12.

Resistant seed vs. trypsin inhibitor

Trypsin inhibitor activity had highest positive direct effect (0.494) on resistant seed among all the characters studied. Trypsin inhibitor activity had indirect effect via. peroxidase activity (0.361), protein content (0.106), tannic acid (0.051) and polyphenol oxidase activity (0.012) were positive. Remaining all characters had negative indirect effect. Trypsin inhibitor activity showed positive and highly significant correlation with resistant seed (0.866).

Resistant seed vs. protein content

It had a negative direct effect (-0.204) on resistant seed. It's indirect effect via. ∝-amylase inhibitor was positive (0.147) followed by polyphenol oxidase activity (0.065) and negligible positive indirect effect through total phenol (0.004). It rendered high negative indirect effect through peroxidase activity (-0.402) followed by trypsin inhibitor activity (-0.257) and tannic acid (-0.039). The total correlation with resistant seed was highly significant and negative (-0.687).

Resistant seed vs. polyphenol oxidase activity

Polyphenol oxidase activity showed positive direct effect (0.274) on resistant seed. It's indirect effect through peroxidase activity (-0.218) and protein content (-0.048) was negative. Polyphenol oxidase activity showed negative and negligible negative indirect effect through tannic acid (-0.008) and total phenol (-0.001). It had positive indirect effect through trypsin inhibitor activity (0.021) and ∝-amylase inhibitor (0.006). The total correlation with resistant seed was leading to non-significant positive (0.025).

Resistant seed vs. tannic acid

It evinced less but positive direct effect (0.065). It had high positive indirect effect through trypsin inhibitor activity (0.390) followed by peroxidase activity (0.355) and protein content (0.123), where as negative indirect effect through ∝-amylase inhibitor (-0.115), polyphenol oxidase activity (-0.036). Tannic acid showed negligible negative indirect effect through total phenol (-0.005). The total correlation with resistant seed was positive and highly significant (0.777) due to high positive indirect effect via trypsin inhibitor activity and peroxidase activity.

Resistant seed vs. total phenol

Total phenol showed negligible negative direct effect (-0.007) on resistance seed. It had positive and highest indirect effect through trypsin inhibitor (0.472) followed by peroxidase activity (0.345), protein content (0.121), polyphenol oxidase (0.047) and tannic acid (0.047). While the ∝-amylase inhibitor showed negative indirect effect (-0.177). The total correlation with resistant seed was positive and highly significant (0.848).

Resistant seed vs. peroxidase activity

It has a high negative direct effect (-0.520) on resistant seed. It's indirect effect via. ∝-Amylase inhibitor (0.143), polyphenol oxidase activity (0.115) and total phenol (0.004) were positive. Remaining all the characters had negative indirect effect. It showed negative and highly significant correlation with resistant seed (-0.803).

4.2.10 Correlation coefficient between biochemical parameters and resistant seed to bruchid in black gram

∝-amylase inhibitor

Alpha amylase inhibitor showed positive and highly significant correlations with trypsin inhibitor (r=0.678) and total phenol (r=0.655), while it was highly significant and negative correlation with peroxidase activity (r=-0.648) presented in Table 17.

Trypsin inhibitor

Trypsin inhibitor had highly significant and positive correlation with total phenol (r=0.818), while there was significant negative correlation with peroxidase activity (r=-0.713).

Protein content

Protein content was significant negatively correlated with resistant seed (r=-0.537), total phenol (r=-0.546) and tannic acid (r=-0.528).

Polyphenol oxidase activity

Polyphenol oxidase activity exhibited significant negative association with tannic acid (r=-0.528) and peroxidase activity (r=-0.477).

Tannic acid

The correlation between tannic acid and resistant seed was highly significant and positive (r=0.775). Tannic acid showed significant positive correlation with total phenol (r=0.532).

Table 17 : Correlation coefficient between biochemical parameters and resistant seed to bruchid in black gram.

Sr. No.	Characters	Trypsin inhibitor	Protein (%)	Polyphenol oxidase	Tannic acid (%)	Total phenol (mg/100 gm)	Peroxidase	Resistant seed
1	Alpha amylase inhibitor	0.678**	-0.313	0.175	0.284	0.655**	-0.648**	0.323
2	Trypsin inhibitor		-0.405	0.314	0.262	0.818**	-0.713**	0.384
3	Protein (%)			0.103	-0.528*	-0.546*	-0.088	-0.537*
4	Polyphenol oxidase				-0.528*	0.193	-0.477*	-0.366
5	Tannic acid (%)					0.532*	0.078	0.775**
6	Total phenol (mg/100 gm)						-0.429	0.615**
7	Peroxidase							0.070

** Significant at 1% level
* Significant at 5% level

Total phenol

Total phenol exhibited positive and highly significant association with resistant seed (r=0.615).

4.2.11 Direct and indirect effect of biochemical parameters on resistant seed to bruchid in black gram

Resistant seed vs. ∝-amylase inhibitor

It had small positive direct effect (0.081) on resistant seed. It rendered positive indirect effects through trypsin inhibitor activity (0.206), tannic acid (0.132) and total phenol (0.180). It rendered negative indirect effect via. peroxidase activity (-0.242), polyphenol oxidase ∝-amylase (-0.019) and protein content (-0.016) presented in Table 18 and Fig. 13.

Resistant seed Vs. Trypsin inhibitor

Trypsin inhibitor activity showed positive effect (0.303) on resistant seed. It's indirect positive contribution towards resistant seed come through total phenol (0.225), tannic acid (0.122) and alpha amylase inhibitor activity (0.055). The indirect effects through peroxidase activity, polyphenol oxidase activity and protein content were negative.

Resistant seed vs. protein content

Protein content showed small positive direct effect (0.052) on resistant seed. It's indirect effect via. all the characters was negative. The total correlation with resistant seed was negative and significant (-0.538).

Resistance seed vs. polyphenol oxidase activity

Polyphenol oxidase activity showed negative direct effect (-0.109) on resistant seed. It showed positive indirect effect

Table 18 : Direct and Indirect effect of biochemical parameters and resistant seed to bruchid in black gram.

Sr. No.	Characters	Alpha amylase inhibitor	Trypsin inhibitor	Protein (%)	Polyphenol oxidase	Tannic acid (%)	Total phenol (mg/100 gm)	Peroxidase	Correlation Coefficient (r)
1	Alpha amylase inhibitor	0.081	0.206	-0.016	-0.019	0.132	0.180	-0.242	0.323
2	Trypsin inhibitor	0.055	0.303	-0.021	-0.034	0.122	0.225	-0.267	0.384
3	Protein (%)	-0.025	-0.123	0.052	-0.011	-0.246	-0.150	-0.033	-0.537*
4	Polyphenol oxidase	0.014	0.095	0.005	-0.109	-0.246	0.053	-0.178	-0.366
5	Tannic acid (%)	0.023	0.079	-0.027	0.057	0.466	0.146	0.029	0.775**
6	Total phenol (mg/100 gm)	0.053	0.248	-0.028	-0.021	0.248	0.275	-0.160	0.615**
7	Peroxidase	-0.053	-0.216	-0.004	0.052	0.036	-0.118	0.374	0.070

Under line figure indicate direct effect
Residual effect = 0.5357
** Significant at 1% level
* Significant at 5% level

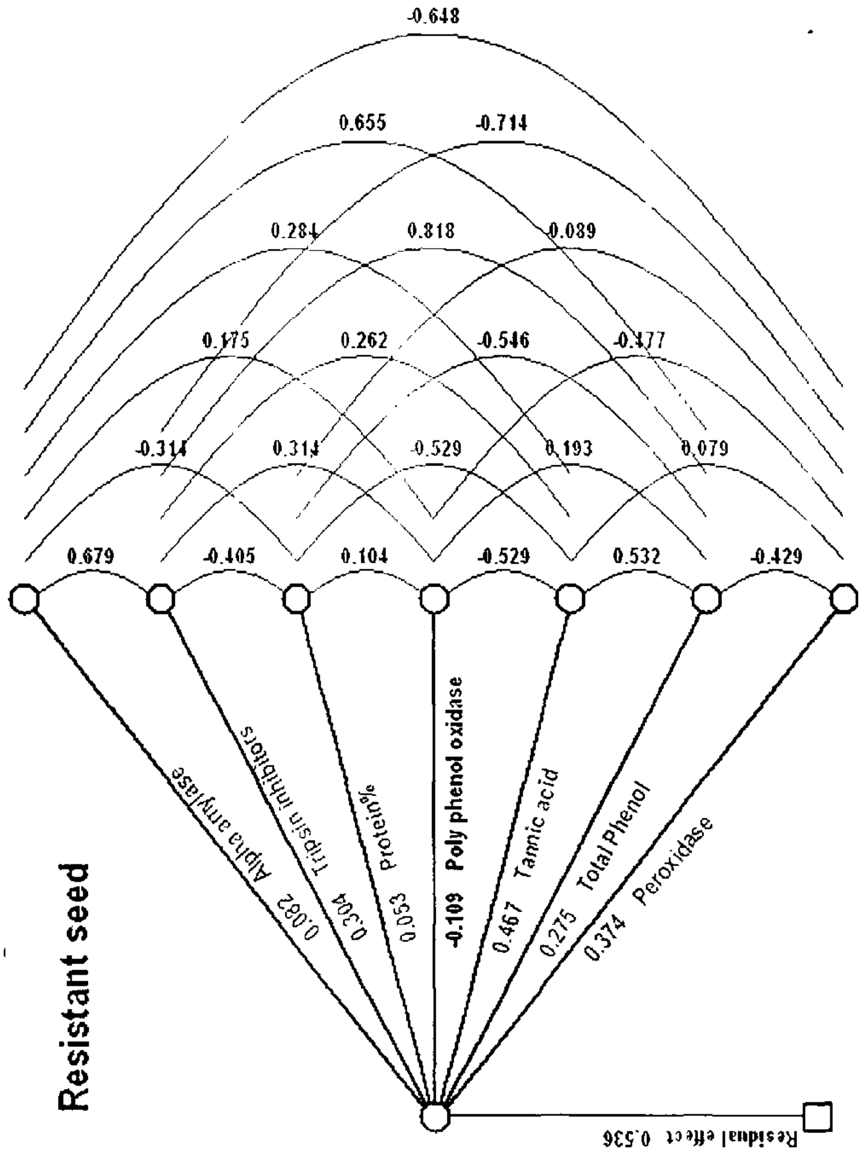


Fig. 13 : Path Diagram (BG)

via. trypsin inhibitor (0.095), total phenol (0.053), alpha-amylase inhibitor (0.014) and protein content (0.005), while tannic acid (-0.246) and peroxidase activity (-0.178) exhibited negative indirect effect on resistant seed.

Resistant seed vs. tannic acid

It had highest positive direct effect (0.466) on resistant seed among all the characters studied. It's indirect effect via. Total phenol (0.146), trypsin inhibitor (0.079), polyphenol oxidase activity (0.057), peroxidase activity (0.029) and ∝-amylase inhibitor (0.023) were positive. Remaining all the characters had negative indirect effect.

Resistant seed vs. total phenol

Total phenol showed positive and highly significant correlation with resistant seed (0.615). It had positive direct effect on resistant seed (0.275). The indirect effect through trypsin inhibitor, tannic acid and alpha amylase inhibitor were positive. Remaining all the characters had negative indirect effect.

Resistant seed vs. peroxidase activity

Peroxidase activity had positive direct effect (0.374) on resistant seed. Its indirect effect via polyphenol oxidase activity (0.052) and tannic acid (0.035) were positive, where as remaining all the characters had negative indirect effects.

4.3 Electrophoresis studies

4.3.1 Electrophoresis of total soluble protein

The systematic diagram of electrophoresis pattern of seed proteins of green gram, black gram and rice bean are presented in Fig 14, 15 and 16 and depicted in Plates 5, 6, and 7.

The classification of banding pattern and characteristics of each genotypes of green gram, black gram and rice bean are presented in Table 19,22 and 25; and 20, 23 and 26 respectively. The results revealed that the electrophoresis patterns of green gram, black gram and rice bean genotypes under study were very unique and the genotype differences were clearly revealed by the presence or absence of particular band in each zymograms.

The SDS- polyacrylamide gel electrophoresis was carried out into three groups according to crop each consisting of *five genotypes viz.*

Sr. No.	**Group- I**	**Group-II**	**Group-III**
	Green gram	**Black gram**	**Rice bean**
1	PM-2	TAU-1	RBL-36
2	PM-9339	TAU-2	RBL-50
3	AKM-8802	TPU-4	RBL-99
4	TARM-18	PU-9503-8	LRB-199
5	Kopergaon	PU-9205-11-3	LRB-202

Green gram

The total of 30 bands was recognized in SDS-PAGE of green gram of five genotypes i.e. PM-2, PM-9339, AKM-8802, TARM-18 and Kopergaon under study (Fig. 14). Out of which band number 1, 3, 5 (Rm 0.18, 0.22 and 0.26, respectively) weak, band number 2,4,6,15,17,18,23,31 (Rm 0.19, 0.23, 0.28, 0.52, 0.57, 0.59, 0.77, 0.91) light, band number 7, 8, 19, 21 (Rm 0.30, 0.34, 0.61 and 0.68 respectively) medium were found as common in all the genotypes. Where as, in TARM-18 band number 23 and 31 showed medium and band number 7,8,19,21 showed dense. Band *no. 10 (Rm* 0.37) and band no. 5 (Rm 0.26) were absent in TARM-18 and PM-2, respectively.

Genotype PM-2 showed total 24 bands, out of which two bands were dense, 4 medium, 11 light and 7 were weak. The genotype PM-2 could be discriminated from other genotypes by band number 5 (Rm 0.26) and band number 22 (Rm 0.72) were weak band present in all genotypess except PM-2.

Genotype PM-9339 had total 25 bands out of which one dense, 5 medium, and 10 light and 9 weak. The genotypes PM-2 and PM-9339 discriminated from each other by the absence of band no. 5 and 22 in PM-2. Band no. 25 (Rm 0.83) was present in PM-2 but it was absent in genotype PM-9339. Band number 13 present in both the genotypes, where as PM-2 showed light and PM-9339 showed weak. In the genotype PM-2 band no. 11 (Rm 0.40) was dense while PM-9339 showed medium.

The genotype, AKM-8802 showed 1 dense, 5 medium, 10 light and 6 weak bands over all 22 bands. The distinguishing band in this was band number 13, 20 and 24 (Rm 0.45, 0.64 and 0.80, respectively), which were absent in AKM-8802.

Genotype TARM-18 showed 28 bands in its electrophoresis pattern. It had 6 dense, 4 medium, 9 light and 9 weak. This genotype had maximum number of dense bands i.e. 6. It had two characteristic band, band number 19 (Rm 0.61) and band number 21 (Rm 0.68), which was present in all genotypes as medium but observed as dense in TARM-18. Band number 9 (Rm 0.36) were present as dense in TARM- 18, where as, it was absent in all genotypes. The presence of four continuous dense bands corresponding to Rm value 0.30, 0.34, 0.36, and 0.40 was the characteristic creature of TARM-18. Band No. 16 (Rm 0.53) was absent in all genotypes while present in TARM-18 as weak.

There were total of 27 bands in the electrophoresis pattern of Kopergaon i.e. 1 dense, 5 medium, 12 light and 9 weak. Presence of continuous 6 light bands corresponding to Rm value

0.42, 0.45, 0.49, 0.52 and 0.59 was the characteristic, bands of Kopergaon.

Table: 19 Classification of green gram genotypes according to number of protein bands and their intensities

Sr. No.	Genotypes	Number of bands	Dense	Medium	Light	Weak
1	PM-2	24	2	4	11	7
2	PM-9339	25	1	5	10	9
3	AKM-8802	22	1	5	10	6
4	TARM-18	28	6	4	9	9
5	Kopergaon	27	1	5	12	9

Table: 20 The characteristics of protein banding pattern of green gram genotype

Sr. No.	Name of genotype	Banding pattern
1	PM-2	**P :** 1,2,3,4,6,7,8,10,11,12,13,14,15,17,18, 19, 20, 21,23,24,25,27,29,31 **A :** 5,9,16,22,26,28,30
2	PM-9339	**P :** 1,2,3,4,5,6,7,8,10,11,12,13,14,15,17,18, 19,20,21,22,23,24,27,29,31 **A :** 9,16,25,26,28,30
3	AKM-8802	**P :** 1,2,3,4,5,6,7,8,10,11,12,14,15,17,18, 19,21,22,23,27,29 **A :** 9,13,16,20,24,25,26,28,30
4	TARM-18	**P :** 1,2,3,4,5,6,7,8,9,11,12,13,14,15,16,17, 18,19,20,21,22,23,24,25,26,28,30,31 **A :** 10,27,29
5	Kopergaon	**P :** 1,2,3,4,5,6,7,8,10,11,12,13,14,15,17,18, 19,20,21,22,23,24,25,26,28,30,31 **A :** 9,16,27,29

P = Present A = Absent

Table 21 : Rm values for different protein bands from each genotypes observed in electrophoresis

Position of bands from top of individual green gram genotypes	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon
1	0.18	0.18	0.18	0.18	0.18
2	0.19	0.19	0.19	0.19	0.19
3	0.22	0.22	0.22	0.22	0.22
4	0.23	0.23	0.23	0.23	0.23
5	0.28	0.26	0.26	0.26	0.26
6	0.30	0.28	0.28	0.28	0.28
7	0.34	0.30	0.30	0.30	0.30
8	0.37	0.34	0.34	0.34	0.34
9	0.40	0.37	0.37	0.36	0.37
10	0.42	0.40	0.40	0.40	0.40
11	0.45	0.42	0.42	0.42	0.42
12	0.49	0.45	0.49	0.45	0.45
13	0.52	0.49	0.52	0.49	0.49
14	0.57	0.52	0.57	0.52	0.52
15	0.59	0.57	0.59	0.55	0.57
16	0.61	0.59	0.61	0.57	0.59
17	0.64	0.61	0.68	0.59	0.61
18	0.68	0.64	0.72	0.61	0.64
19	0.77	0.68	0.77	0.64	0.68
20	0.80	0.72	0.87	0.68	0.72
21	0.83	0.77	0.89	0.72	0.77
22	0.87	0.80	0.92	0.77	0.80
23	0.89	0.87	-	0.80	0.83
24	0.92	0.89	-	0.83	0.85
25	-	0.92	-	0.85	0.88
26	-	-	-	0.88	0.90
27	-	-	-	0.90	0.92
28	-	-	-	0.92	-

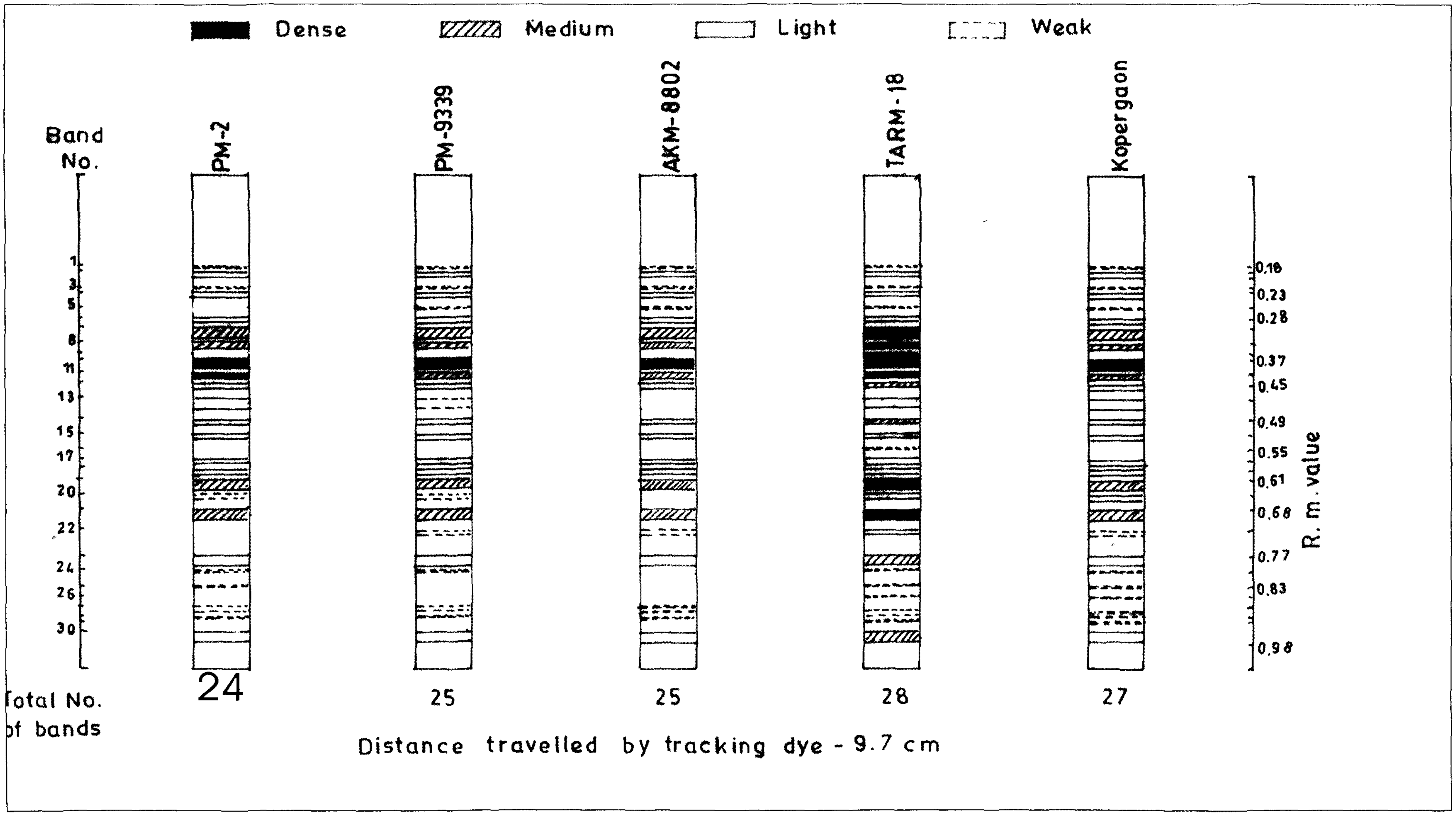


Fig. 14 : Electrophorograms of green gram genotypes

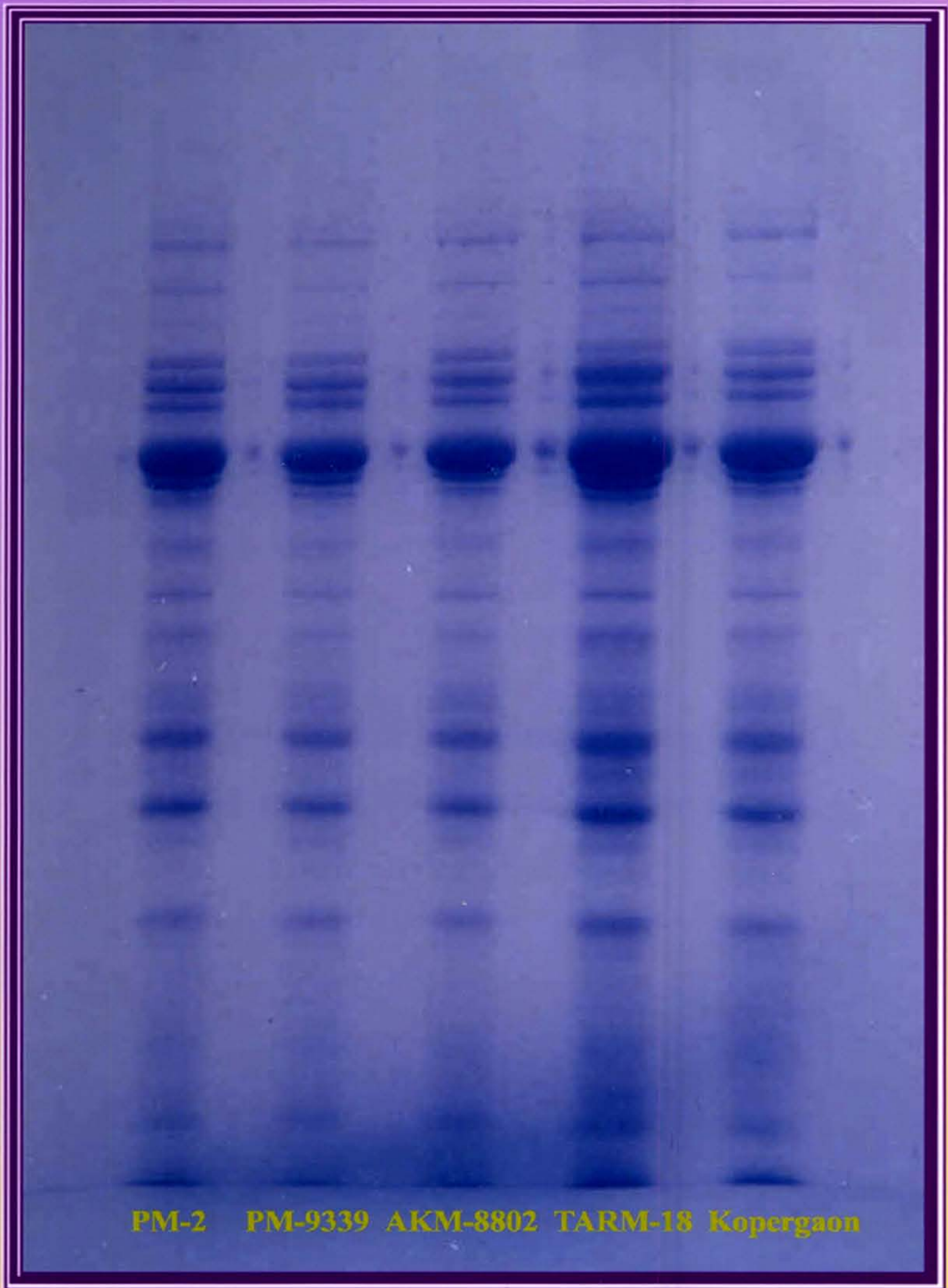


PLATE 5 : Electrophoregrams of total soluble protein in green gram genotypes

Black gram

The total number of 37 bands were recognized in the SDS-PAGE of the soluble protein of seed in different genotypes of black gram under study (Fig. 15) out of which the band number 13 and 14 (Rm 0.38 and 0.14 respectively) dense, band number 15 and 22 (Rm 0.44 and 0.56 respectively) light and band number 6,7,8,9,10 and 21 (Rm 0.22, 0.23, 0.25 0.26, 0.29 and 0.54 respectively) weak were found as common in all the genotypes.

TAU-1 showed 3 dense, 4 medium, 6 light and 17 weak bands thus total 30 bands. The bands corresponding to Rm value 0.15 (light), 0.67 (weak) and 0.091(weak) were characteristics bands that were absent in remaining four genotypes. Band number 13 (Rm 0.38) was found common in all genotypes, which was dense band in TAU-1. Band number 5 (Rm 0.18) was present in all the genotypes as light band but in TAU-1 showed dense band.

The genotypes TAU-1 and TAU-2 could be discriminated from each other by the absence of band no. 1,3,16,19,20,23,27 (Rm 0.091,0.15,0.46,0.52,0.53,0.58 and 0.67 respectively) in TAU-2. Genotype TAU-2 showed total 25 bands out of which 2 dense, 2 medium, 8 light and 13 weak. Band No. 11 (Rm 0.32) showed light intensity band in TAU-2, while in TAU-1 showed medium intensity band. The band 18 and 28 (Rm 0.51 and 0.68 respectively) that was absent in TAU-1 cv. and present in TAU-2.

The electrophoresis pattern of genotype TAU-4 showed 24 bands including 2 dense, 2 medium, 9 light and 11 weak. Band number 19 (Rm 0.52) with light intensity was the characteristics bands for identifying the TPU-4 genotype, where as in other genotype it was weak band except TAU-2. Band number 12 (Rm

0.36) was found as medium in TAU-4 and TAU-2 while in remaining all genotypes it was darkness.

In PU-9503-3, there were 26 bands out of which 3 dense, 2 medium, 8 light and 13 weak. The band number 29 (Rm 0.69) was weak the characteristics band, where as it was absent in all the four genotypes. Band number 16 (Rm 0.46) was weak intensity band in PU-9503-8 and TAU-1 and light in PU-9205-11-3, while it was absent in remaining two genotypess.

Electrophoresis pattern of PU-9205-11-3 showed over all 31 bands, which contain 5 dense, 4 medium, 9 light and 13 weak bands. This genotype could be identified from other genotype ars due to highest number of dense band (Band No. 12, 13,14,26,37). The highest distance traveling band in this genotype was the band number 37 (Rm 0.93) dense, where as in other genotype it was light. Band no. 15(Rm 0.44) was dense in PU-9205-11-3, where as in remaining genotype itr was light. Band number 35 and 36 (Rm 0.91 and 0.92 respectively) was absent in all four genotypes. Band number 1 and 2 (Rm 0.091 and 0.11) were present only in PU-9205-11-3 and TAU-1.

Table: 22 Classification for black gram genotypes according to number of protein bands and their intensities.

Sr. No.	Genotypes	No. of bands	Dense	Medium	Light	Weak
1	TAU-1	30	3	4	6	17
2	TAU-2	25	2	2	8	13
3	TPU-4	24	2	2	9	11
4	PU-9503-8	26	3	2	8	13
5	PU-9205-11-3	31	5	4	9	13

Table : 23 The characteristic of protein banding pattern of black gram genotypes

Sr. No.	Name of genotype	Banding pattern
1	TAU-1	**P :** 1,2,3,4,5,6,7,9,10,11,12,13,14,15, 16, 17,19,20,21,22,23,24,26,27,30,32,33, 34, 37 **A :** 18,25,28,29,31,35,36
2	TAU-2	**P :** 2,4,5,6,7,8,9,10,11,12,13,14,15,17,18, 21,22,24,26,28,30,32,33,34,37 **A :** 4,3,16,19,20,23,25,27,29,31,35,36
3	TPU-4	**P :** 5,6,7,8,9,10,11,12,13,14,15,17,18,19, 21, 22,24,28,30,32,33,34,37 **A :** 1,2,3,4,16,20,23,25,27,29,31,35,36
4	PU-9503-8	**P :** 5,6,7,8,9,10,11,12,13,14,15,16,17,19, 20, 21,22,23,24,26,28,29,32,33,34,37 **A :** 1,2,3,4,18,25,27,30,31,35,36
5	PU-9205-11-3	**P :** 1,2,5,6,7,8,9,10,11,12,13,14,15,16,17, 19,20,21,22,23,24,25,26,28,29,31,32,33, 34,35,36,37 **A :** 3,4,18,27,29,30

Table : 24 Rm values for different protein bands from each genotype observed in Electrophoresis

Position of bands from top of individual black gram genotype	TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3
1	0.091	0.11	0.18	0.18	0.091
2	0.11	0.16	0.22	0.22	0.11
3	0.15	0.18	0.23	0.23	0.18
4	0.16	0.22	0.25	0.25	0.22
5	0.18	0.23	0.26	0.26	0.23
6	0.22	0.25	0.29	0.29	0.25
7	0.23	0.26	0.32	0.32	0.26
8	0.25	0.29	0.36	0.36	0.29
9	0.26	0.32	0.38	0.38	0.32
10	0.29	0.36	0.41	0.41	0.36
11	0.32	0.38	0.44	0.44	0.38
12	0.38	0.41	0.49	0.46	0.41
13	0.41	0.44	0.51	0.49	0.44
14	0.44	0.49	0.52	0.52	0.46
15	0.46	0.51	0.54	0.53	0.49
16	0.49	0.54	0.56	0.54	0.52
17	0.52	0.56	0.61	0.58	0.53
18	0.53	0.61	0.64	0.61	0.54
19	0.54	0.64	0.68	0.64	0.56
20	0.56	0.68	0.70	0.68	0.58
21	0.58	0.70	0.77	0.69	0.61
22	0.61	0.77	0.83	0.77	0.62
23	0.64	0.83	0.93	0.83	0.64
24	0.67	0.87	-	0.87	0.68
25	0.70	0.93	-	-	0.71
26	0.77	-	-	-	0.77
27	0.84	-	-	-	0.83
28	0.87	-	-	-	0.87
29	0.93	-	-	-	0.91
30	-	-	-	-	0.92
31	-	-	-	-	0.93
32	-	-	-	-	-

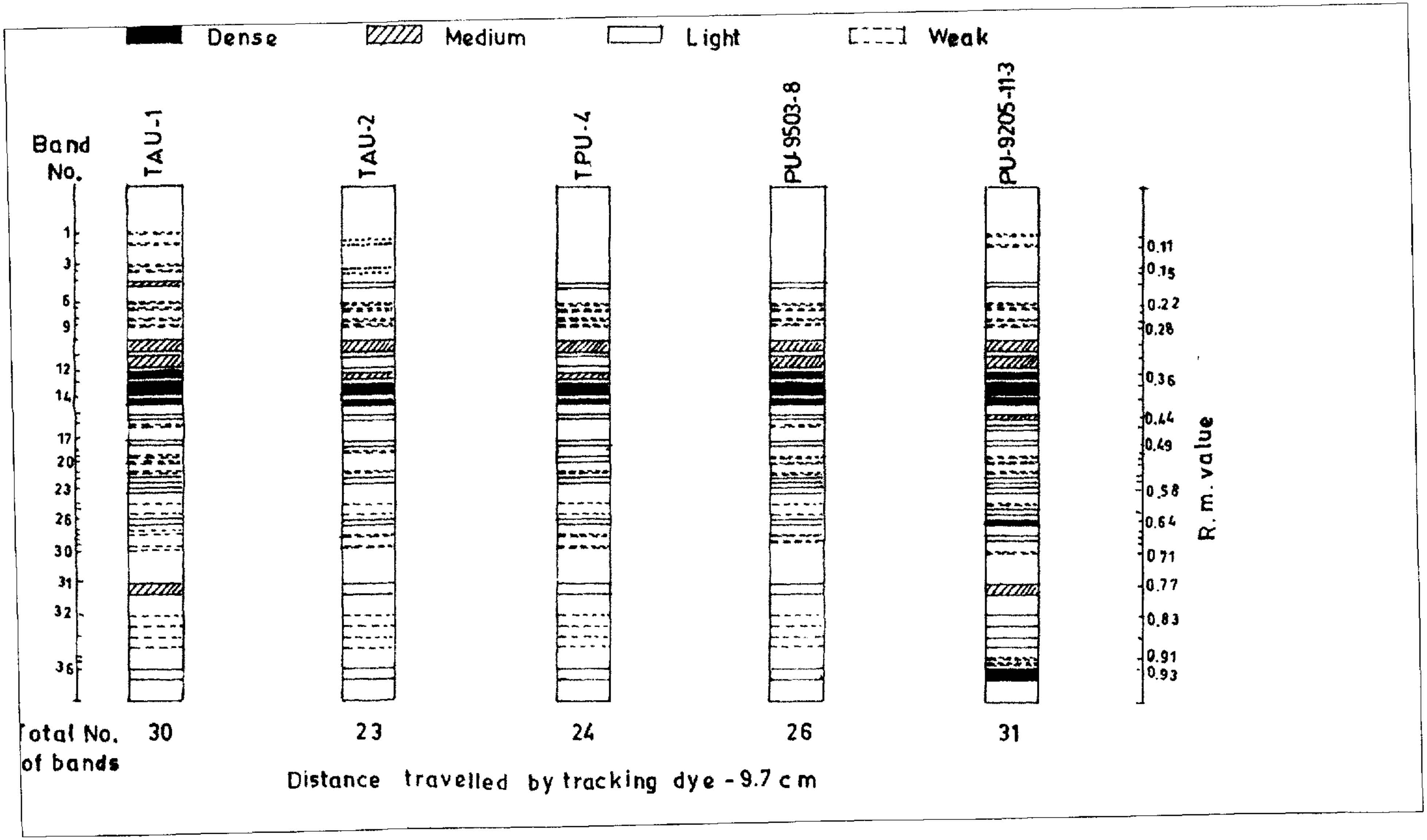


Fig. 15 : Electrophorograms of black gram genotypes

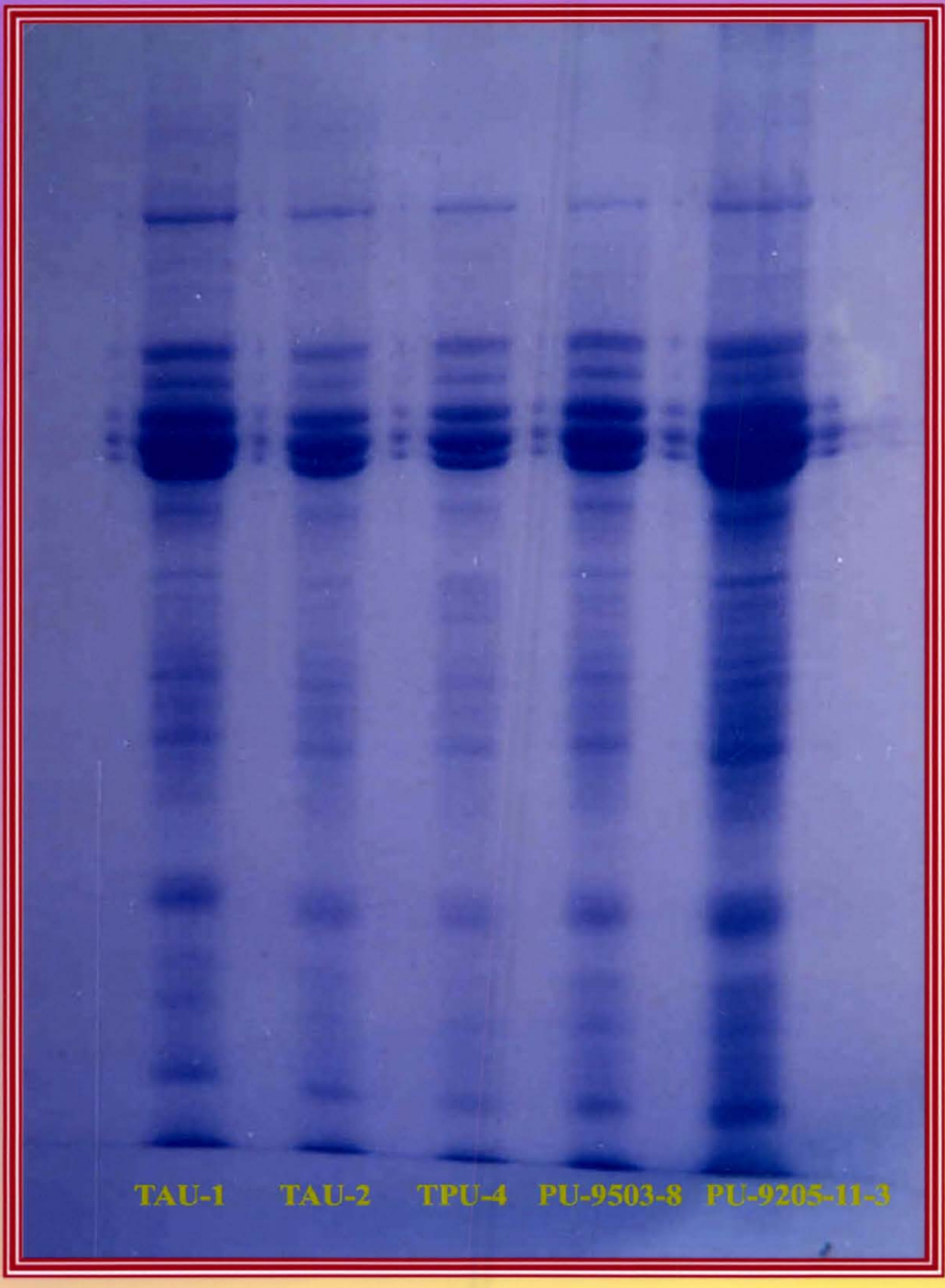


PLATE 6 : Electrophoregrams of total soluble protein in black gram genotypes

Rice bean

A total of 35 bands were recognized in SDS-PAGE electrophoresis pattern of rice bean genotypes i.e. RBL-36, RBL-50, RBL-99, LRB-199 and LRB-202 under study (Fig 16). Out of which band number 10 (Rm 0.32) dense, band number 8 and 9 (0.29 and 0.30 respectively) medium, band number 21 and 26 (Rm 0.56 and 0.65 respectively) light and band number 1,3,12 and 24 (Rm 0.16, 0.20, 0.38 and 0.61 respectively) weak were common in all the genotypes except RBL-99. The number of bands in individual genotype ranged from 24 and 31. Band number 1 corresponding to Rm value of 0.18 was observed in all genotypes except RBL-99, while band number 33 corresponding to Rm of 0.94 representing highest distance traveled by protein.

The electrophoresis pattern of genotypes RBL-36 showed 31 bands including 1 dense, 7 medium, 9 light and 14 weak bands, where as genotype RBL-50 showed total 30 bands including dense, 12 medium, 9 light and 8 weak bands. RBL-50 genotype showed maximum number of medium bands (12). The genotypes RBL-36 and RBL-50 were discriminates from each other by band number 4 (Rm 0.21) which was weak present in RBL-36 and absent in RBL-50. Bands corresponding to Rm value 0.30, 0.50, 0.69, 0.75 and 0.94 (band number 13,18,27,29 and 33 respectively) those were present in RBL-50 as light intensity band, where as in RBL-36 as weak band. In the RBL-36 band numbers 14, 21,22,23,31,32 (Rm 0.41, 0.56, 0.58, 0.59, 0.84 and 0.96 respectively) were light band. While, RBL-50 contain medium band. The high distance traveling 3 medium bands, band number 30, 31 and 32 (Rm 0.77, 0.84 and 0.99) were the characteristic band of RBL-50.

The genotype RBL-99 contained 24 bands including 1 dense, 1 medium, 7 light and 15 weak. RBL-99 showed minimum number of band than the other genotypes. The first band was started from Rm 0.17 and second band at Rm 0.27 which was weak. Band corresponding to Rm value 0.34 (Band number 11) which was dense present only in RBL-99, while in another four genotypes it was absent. Band number 30 (Rm 0.77) was medium in RBL-36, RBL-50 and LRB-199, whereas, RBL-99 and LRB-202 showed light band. Band 1,3,8,9 and 10 absent in RBL-99 and present in all four genotypes.

Electrophoresis pattern of LRB-199 showed overall 31 band out of which one dense, 7 medium, 11 light and 12 weak bands. Band number 5 (Rm 0.22) was weak band present only in genotypes LRB-199 and LRB-202. Band corresponding to Rm value 0.84, 0.90 and 0.94 (band number 31, 32 and 33 respectively) were the long distance traveling 3 continuous light band which was the characteristic bands of the LRB-199.

There were 27 bands observed in electrophoresis pattern of LRB-202, which contained one dense, 2 medium, 8 light and 16 weak band. Band number 18 and 19 (Rm 0.50 and 0.52 respectively) were present in all remaining genotypes, where as, in LRB-202 it was absent. LRB-202 showed four continuous weak bands corresponding to Rm 0.58, 0.59, 0.61 and 0.63 (band number 22, 23,24 and 25 respectively) which were characteristic band of this genotype. Band number 7 and 20 (Rm 0.27 and 0.54 respectively) were present in all genotypes, whereas in LRB-202 showed light intensity band.

Table : 25 Classification of rice bean genotypes according to number of protein bands and their intensities

Sr. No.	Genotypes	No. of bands	Dense	Medium	Light	Weak
1	RBL-36	31	1	7	9	14
2	RBL-50	30	1	12	9	8
3	RBL-99	24	1	1	7	15
4	LRB-199	31	1	7	11	12
5	LRB-202	27	1	2	8	16

Table : 26 The characteristics of protein banding pattern of rice bean genotypes

Sr. No.	Name of genotype	Banding pattern
1	RBL-36	P : 1,2,3,4,5,7,8,9,10,12,13,14,15,16,17, 18, 19,20,21,22,23,24,25,26,27,28,29,30,31, 32,33 A : 5,11
2	RBL-50	P : 1,2,3,6,7,8,9,10,12,13,14,15,17,18,19,20, 21,22,23,24,25,26,27,28,29,30,31,32,33 A : 4,5,11
3	RBL-99	P : 2,7,11,12,13,14,15,16,17,18,19,20,21,22, 23,24,25,26,27,29,30,31,32,33 A : 1,3,4,5,6,8,9,10,28
4	LRB-199	P:1,2,3,4,5,7,8,9,10,12,13,14,15,16,17,18,19, 20,21,22,23,24,25,26,27,28,29,30,31,32,33 A : 6,11
5	LRB-202	P : 1,2,3,4,5,7,8,9,10,12,13,14,15,16,18,19, 21,22,23,24,25,26,27,29,30,31,32,33 A : 6,11,17,19,20,28

Table : 27 Rm value for different protein bands from each genotype of rice bean observed in electrophoresis

Position of bands from top of individual rice bean genotype	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202
1	0.16	0.16	0.17	0.16	0.16
2	0.17	0.17	0.27	0.17	0.17
3	0.20	0.20	0.34	0.20	0.20
4	0.21	0.23	0.38	0.21	0.21
5	0.23	0.27	0.39	0.22	0.22
6	0.27	0.29	0.41	0.27	0.27
7	0.29	0.30	0.43	0.29	0.29
8	0.30	0.32	0.45	0.30	0.30
9	0.32	0.38	0.47	0.32	0.32
10	0.38	0.39	0.50	0.38	0.38
11	0.39	0.41	0.52	0.39	0.39
12	0.41	0.43	0.54	0.41	0.41
13	0.43	0.45	0.56	0.43	0.43
14	0.45	0.47	0.58	0.45	0.47
15	0.47	0.50	0.59	0.47	0.54
16	0.50	0.52	0.61	0.50	0.56
17	0.52	0.54	0.63	0.52	0.58
18	0.54	0.56	0.65	0.54	0.59
19	0.56	0.58	0.69	0.56	0.61
20	0.58	0.59	0.75	0.58	0.63
21	0.59	0.61	0.77	0.59	0.65
22	0.61	0.63	0.84	0.61	0.69
23	0.63	0.65	0.90	0.63	0.75
24	0.65	0.69	0.94	0.65	0.77
25	0.69	0.71	-	0.69	0.84
26	0.71	0.75	-	0.71	0.90
27	0.75	0.77	-	0.75	0.94
28	0.77	0.84	-	0.77	-
29	0.84	0.90	-	0.84	-
30	0.90	0.94	-	0.90	-
31	0.94	-	-	0.94	-
32	-	-	-	-	-
33	-	-	-	-	-
34	-	-	-	-	-

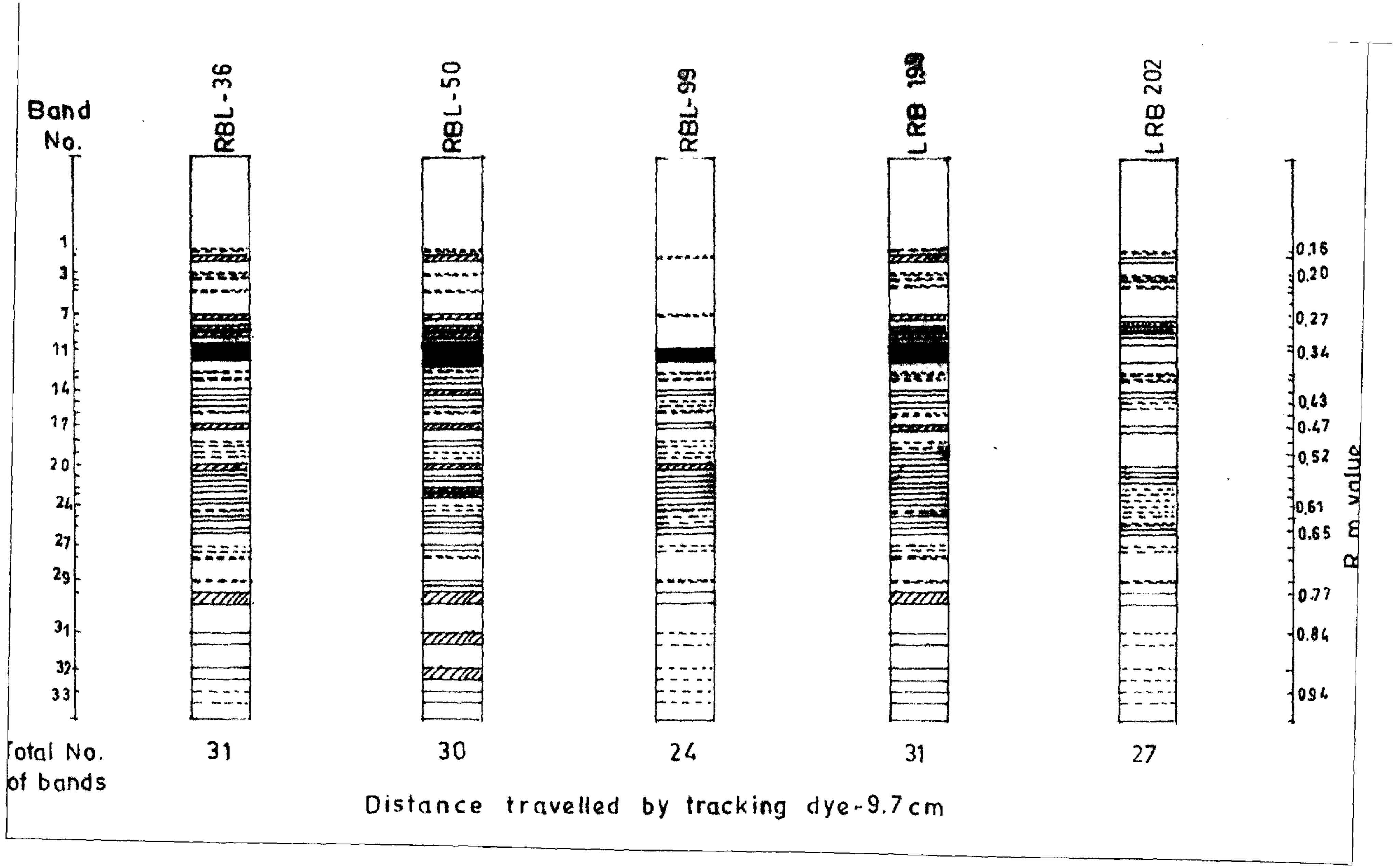


Fig. 16 : Electrophorograms of rice bean genotypes

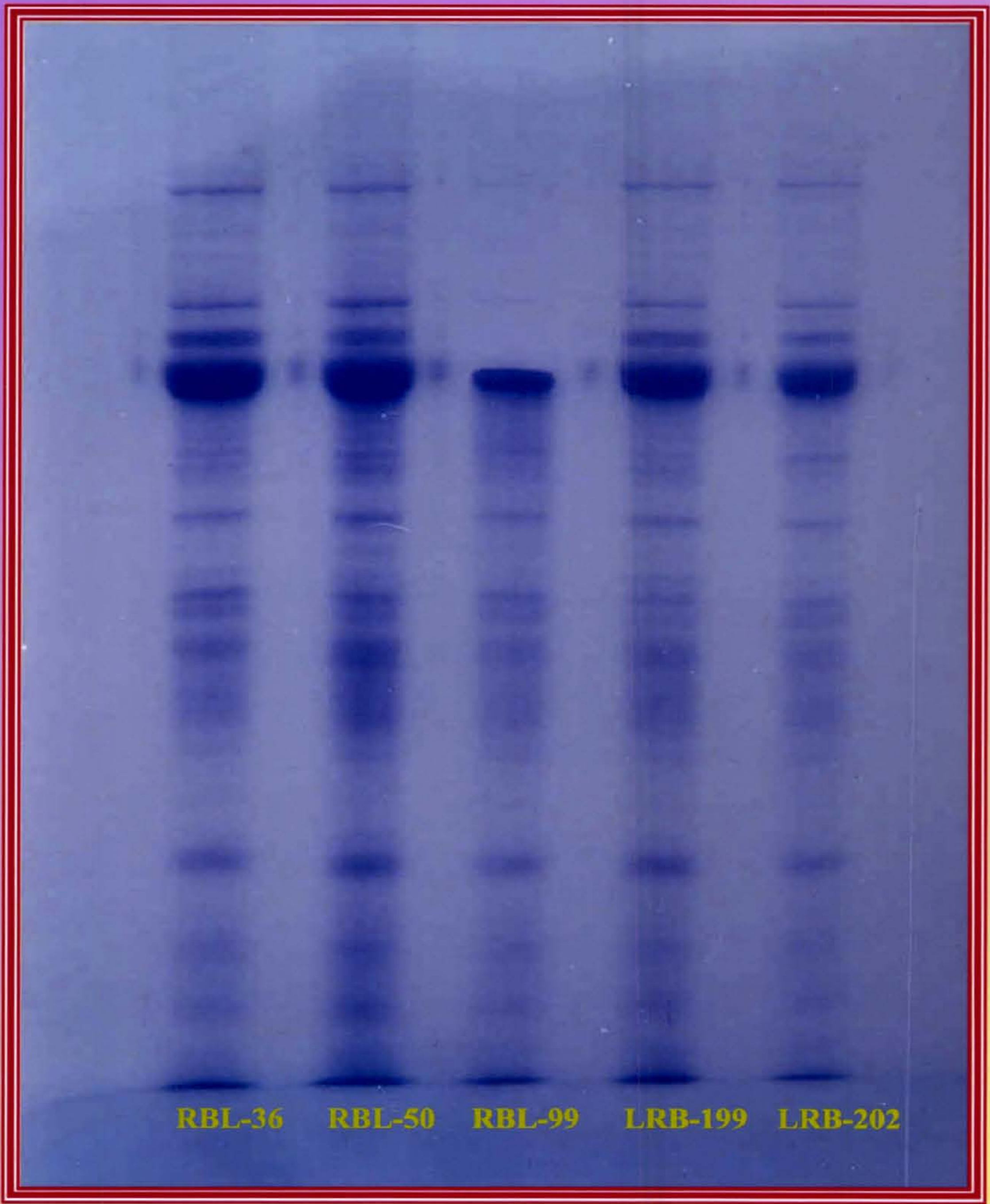


PLATE 7 : Electrophoregrams of total soluble protein in rice bean genotypes

4.3.2 Electrophoresis of peroxidase isozyme

Green gram

A total of 8 bands were recognized in the PAGE of the peroxidase isozyme in different genotypes of green gram viz. PM-2, PM-9339, AKM-8802 and TARM-18 and Kopergaon (Fig. 17). Out of which band number 1 (Rm 0.15, medium) and band number 8 (Rm 0.87, weak) were commonly observed in all the genotypes. The number of band in individual genotypes was ranged from 5 to 8.

Genotype PM-2 showed total 5 bands out of which 2 were medium, 2 light and 1 weak. Band number 2 (Rm 0.24) and band number 7 (Rm 0.78) were present in all the genotypes as different intensity where as in PM-2 it was light. These two bands was the characteristic band of this genotype. Band corresponding to Rm value of 0.42 (band number 4) was present in all genotypes as medium band except TARM-18.

PM-9339 showed 6 bands in its isozyme-banding pattern. It had one dense, 3 medium, 1 light and 1 weak band. Band number 3 corresponding to Rm of 0.30, which was light band, this band was the characteristics band of PM-9339, while in other four genotypes it was absent except Kopergaon. The band number 2 (Rm 0.24) was dense which is also the characteristic band of this genotype.

AKM-8802 showed total 5 bands including 4 medium and 1 weak. Presence of four continuous medium bands viz. band number 1 (Rm 0.13), band number 2 (Rm 0.24), band number 4 (Rm 0.42) and band number 7 (Rm 0.78) were characteristic bands of this genotype.

Electrophoresis pattern of genotype TARM-18 showed 5 bands, 2 medium, 2 lights and 1 weak. The distinguishing band in this genotype was band number 4 (Rm 0.42), which was light in TARM-18 where as in other genotypes it was medium.

There were total of 8 bands in the electrophoresis pattern of Kopergaon including 4 medium, 2 light and 2 weak. Presence of band number 5 (Rm 0.54) and band number 6 (Rm 0.60) which were light and weak respectively were the characteristic band of this genotype. Band number 3 (Rm 0.30) was light band present in only Kopergaon and PM-9339.

Table : 28 Classification of green gram genotypes according to number of peroxidase isozyme bands and their intensity

Sr. No.	Genotype	Total no. of band	Dense	Medium	Light	Weak
1	PM-2	5	-	2	2	1
2	PM-9339	6	1	3	1	1
3	AKM-8802	5	-	4	-	1
4	TARM-18	5	-	2	2	1
5	Kopergaon	8	-	4	2	2

Table 29: Characteristics of peroxidase isozyme banding pattern of green gram genotype.

Sr. No.	Genotypes	Banding pattern
1	PM-2	P : 1,2,4,7,8 A : 3,5,6
2	PM-9339	P : 1,2,3,4,7,8 A : 5,6
3	AKM-8802	P : 1,2,4,7,8 A : 3,5,6
4	TARM-18	P : 1,2,4,7,8 A : 3,5,6
5	Kopergaon	P : 1,2,3,4,5,6,7,8 A : -

P: Present A: Absent

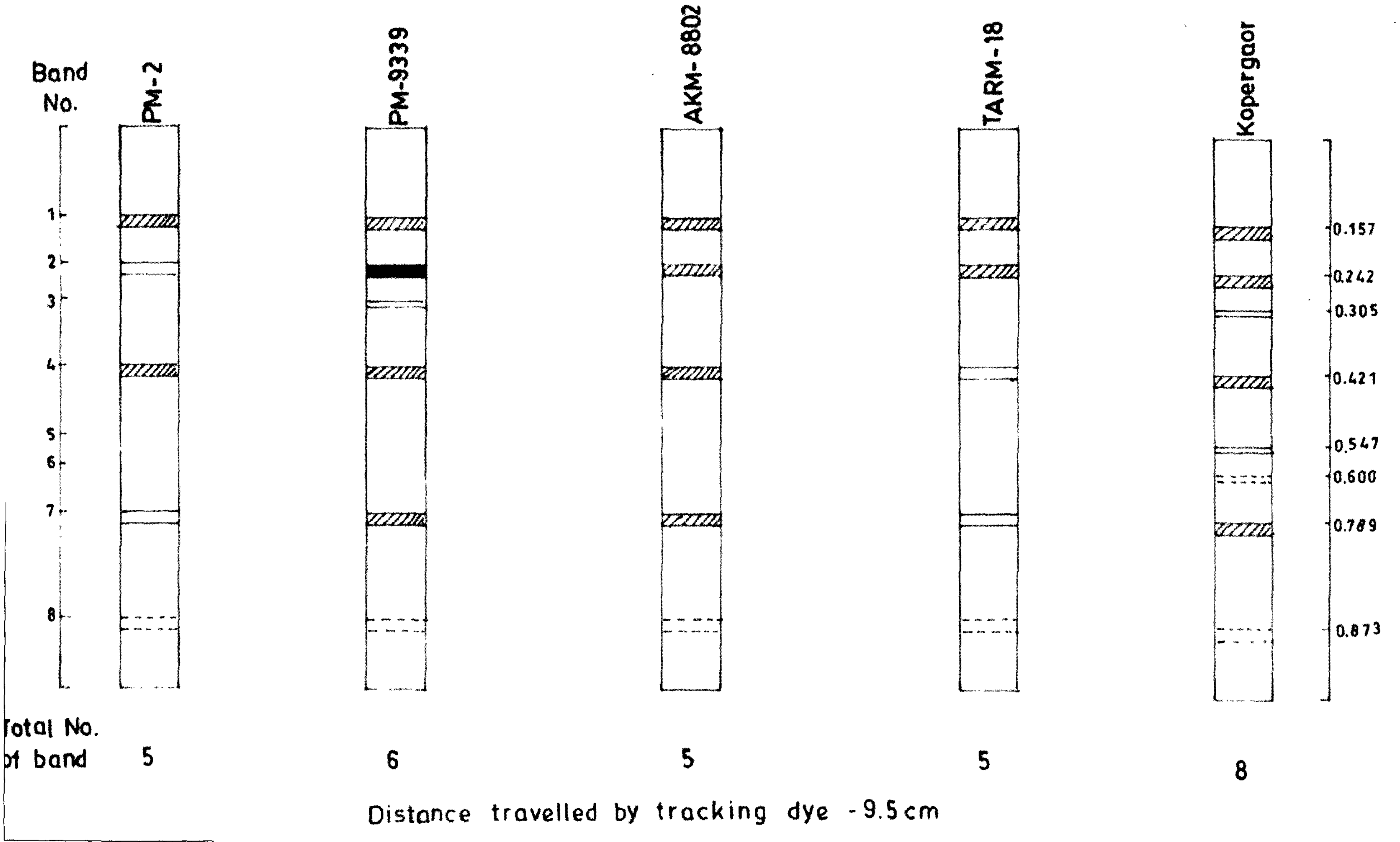


Fig. 17 : Electrophorograms of the PAGE pattern of peroxidase isozyme of green

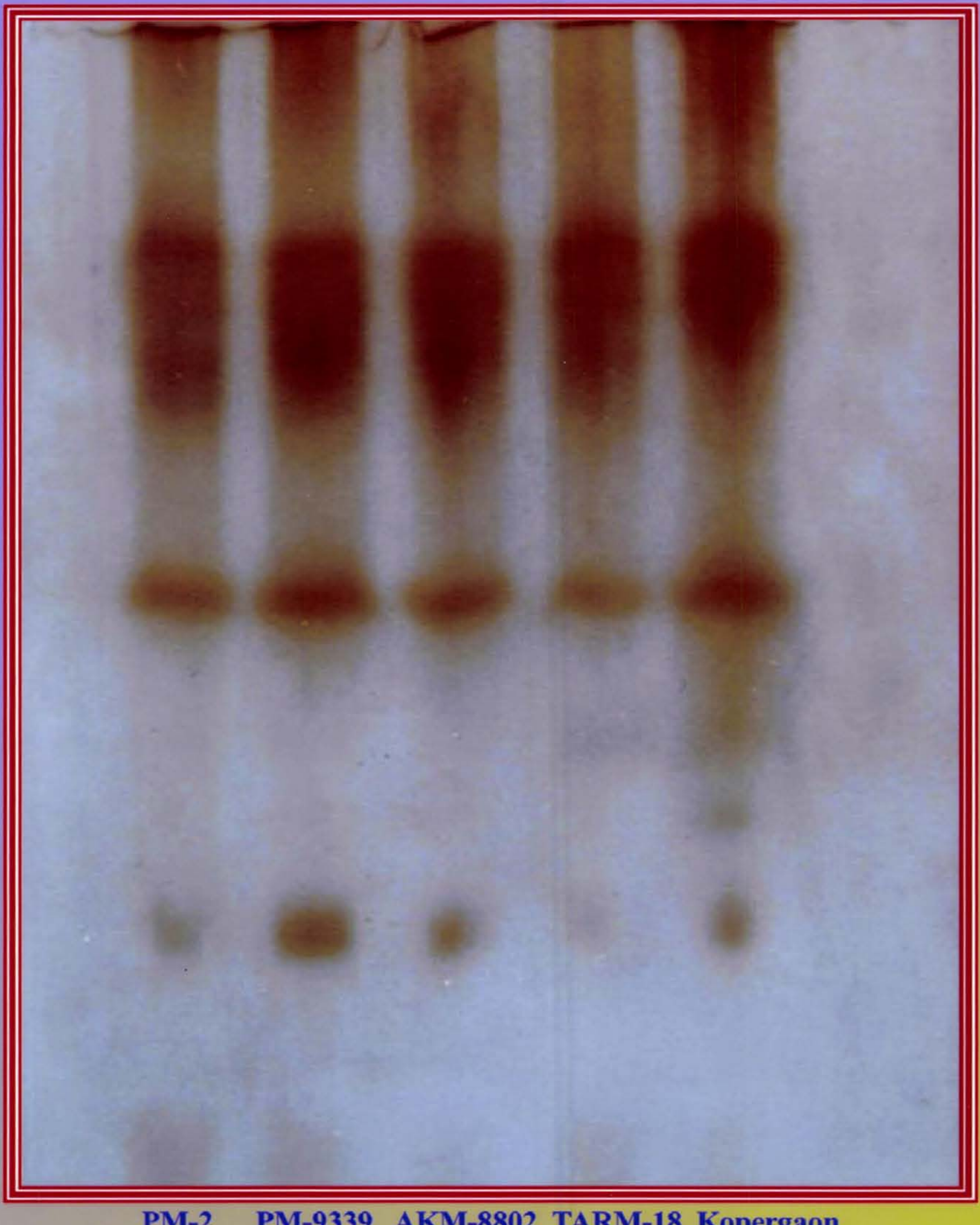


PLATE 8 : Electrophorograms of peroxidase isozymes in green gram genotypes

Table 30 : Rm values of different peroxidase isozyme bands from each genotype of green gram genotypes.

Position of bands from top of individual green gram genotypes	PM-2	PM-9339	AKM-8802	TARM-18	Kopergaon
1	0.15	0.15	0.15	0.15	0.15
2	0.24	0.24	0.24	0.24	0.24
3	0.42	0.30	0.42	0.42	0.30
4	0.78	0.42	0.78	0.78	0.42
5	0.87	0.78	0.87	0.87	0.54
6	-	0.87	-	-	0.60
7	-	-	-	-	0.78
8	-	-	-	-	0.87

Black gram

A total of 8 bands were recognized in the PAGE of the peroxidase isozyme in different genotypes of black gram (Fig. 18). Out of which band number 1 corresponding to Rm of 0.21 was common in all the genotypes except PU-9202-11-3. The number of bands in individual genotype ranged from 7 to 8.

Electrophoresis pattern of TAU-1 showed over all 8 bands, which contained 2 dense, 4 medium, one light and one weak. The most characteristic bands of this genotype were two continuous dense bands (Rm 0.68 and 0.73 respectively) and 3 continuous medium bands (Rm 0.21, 0.36 and 0.45 respectively). The weak band corresponding to Rm of 0.84 was unique band and it was absent in remaining four genotypes. Presence of band number 6 and band 8 was medium and light respectively were the characteristic band of TAU-1 where as in other genotypes showed weak band.

TAU-2 showed total 7 bands including 3 medium, 2 light and 2 weak. Presence of 2 continuous medium band corresponding to Rm of 0.68 and 0.73 (band number 4 and 5 respectively) and 2 continuous light band corresponding to Rm of 0.36 and 0.45 (band number 2 and 3 respectively) were the characteristic bands of this genotype.

There were total 7 bands in the electrophoresis pattern of TPU-4 including 1 medium, 4 light and 2 weak. Presence of four continuous medium bands corresponding to Rm value 0.36 (Band number 2), 0.46 (Band number 0.48), 0.68 (Band number 4) and 0.73 (Band number 5) were the characteristic bands of this genotype.

PU-9503-8 showed total 11 bands out of which four were medium, 1 light and 2 weak. The distinguishing bands in this genotype were continuous four medium bands viz. 1,2,3,4 (Rm 0.21, 0.36, 0.45 and 0.68 respectively). Band number 5 (Rm 0.75) was light in PU-9503-8 and TPU-4 where as in TAU-1 dense, TAU-2 medium and PU-9205-11-3 it was medium.

PU-9205-11-3 showed 7 bands in its electrophoresis pattern. It had 2 dense, 3 medium and 2 weak bands. It had one characteristic band number 1 (Rm 0.21) which was present in all the genotypes as medium band but observed as dense in PU-9205-11-3. Band number 4 (Rm 0.68) was observed as dense in PU-9205-11-3 and TAU-1, where as in TAU-2 it was (medium), TPU-4 light and PU-9503-8 medium. The medium band corresponding to Rm value 0.73 was observed only in PU-9205-11-3 and TAU-2.

Table : 31 Classification of black gram genotypes according to number of peroxidase isozyme bands and their intensity

Sr. No.	Genotype	Total number of bands	Dense	Medium	Light	Weak
1	TAU-1	8	2	4	1	1
2	TAU-2	7	-	3	2	2
3	TPU-4	7	-	1	4	2
4	PU-9803-8	7	-	4	1	2
5	PU-9205-11-3	7	2	3	-	2

Table : 32 Characteristics of peroxidase isozyme banding pattern of black gram genotype

Sr. No.	Genotypes	Banding pattern
1	TAU-1	P : 1,2,3,4,5,6,7,8 A : -
2	TAU-2	P : 1,2,3,4,5,6,8 A : 7
3	TPU-4	P : 1,2,3,4,5,6,8 A : 7
4	PU-9503-8	P : 1,2,3,4,5,6,8 A : 7
5	PU-9205-11-3	P : 1,2,3,4,5,6,8 A : 7

P : Present A : Absent

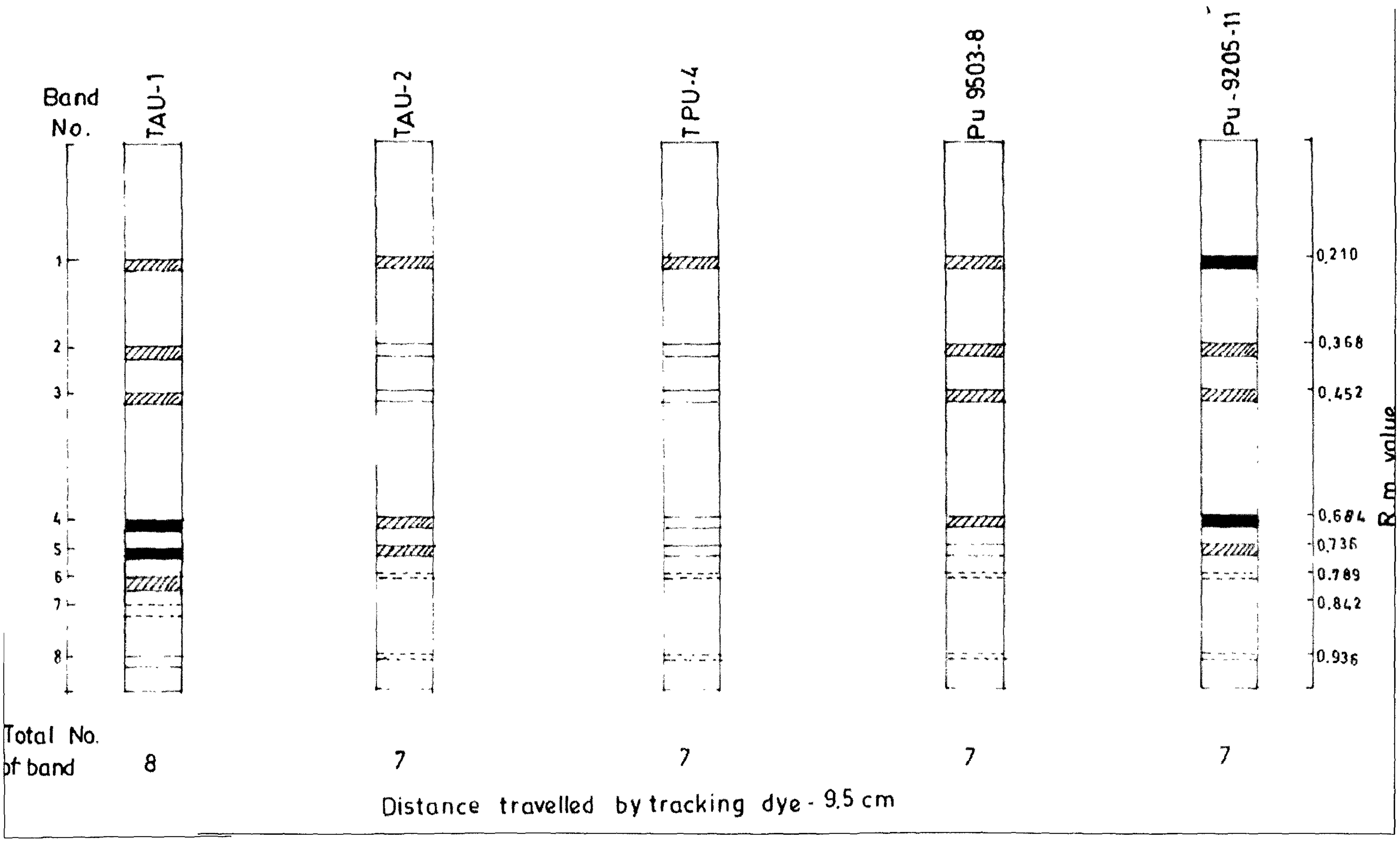


Fig. 18 : Electrophorograms of the PAGE pattern of peroxidase isozyme of black gram genotypes

PLATE 9 : Electrophorograms of peroxidase isozyme in black gram genotypes

Table : 33 Rm values of different peroxidase isozyme bands from each genotype of black gram .

Position of bands from top of individual black gram genotypes	TAU-1	TAU-2	TAU-4	PU-9503-8	PU-9205-11-3
1	0.21	0.21	0.21	0.21	0.21
2	0.36	0.36	0.36	0.36	0.36
3	0.45	0.45	0.45	0.45	0.45
4	0.68	0.68	0.68	0.68	0.68
5	0.73	0.73	0.73	0.73	0.73
6	0.78	0.78	0.78	0.78	0.78
7	0.84	0.84	0.84	0.84	0.84
8	0.93	-	-	-	-

Rice bean

A total of 8 bands were recognized in the PAGE of the peroxidase isozyme banding pattern in different genotypes of rice bean viz. RBL-36, RBL-50, RBL-99, LRB-199 and LRB-202 (Fig. 19). Out of which four bands (Rm 0.21, 0.47, 0.64 and 0.74) were common in all the genotypes. Band number 1 corresponding to Rm value of 0.17 was observed in genotype RBL-99 and LRB-202 while band number 8 (Rm 0.92) representing high distance traveled by peroxidase isozyme in all genotypes.

Genotype RBL-36 showed 6 bands out of which 1 was dense, 4 medium and 1 weak. It had one characteristic band number 8 (Rm 0.92), which was present in all the genotypes but observed as medium in RBL-36.

The electrophoresis pattern of the RBL-50 showed 6 bands that contained 1 dense, 3 medium, 1 light and 1 weak. The band number 8 (Rm 0.92) was light intensity, the characteristic band of RBL-50. The dense band corresponding to Rm of 0.25 present in all the genotypes except RBL-99 but RBL-36 and RBL-50 was showed high intensity and width than other genotypes.

There were total 8 bands in the electrophoresis pattern of RBL-99 including 3 medium, 3 light and 2 weak. Band corresponding to Rm value 0.56 (band number 5) was characteristic band representing light intensity band that was absent in remaining four genotypes. Band number 3 (Rm 0.25) was represented as light band in RBL-99, where as in other genotypes found as dense intensity band. Band number 8 (Rm 0.90) was also the characteristic band of RBL-99.

LRB-199 contained 6 bands including 1 dense, 2 medium and 2 light. Band number 4 corresponding to Rm value 0.47 was common in all the genotypes as medium intensity band where as here present as light intensity band. Band number 7 (Rm 0.74) was present in all genotypes, while absent in LRB-199.

LRB-202 showed total 7 bands in its electrophoresis pattern, which includes 1 dense, 3 medium, 2 light and 1 weak band. Presence of band number 1(Rm 0.17) and 8 (Rm 0.92) of light intensity was the characteristic band of LRB-202. Band number 1 corresponding Rm value of 0.17 was present in LRB-202 and RBL-99 where as in other genotypes it was absent.

Table : 34 Classification of rice bean genotypes according to number of peroxidase isozyme bands and their intensity

Sr. No.	Genotype	Total No. of bands	Dense	Medium	Light	Weak
1	RBL-36	6	1	4	-	1
2	RBL-50	6	1	3	1	1
3	RBL-99	8	-	3	3	2
4	LRB-199	5	1	2	2	0
5	LRB-202	7	1	3	2	1

Table : 35 Characteristics of peroxidase isozyme banding pattern of rice bean genotypes

Sr. No.	Genotype	
1	RBL-36	P : 2,3,4,6,7,8 A : 1,5
2	RBL-50	P : 2,3,4,6,7,8 A : 1,5
3	RBL-99	P : 1,2,3,4,5,6,7,8 A : -
4	LRB-199	P : 2,3,4,6,8 A : 1,5,7
5	LRB-202	P : 1,2,3,4,6,7,8 A : 5

P : Present A : Absent

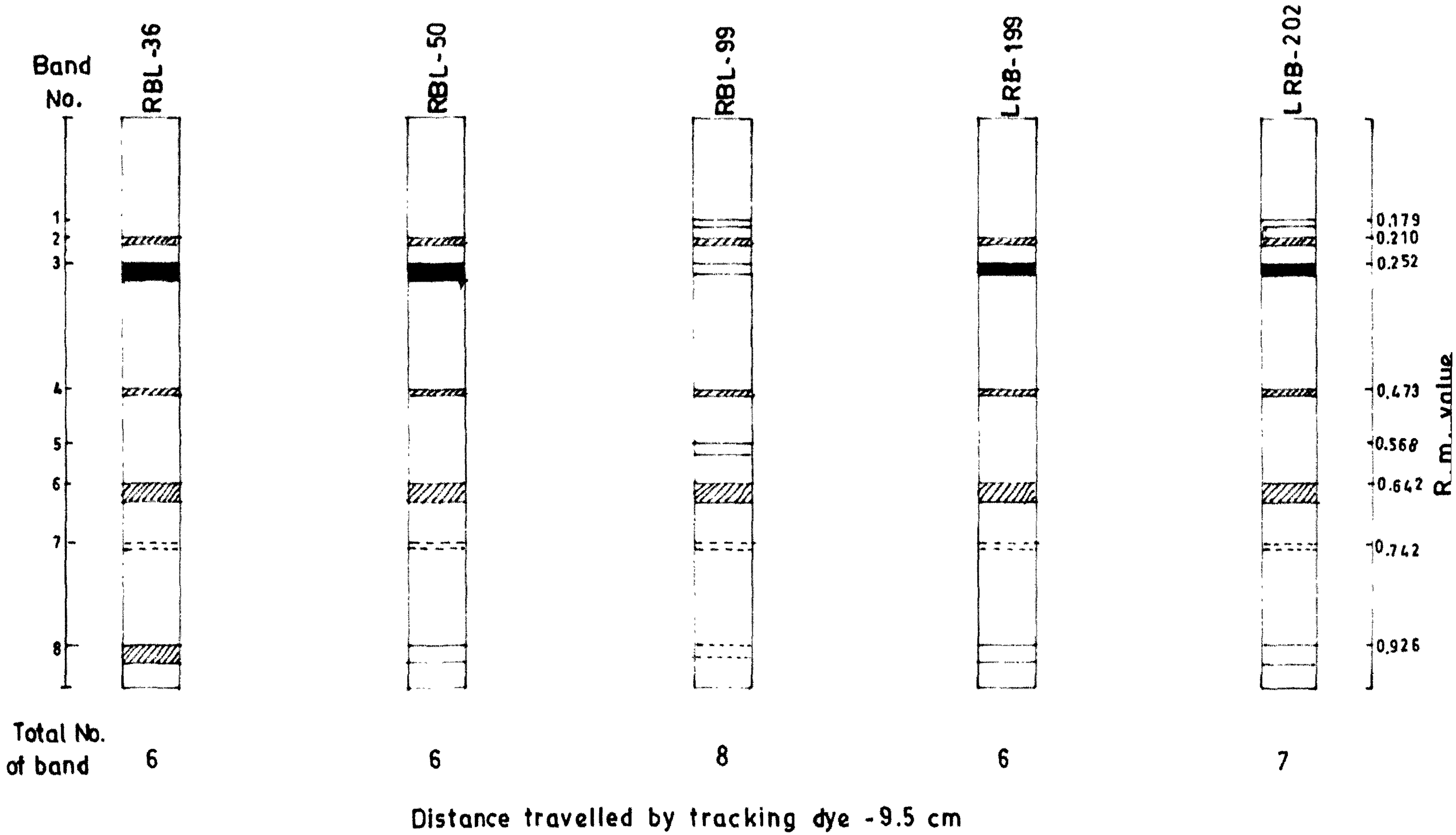


Fig. 19 : Electrophorograms of the PAGE pattern of peroxidase isozyme of rice bean genotypes

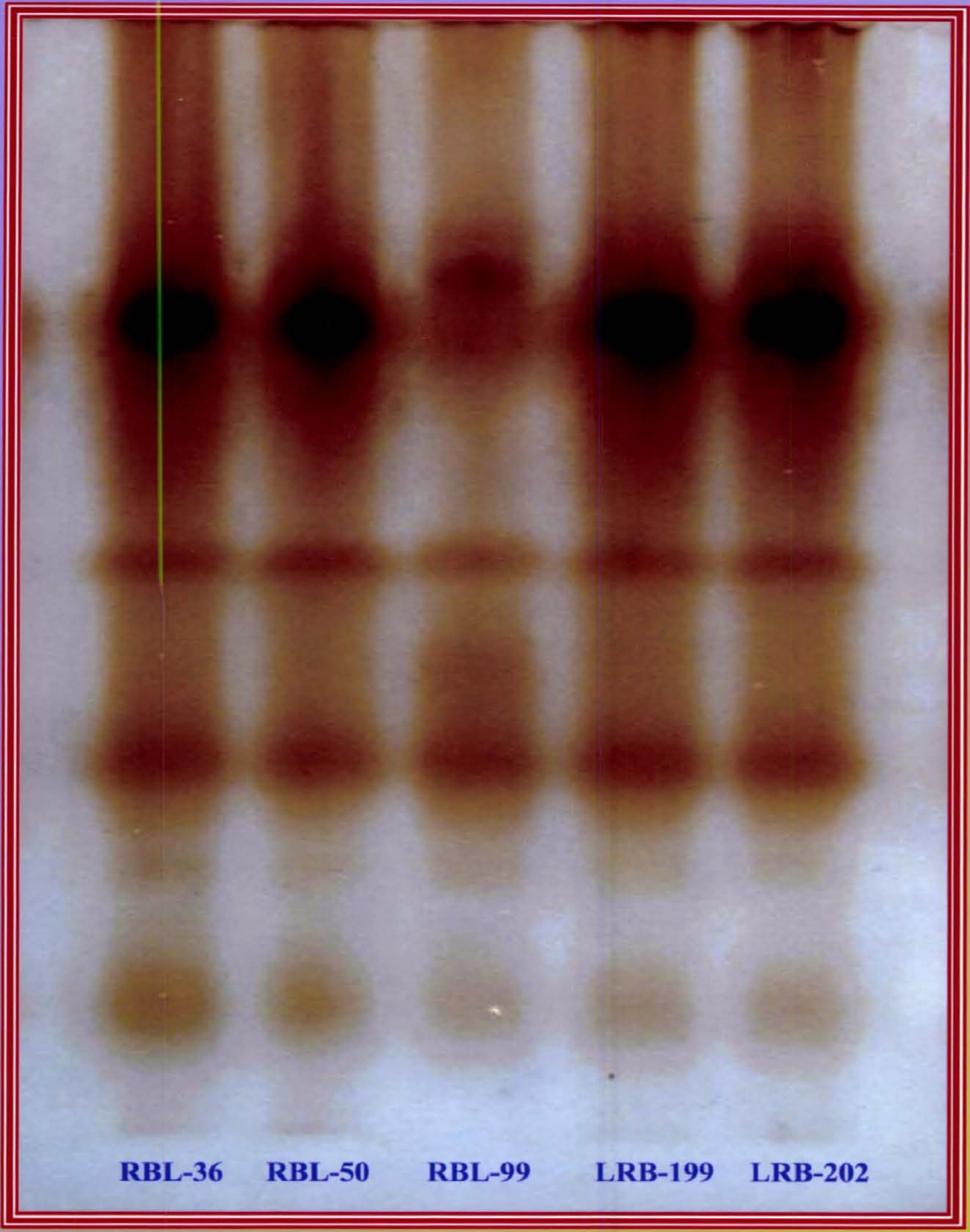


PLATE 10 : Electrophorograms of peroxidase isozymes in rice bean genotypes

Table : 36 Rm values of different peroxidase isozyme bands from each genotype of rice bean genotypes.

Position of bands from top of individual rice bean genotypes	RBL-36	RBL-50	RBL-99	LRB-199	LRB-202
1	0.21	0.21	0.17	0.21	0.17
2	0.25	0.25	0.21	0.25	0.21
3	0.47	0.47	0.25	0.47	0.25
4	0.64	0.64	0.47	0.64	0.47
5	0.74	0.74	0.56	0.92	0.64
6	0.92	0.92	0.64	-	0.74
7	-	-	0.74	-	0.92
8	-	-	0.92	-	-

4.4 Seed mycoflora study

Green gram

Seven seed borne fungi were found associated with the seeds of five genotypes of green gram. These were *Alternaria alternata, Fusarium monoliforme, Aspergillus* sp., *Penicillium* spp., *Rhizopus stolonifer, Macropheomina phasolina* and *Curvularia lunata* (Table 37).

The lowest incidence of seed borne fungi was observed in TARM-18 (9 %). This genotype showed the incidence of *Aspergillus* spp. (4 %), *Penicillium* spp. (2 %), *Rhizopus stolonifer* (2 %), and *Curvularia lunta* (1 %). The highest incidence of seed borne fungi was observed in the genotype PM-2 (19 %) followed by AKM-8802 (18 %).

Black gram

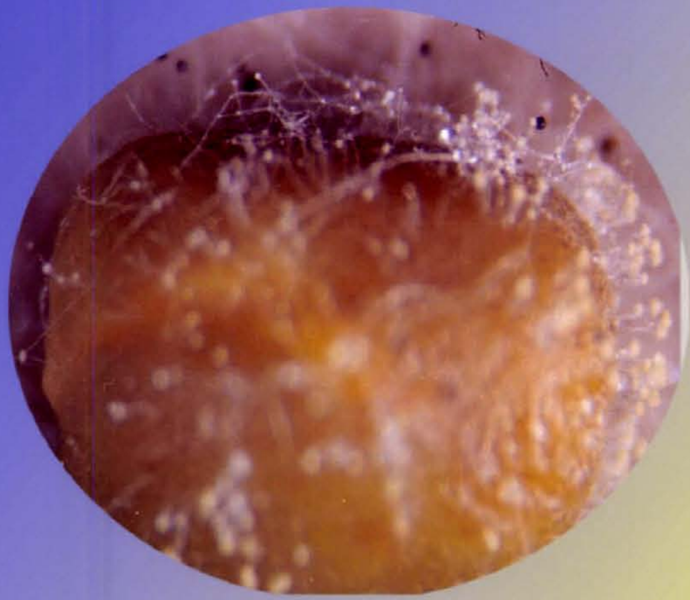
Six seed borne fungi were found associated with seeds of five genotypes of black gram. These were *Alternata alternata, Fusarium monoliforme, Aspergillus* spp., *Penicillium* spp., *Rhizopus stolonifer,* and *Macrophomina phasolina* (Table 38).

The lowest incidence of seed borne fungi was observed on TPU-4, (16 %). This genotype showed the incidence of *Fusarium monoliforme* (2 %), *Aspergillius* spp. (5 %), *Penicillium* sp. (6 %) and *Rhizopus stolonifer* (3 %). The highest incidence of seed borne fungi was observed in the genotype TAU-2 (24 %) followed by PU-9503-8 (22 %).

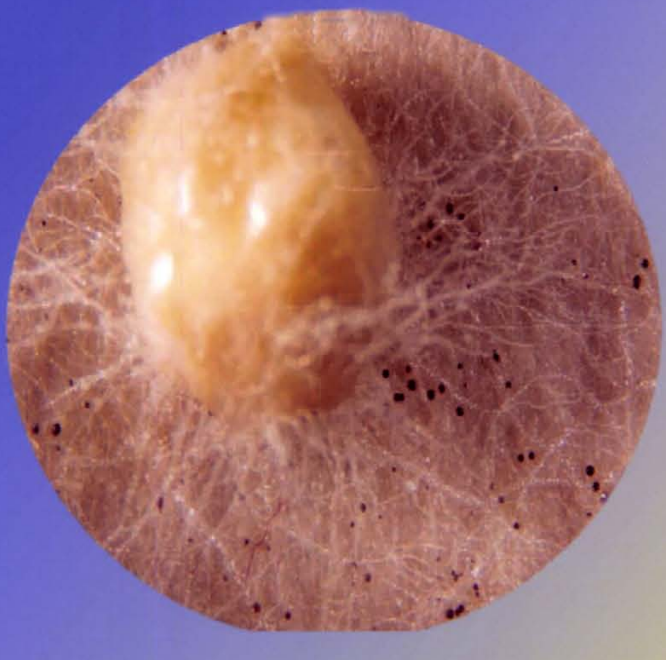
Rice bean

Alternaria alternata, Fusarium monoliforme, Aspergillus spp, *Penicillium* sp. and *Rhizopus* sp. were observed to be associated with the seeds of five genotypes of rice bean presented in Table 39.

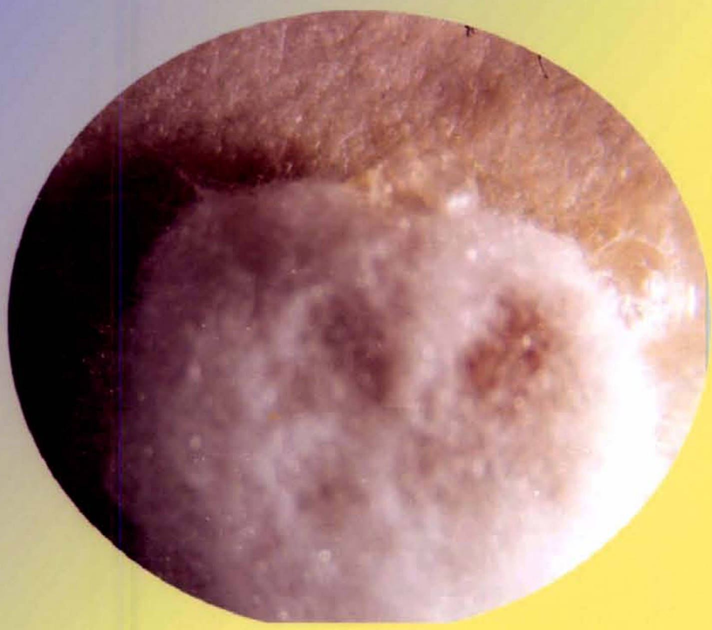
Alternaria alternata



Aspergillus species



Rihizopus stolonifer



Fusarium monoliforme

PLATE 11 : Seed borne fungi assciated with different *vigna* species

Table 37 : Incidence of seed borne fungi on green gram genotypes

Sr. No.	Name of seed borne fungi	Incidence on green gram genotypes (%)				
		PM-2	**PM 9339**	**AKM 8802**	**TARM-18**	**Kopergaon**
1	*Alternaria alternata*	3	0	0	0	0
2	*Fusarium monoliforme*	2	2	3	0	0
3	*Aspergillus spp.*	5	6	7	4	5
4	*Penicillium* spp.	2	1	4	2	2
5	*Rhizopus stolonifer*	5	4	2	2	3
6	*Macrophomina phaseolina*	2	2	2	0	0
7	*Curvularia lunata*	0	0	0	1	0
	Total incidence (%)	19	15	18	9	10

Table 38 : Seed borne fungi incidence (%) associated with black gram genotypes

Sr. No.	Name of seed borne fungi	Incidence on black gram genotypes (%)				
		TAU-1	TAU-2	TPU-4	PU-9503-8	PU-9205-11-3
1	*Alternaria alternata*	0	3	0	0	0
2	*Fusarium moniliforme*	5	3	2	2	4
3	*Aspergillus* spp.	2	5	5	5	6
4	*Penicillium* spp.	5	3	6	4	5
5	*Rhizopus stolonifer*	0	5	3	6	3
6	*Macrophomina phaseolina*	6	5	0	5	0
	Total incidence (%)	18	24	16	22	18

Table 39 : Seed borne fungi incidence (%) associated with rice bean genotypes

Sr. No.	Name of seed borne fungi	Incidence on rice bean genotypes (%)				
		RBL-36	**RBL-50**	**RBL-99**	**LRB-199**	**LRB-202**
1	*Alternaria alternata*	6	0	10	0	0
2	*Fusarium monoliforme*	0	2	0	0	2
3	*Aspergillus* spp.	14	8	4	11	12
4	*Penicillium* spp.	3	1	2	6	4
5	*Rhizopus stolonifer*	3	1	3	4	1
	Total incidence (%)	26	12	19	21	19

Rice bean genotypes i.e. RBL-50 showed the lowest incidence of seed borne fungi (12 %). There was *Fusarium monoliforme* (2 %), *Aspergillium* sp. (3 %), *Penicillium* sp. (1 %) and *Rhizopus stolonifer* (1 %). The highest incidence of seed borne fungi was observed in case of RBL-36 (26 %) followed by LRB 199 (21 %).

DISCUSSION

5. DISCUSSION

The seeds of plant are rich source of protein, carbohydrates and lipids and are therefore used as valuable food sources. Humans use seed as a major food source and have learned, through agricultural practices, how to increase the levels and the quality of their components. They have also learned how to deal with the multiplicity of toxic and antinutritional compounds present in seeds. It is believed that these seeds most of which are not essential for the establishment of the new plant following germination contribute to the protection and define of seeds against pathogens and predators. However, insects, fungi and bacteria have also learned how to cope with detrimental compounds in order to take advantage of the high nutritional value of seeds.

Coleopteran insects of family bruchidae, the seed weevils, have been associated with the seed of leguminous plants through co-evolutionary processes. These processes have permitted the weevils to thrive on seeds full of toxic compounds, in contrast to the majority of the other potential aggressors, which are incapable of dealing with them. The association between bruchids and legume seeds is highly specific with only seeds of a very few species being attacked by any one insect species (Sales *et al.*, 2000).

Among our food sources, plants of the legume family contribute some of the most important protein rich seeds. Green gram (*Vigna radiata* (L.)) and black gram (*Vigna mungo*) which originated in India are the major sources of protein in Indian diet. They are heavily attacked by bruchids (*Callosobruchus chinensis*) both in the field and storage. Infestations are commonly so heavy that the seeds are unsuitable for use as food, feed or planting.

Control of bruchid infestation is generally done by treating stored seeds with methyl bormide, carbon disulfide, zinc phosphate and several other chemicals. These are considered environmentally undesirable and are too expensive for subsistence farmers. To increase the insect resistance in cultivated genotypes, plant breeders are interested in understanding resistance mechanisms that operates in wild genotypes or why certain bruchids attack one cultivated species but not another. Most of the land races of rice bean (*Vigna umbellata*) that produce seed resistance to bruchid have been discovered. The structural and biochemical basis of the resistance of these *Vigna* species seeds to *Callosobruchus chinensis* is discussed in this chapter.

5.1a Evaluation of level of resistance to bruchid

The resistance to the bruchid in green gram, black gram and rice bean has been evaluated by using the parameters oviposition, number of adult emerged, number of damaged seed, loss in seed weight, seed hardness and number of resistant seed, reduction in germination and 100 seed weight.

5.1a.1 Oviposition

Green gram was found to be most preferred host for oviposition followed by black gram and rice bean. Similar results have been reported by Obaidullah *et al.,* (2003), Ghosal *et al.,* (2004). The genotypes Kopergaon and AKM-8802 of green gram having lustrous green bold seeds favored the insect for oviposition followed by PM-9339. Choudhary *et al.,* (1989) also reported that the genotype Kopergaon as the most preferred for oviposition.

The black gram genotypes TAU-1 and TAU-2 recorded highest oviposition with list on PU-9205-11-3 followed by PU-9503-

8 and TPU-4. In rice bean, maximum oviposition was observed on the genotype LRB-202 followed by RBL-36 and RBL-99.

5.1a.2 Number of adult emerged

Higher number of adult emergence was recorded in green gram genotypes followed by black gram. No adult emergence was found in case of rice bean as such green gram was found to be most susceptible followed by black gram and rice bean as completely resistance. Maximum number of adult emergence was recorded in genotype kopergaon followed by AKM-8802, PM-9339 and PM-2 of green gram, where as on TARM-18 it was least. TARM-18 was found to be relatively resistance to bruchid than rest of the genotypes.

5.1a.3 Number of damaged seed

Maximum number of damaged seeds were observed in green gram followed by black gram, where as in rice bean no damaged seed was recorded. In green gram, maximum number of damaged seed was found in genotype kopergaon followed by AKM-8802 and PM-9339. The number of damaged seeds were least in TARM-18.

In black gram damaged seeds were highest in the genotype TAU-2 followed by TAU-1, where as the number of damaged seed was very low in genotype PU-9205-11-3. These results are in accordance with the reports of Singh and Sharma (1985), Rasul *et al.,* (1989) and Ramzan *et al.,* (1990).

5.1a.4 Loss in seed weight

Highest loss in seed weight was observed in case of green gram followed by black gram; where as no loss in seed weight was recorded in rice bean. In green gram, the loss in seed weight

(%) was maximum in the genotype Kopergaon followed by AKM-8802 and PM-9339, whereas minimum loss in seed weight occurred in TARM-18.

In black gram, maximum loss in seed weight was observed in genotype TAU-2 followed by TAU-1 and TPU-4. On the other hand minimum loss in seed weight was recorded in genotype PU-9205-11-3 and PU-9503-8. Asrof *et al.,* (1999) and Rasul (1989) reported maximum loss in seed weight in mung bean and udid bean due to bruchids.

5.1a.5 Seed hardness

The hardness of the seed coat has reported as one of the major factor in providing the resistance to the bruchid attack (Gokhale 1973, Singh *et al.,* 1977 and Raina, 1971).

The newly hatched larvae find it difficult to penetrate through hard seed coat and offen perish before reaching cotyledon. Highest seed hardness was recorded in rice bean followed by black gram and it was least in green gram. It is interesting to note the linear response of seed hardness with oviposition, number of adult emerges, and number of damaged seed, number of resistant seed and 100 seed weight. The hardness of the seed coat has been implicated in the failure of burchid larva to enter the seed (Podoler and Applebaum 1968, Thiery, 1984). Simmonds *et al.,*(1989) associated this type of resistance to unusually thick testa and the presence of toxic compounds.

In green gram, highest seed hardness was recorded in the genotype TARM-18 followed by PM-2. The lowest seed hardness (3.4 kg/cm^2) was observed in the most bruchid susceptible genotype kopergaon followed by AKM-8802. In general seed hardness was maximum in black gram than green gram. In black gram, it was the highest in the genotype PU-9205-11-3 followed by

PU-9503-8, which recorded low adult emergence. No significant differences were recorded in seed hardness of rice bean genotypes. However it is highest among the three species, which could be directly related to oviposition, number of adult emerged, loss in weight and may be treated as principle component of resistance to bruchid.

5.1a.6 Number of resistant seeds

Rice bean was found to be resistant to bruchid in which all seeds were found to be free from adult emergence, where as black gram was relatively but marginally resistant than green gram. In green gram, the genotype TARM-18 recorded highest number of resistant seed followed by PM-2 and PM-9339, where as the resistant seed recorded in the genotype Kopergaon was least.

In black gram, maximum number of resistant seeds was recorded in genotype PU-9205-11-3 followed by PU-9503-8; where as minimum number of resistant seeds were recorded in TAU-2 and TAU-1.

5.1a.7 Reduction in germination (%)

Maximum reduction in germination percentage was noticed in green gram and black gram, where as in rice bean it was negligible. This may be due to destruction of embryo as evident by adult emergence from respective species. In green gram more reduction in germination was observed in genotype Kopergaon followed by AKM-8802, where as it was least in TARM-18.

In black gram, more reduction in germination was recorded in genotype TAU-2 followed by TAU-1 and TPU-4, where as it was least in PU-9205-11-3. In case of rice bean, the reduction in germination in all genotypes was negligible.

5.1a.8 100 seed weight

At species level rice bean recorded highest seed weight (5.35 g) followed by black gram (5.13 g) and least in the case of green gram (3.79 g). The resistance to bruchid in these species also followed that order of merit. However, in green gram more seed weight relates to more susceptibility to bruchid in all genotypes. The genotype Kopergaon has more 100 seed weight (4.66 g) showed highest adult emergence followed by AKM-8802. The genotype TARM-18 which has low 100 seed weight (3.19 g) show least adult emergence.

In black gram, the genotype TAU-2, TAU-1 and TPU-4 showed higher 100 seed weight (above 5.0 g) recorded maximum number of adult emergence, where as the genotype PU-9503-8 and PU-9205-11-3 seed weight recorded low adult emergence.

In rice bean, 100 seed weight in all genotypes exceeds 5.0 g however there was no adult emergence.

Kashiwaba *et al.,* (2003) reported that cultivated rice bean has longer seeds than mung bean and udid bean. Further the 100 seed weight of rice bean is greater than that of susceptible green gram and black gram. In addition, the shape of cultivated and wild rice bean is similar to mung bean and udid bean. Thus among four cases of resistance proposed by Southgate (1979), it is considered that some chemical in the embryo and /or cotyledon in cultivated rice bean was responsible.

Southgate (1979) stated that bruchid resistance might be the result of one more four causes (1) the seed may be too hard for newly hatched larva to penetrate (2) the seed may physically be too small or of an inconvenient shape for the larvae to reach full size (3) the seed may contain to little food to support a larva (4) the seed may contain toxin or other substance in cotyledons or it's enveloping seed coat that inhibits the development of larvae.

5.1b Correlation coefficient among the various parameters and resistance seed to bruchid

In green gram, most of the parameters of resistance to bruchid were found to be negatively associated with resistant seed except seed hardness. Seed hardness has highly significant positive correlation with resistant seed. Where as number of adult emerged, loss in seed weight, 100 seed weight and oviposition have significant negative association with resistant seed. Number of adult emerged have highly significant positive correlation with loss in seed weight and 100 seed weight. Oviposition and seed hardness had significant negative correlation.

Significant positive correlation of 100 seed weight with number of adult emerged and loss in seed weight was reported by Muhammad (1997) in mung bean.

High positive correlation between weight loss and number of adult emerged, percent adult emerged was reported by Babu, *et al.* (2000). They also reported high positive correlation between the percent adult emergences with seed colour; size, texture, percent weight loss and number of adults emerged.

The significant positive correlation between susceptibility parameter *viz.*, percentage of affected seeds, number of eggs laid, number of adult emerged and percent of weight loss with seed weight was recorded by Chakraborty *et al.*,(2004) in mung bean. Highly significant positive correlation between adult emerged with weight loss and damaged seed in mung bean were recorded by Khattak *et al.*, (1987).

The path analysis further clarifies the extent of role of independent characters on the dependent characters association.

Significant negative correlation of resistant seed (-0.486) with oviposition was mainly due to the direct effect of oviposition. Number of adult emerged have highest negative indirect effect. The substantial amount of negative indirect effect *via.* number of adult emerged is also important.

The number of adult emerged had very high negative direct effect (-0.516) on resistant seeds, which is responsible for very high level of highly significant negative correlation (-0.953). The indirect effect *via.* Loss in seed weight, 100 seed weight and seed hardness have played major role in this association.

The correlation between seed hardness and resistant seed was highly significant positive (0.757), which is due to positive direct effect of seed hardness (0.143) and very high indirect effect *via.,* number of adult emerged and loss in seed weight.

The negative direct effect of loss in seed weight and very high indirect effect via. number of adult emerged (-0.465) were responsible for highly significant negative correlation (-0.874) between loss in seed weight and resistant seed.

Negative direct effect of 100 seed weight and negative indirect effect via. number of adult emerged (-0.336) played major role in the correlation of these traits.

In black gram, the parameters of resistance to bruchid *viz.,* number of adult emerged; loss in seed weight and 100 seed weight were negatively correlated with resistant seed. Where as seed hardness have significant positive correlation with resistance. Number of adult emerged was highly significant positively associated with loss in seed weight and significant positively correlated with 100 seed weight. Loss in seed weight and 100 seed weight were highly significant positively associated.

Seed hardness is the principle component of resistance to bruchid, which has reflected through a very high direct effect (0.579), observed in path analysis. Number of adult emerged had negative direct effect on resistant seed. The indirect effect via. 100 seed weight and loss in seed weight are responsible for a highly significant negative correlation of number of adult emerged with resistant seed. Similarly in case of loss in seed weight, negative indirect effect via. number of adult emerged, 100 seed weight and seed hardness are more important.

5.2a Biochemical parameters

The knowledge of biochemical mechanisms of resistance to insects enables the plant breeders to assess their potential and to exploit such traits in plant breeding programs in a more directed fashion. Legume seeds are a rich and varied source of secondary plant compounds, many of which are toxic to or antimetabolic toward predators. *Vigna* spp. contains a family of evolutionarily related plant defense proteins, composed of phytohemagglutinin, arcelin and alpha amylase inhibitor (Chrispeels and Raikhel, 1991). This protein family is encoded by homologous genes found at one complex locus in the bean genome and it is therefore likely that these genes arose by duplication of a single ancestral gene (Nodari *et al.,* 1993).

The three proteins have quite different biochemical and physiological properties. Phytohemagglutinin is a lectin that binds to glycoproteins in the intestinal mucosa of mammals and birds and it's toxicity results from this binding (Liener, 1986). Alpha amylase inhibits the alpha amylase in the digestive tract of mammals and coleoptetra (Powers and Whitaker 1977, Powers and Culberton, 1983). Arcelin was identified in a few wild bean accessions from mexico (Osborn *et al.,* 1986) and the arcelin phytohemagglutinin alpha amylase inhibitor locus was identified into cultivated lines (Osborn *et al.,* 1988, Cardona *et al.,* 1990, Hartweck *et al.,* 1991).

The presence of proteins characteristically inhibiting digestive enzymes has been detected in different tissues of several vegetables species, cereals, legumes, tubers and fruits (Liener, 1980). The physiological role of such proteins, though not yet fully understood, can be described as the elimination of unwanted hydrolysis of the macromolecules viz. proteins, starch and lipids (Rackis, 1986). In dormant seeds, in facts, the inhibitors might be able to control the breakdown of stored reserves. On the other hand, the inhibitors occurring in the storage tissues might themselves function as safe storage forms of proteins which had been immune to digestion for a long as required during the germination stage (Gupta, 1987).

Callosobruchus chinensis is the most damaging stored grain pest of the *Vigna radiata* and *Vigna mungo*, which are indigenous legumes of India. Therefore the aim of this study is to investigate whether there are any relations between the different biochemical parameters with the resistance to storage pest of *Vigna* species. The identification of one or more antimetabolites related to degree of resistance seeds may provide the basis on which to breed plant genotypes inheritably can naturally resistant to bruchid attack.

5.2a.1 ∝- amylase inhibitor

Alpha amylase inhibitor activity (Alpha AI) was found to be highest in rice bean followed by black gram and green gram. Interestingly these pulses follow the same order of merit in respect of resistance to bruchid. In green gram, the alpha amylase inhibitor activity was high in the resistant genotype TARM-18 followed by PM-2 and PM-9339. It was least in susceptible genotype Kopergaon. Similar in case of black gram, maximum activity of alpha amylase inhibitor was observed in the genotype PU-9503-8 and PU-9205-11-3, where as in rice bean almost all the genotypes have high level of alpha amylase inhibitor activity.

Ishimoto and Kitamura (1989) demonstrated that a seed alpha amylase inhibitor protein in several cultivars of bean plays a protective roles against bruchid. A similar result has also been reported by Naik and Jadhav (2001).

∝- amylase are valuable components for transgenic approaches when using multiple mechanisms of resistance, introgression of common bean alpha amylase inhibitor gene into peas makes them resistant to *Callosobruchus maculatus* and *Callosobruchus chinensis* (L.) (Shade *et al.,* 1994) and *Bruchus pisorum* (L.) (Schroeder *et al.,* 1995), which are not pest of common beans. Alpha amylase may be useful even if presenting low levels of inhabitation, because by using transgenic approaches the level of expression of a protein can be increased. To select potential genes, it would be desirable to understand the molecular basis of inhibition of alpha amylase by ∝- amylase inhibitor. Eight electrophoretic variants of ∝-amylase inhibitor have been reported in a comprehensive analysis on the presence or absence of the different patterns across Latin American genotypes (Ishimoto *et al.,* 1995).

Resistance of wild and cultivated rice bean (*Vigna umbellata* [Thunberg) Ohwi and Ohashi) to three bruchid species, (*Callosobruchus chinensis* (L.), *Callosobruchus maculatus* (F.) and *Callosobruchus analis* (F.)), was evaluated (Kashiwaba *et al.,* 2003). All three accessions of cultivated and all wild rice bean accessions tested, exhibited complete resistance to the three bruchid species. Results indicate that physical attributes and or chemical (s) present in the seed coat of rice bean are not the main factors responsible for resistance. Feeding tests were performed by using artificial bean prepared with varying proportions of rice bean (resistant) and azuki bean (susceptible) flour. Number of bruchid adults that emerged decreased and larval developmental period (days) was extended, when artificial bean with an increasing proportion of rice bean flour were used. These tests revealed that a chemical compound (s) contained in the cotyledon of rice bean has an inhibitory effect on the growth of these bruchid species. The results also indicate that the chemical (s) in rice bean cotyledon is most effective against *Callosobruchus maculatus*.

5.2a.2 Trypsin inhibitor

Trypsin inhibitors is widely distributed in legume seeds and have been investigated extensively because of possible adverse effects on protein digestion when ingested by animals. Although their function in plant are obscure. Several roles have been proposed from them including control of protein hydrolysis of bacteria and insect pests (Chen and Mitchell, 1973).

Highest activity of trypsin inhibitor was recorded in rice bean, which was resistant to *Callosobruchus chinensis* where as this activity was low in green gram and black gram. In green gram, trypsin inhibitor activity was very high in the genotype TARM-18, where as it was very low in Kopergaon. As TARM-18 was found to be resistant and Kopergaon as susceptible, shows the major role of trypsin inhibitor in resistance to bruchid.

The bruchid beetle cause extensive damage to seeds of cowpea during storage. The resistant genotype contained a significantly higher level of inhibitors, about twice as much as any other genotypes. The antimetabolic nature of cowpea trypsin inhibitor was conformed by insect feeding trails in which various protein fractions were added to a basic meal and the albumin protein of cowpea (containing trypsin inhibitor) at a level of 10 per cent was toxic larva of bruchid beetle (Gatehouse, 1979).

Gatehouse and Boutler (1983) reported high content of trypsin inhibitor seems to be related to the mechanism of resistance to bruchid beetle.

In black gram also the resistance genotype PU-9503-8 and PU-9505-11-3 were having high values of trypsin inhibitor, where as in susceptible genotype TAU-1 and TAU-2 were low.

5.2a.3 Protein content

The average protein content of green gram was high followed by rice bean and black gram. In green gram the protein content was positively associated with the susceptibility to bruchid. The susceptible genotype Kopergaon is having very high protein content followed by AKM-8802. On the other hand, resistance genotype TARM-18 have low protein content followed by PM-2 and PM-9339.

In black gram also, the resistance genotype PU-9503-8 and PU-9205-11-3 have low protein content where as the susceptible once (TAU-1 and TAU-2) have high protein content.

Umrao and Verma (2004) reported that the increase protein content of pea genotype increase the infestation of bruchid. These observations indicate that higher protein content lines were most preferred by bruchid.

5.2a.4 Total phenols

Polyphenols present in legume are known to from the complexes with the digestive enzymes, dietary proteins, minerals and vitamins and reduce their bioavliability (Hulse, 1979). These are primarily located in seed coat with low or negligible amount in cotyledons. The phenolic play a role in healthy plants as preformed resistant factors (Goodman *et al.,* 1967). The phenolic compounds (Phenolic acid, flavonide) and enzyme related to their metabolism (Peroxidase, polyphenol oxidase) are widely implicated in resistance to infection of insect (Jambunathan *et al.,* 1986).

Highest phenol content was recorded in rice bean followed by black gram and green gram. In green gram, it was lowest in the highly susceptible genotype Kopergaon, where as high in resistant genotype TARM-18 followed by PM-9339 and PM-2. In black gram, highest phenol content was recorded in resistant genotype PU-9205-11-3 and PU-9503-8, where as it was minimum in susceptible genotype TAU-1 and TAU-2. Phenol content was very high in the genotype of rice bean.

Phenol content of stored legumes seed (*Vigna mungo, Vigna radiate*) was reported to be the most important and significant criteria responsible for the susceptibility and persistency of *Callosobruchus chinensis*. The phenol content > 0.92 mg/g of stored legume seeds drastically retarded the growth and development of *Callosobruchus chinensis* (Ghosal *et al.,* 2004).

5.2a.5 Tannic acid

Tannin is a condensed polyphenolic compound, which are widely distributed in plants especially in seed coat. They act as defense mechanisms in plants against insects, pathogens and herbivores. Janzen *et al.,* (1977) and Boughdad *et al.,* (1986) demonstrated that the tannins were highly toxic for bruchid larvae in cowpea. However the bruchids present high capacities for adaptation and are capable of detoxifying antinutritional factors as has been shown by Rosenthal *et al.* (1977).

Tannic acid was found to be highest in rice bean followed by black gram and green gram. Interestingly these pulses follow the same order of merit in respect of resistance to bruchid. In green gram, the tannic acid was high in the resistant genotype TARM-18 followed by PM-2 and PM-9339. It was least in susceptible genotype Kopergaon. Similar is the case of black gram in which maximum tannic acid content was observed in the resistant genotype PU-9503-8 and PU-9205-11-3, where as in rice bean almost all the genotypes have high tannic acid content.

5.2a.6 Peroxidase and polyphenol oxidase activity

Peroxidase and polyphenol oxidase are known to accelerate the oxidation of phenolic compounds which intern are responsible for resistance against biotic stresses. The peroxidase acitivty of rice bean genotypes was significantly higher than black gram and green gram. Where as the polyphenol oxidase activity is comparatively lower in rice bean than green gram and black gram. The higher activity of peroxidase might be responsible for higher accumulation of phenol oxidation products which inturn can impart resistance. A positive correlation between peroxidase activity and resistance to injury and diseases as well as better storability has been reported. (Aluko and Oghodu, 1986, Karla *et al.,* 1986).

5.2b Correlation coefficient among the biochemical parameters and resistant seed to bruchid

The bruchid resistance is related to some class of antinutritionals. The importances of each biochemical mechanisms the activities of tested inhibitor were submitted to correlation analysis.

In green gram, most of the biochemical parameters associated with the resistance to bruchid were found to be strong positively associated with resistant seed except protein content and peroxidase activity. Protein content and peroxidase activity had highly significant negative correlation with resistant seed. Where as alpha amylase inhibitor activity, trypsin inhibitor activity, tannic acid and total phenol have significant positive association with resistant seed.

Alpha amylase inhibitor activity has highly significant positive correlation with trypsin inhibitor activity, tannic acid and total phenol, where as highly significant activity with protein and peroxidase activity. Highly significant positive correlation of trypsin inhibitor activity was observed with total phenol and tannic acid. On the other hand trypin inhibitor activity has highly significant negative association with protein (%) and peroxidase activity. Protein (%) and peroxidase activity had significant positive correlation, where as protein (%) had highly significant negative correlation with tannic acid and total phenol. Tannic acid and total phenol had highly significant positive correlation.

The extent of role of dependent characters on the independent characters association further clarified through path analysis.

The correlation between alpha amylase inhibitor activity and resistant seed was highly significant positive (0.566) which is due to very high positive indirect effect via. trypsin inhibitor activity, peroxidase activity and protein (%).

Trypsin inhibitor activity had very high positive direct effect (0.494) on resistant seeds, which is responsible for very high level of highly significant positive correlation (0.866). The indirect effect through peroxidase activity, protein (%) and tannic acid has play major role in this association.

The negative direct effect of protein (%) and high negative indirect effect via. peroxidase activity was responsible for highly significant negative correlation (-0.687) between protein (%) and resistant seed.

The very high level of correlation between tannic acid and resistant seed (0.777) is due to positive direct effect of tannic acid (0.065) and very high positive indirect effect via. trypsin inhibitor activity, peroxidase activity and protein (%).

Positive correlation (0.848) of total phenol and resistant seed was mainly due to the high positive indirect effect via. trypsin inhibitor activity, peroxidase activity and protein (%).

Peroxidase activity had very high negative direct effect (-0.520) on resistant seed, which is responsible for highly significant negative correlation. The indirect effect through trypsin inhibitor activity, protein (%) and tannic acid have played major role in this association.

In black gram, the biochemical parameters of resistance to bruchid *viz.,* tannic acid and total phenol were positively correlated with resistant seed, whereas protein (%) has significant negative correlation with resistant seed. Alpha amylase inhibitor activity was highly significant positively correlated with trypsin inhibitor activity and total phenol. Trypsin inhibitor activity and total phenol had highly significant correlation. Tannic acid had highly significant positive correlation with total phenol.

Tannic acid had very high positive direct effect (0.466) on resistant seed, which is responsible for very high level of highly significant positive correlation (0.775). The substantial amount of positive indirect effect through total phenol, trypsin inhibitor activity, polyphenol oxidase activity, peroxidase activity and alpha amylase inhibitor activity are also important traits.

Positive direct effect of total phenol and positive indirect effect through trypsin inhibitor activity, tannic acid and alpha amylase inhibitor activity played major role in the correlation of these traits.

The negative indirect effect through alpha amylase activity, trypsin inhibitor activity, polyphenol oxidase activity, tannic acid, total phenol and peroxidase activity were responsible for negative correlation (-0.537) between protein (%) and resistant seed.

Significant correlations were found between the resistance degree and both trypsin inhibitor and alpha amylase inhibitor. These results suggested that the selection of the genotype characterized by high activities of both trypsin inhibitor and ∝-amylase inhibitor could be an effective way to reduce the storage pest attack. However a breeding programme directed to the selection of high level of these antimetabolites cannot be developed independently from the evaluation of the effects that such compound can have on human being. Of course, an appreciable reduction of inhibitor activities through domestic treatment (soaking, sprouting and cooking) before eating is an essential characteristic as has been reported by Piergiovanni, 1994).

Consequently if breeding for high trypsin inhibitor level does not constitute any problem, the selection for alpha amylase inhibitor should be addressed towards intermediate values of activity. Infect very high level of such inhibitor could be dangerous for human beings, while very low content are less effective to confer resistance.

Significant positive correlations between seed resistance to bruchid and trypsin inhibitor and tannin content were reported by Marconi et al; (1997).

5.3 Electrophoresis studies

Classical identification of genotypes and more so the germplasm diversity based on standard morphological markers has proved to be inadequate because of the wide spectrum of phenotypic variation and their interaction with environment. In such instances electrophoretic patterns of seed protein can be used effectively to decipher the similarities and differences between cultivars and genotypes. Protein markers have been successfully used for varietal identification for several crops. Isozymes also provide useful evidences in the study of variation within cultivars in terms of intensity of common bands and presence or absence of other bands.

In this study, seed proteins of green gram, analyzing seed protein fractions and studying protein polymorphism by SDS-PAGE characterized black gram and rice bean genotypes. This was supported by the analysis of peroxidase isozymes by polyacrylamide gel electrophoresis (PAGE). The result obtained in this study was discussed below.

5.3a Electrophoresis of total soluble protein

In the present study, the banding pattern in green gram, black gram and rice bean seed storage proteins represented in Fig. 14, Fig. 15 and Fig. 16. The banding pattern in green gram for seed protein shown in Fig. 15 revealed variation in the number of band and staining intensity of bands among the different green gram genotypes. The number of bands in each genotype ranged from 22-28 and a total of 30 bands were recognized in the material studied. All genotypes recorded 16 common bands 1, 3, 5 (weak), 2, 4, 6, 15, 17, 18, 23 and 31 (light), 7, 8, 19, 21 (medium) and 10 (dense). Some bands differed in their band intensities and Rm values, thus helpful in identifying them individually. Every genotype exhibited almost a unique banding pattern.

Genotype PM-2 showed 2 characteristics and weak bands 5 and 22. The genotypes PM-2 and PM-9339 discriminate from each other by the absence of band 5, 15 and 22 in PM-9339. Band number 11 (medium) was the characteristics band in PM-9339. Band numbers 13, 20 and 24 were present in all genotypes and absent in AKM-8802 being the characteristic because of this. TARM-18 had maximum number of dense bands. The presence of four continuous dense bands 7, 8, 9 and 11 were the characteristics features of TARM-18. TARM-18 showed bands 16 (weak) as specific band, which was not present in other genotype. Kopergaon was characterized by the presence of six continuous light bands 12, 13, 14, 15, 17 and 18 (Fig. 14).

The banding pattern of black gram seed protein represented in Fig. 15 reveals variation in the number and staining intensity bands among different black gram genotypes. The number of bands in each genotype ranged for 23-31 and a total of 37 bands were recognized in the material studied. All the genotypes recorded 10 common bands, band number 13 and 14 (Dense), band number 15 and 22 (light) and band number 6, 7, 8, 9, 10 and 21 (weak). However certain bands were differed which were helpful in identifying them individually. Every genotype exhibited almost a unique banding pattern. The qualitative polymorphism was sufficient to discriminate the genotype.

The genotype TAU-1 showed characteristic bands like band number 0.091 (weak), 0.15 (light), 0.18 (medium) and 0.67 (weak). The genotypes TAU-1 and TAU-2 were discriminated from each other by the absence of band number 1,3,16,19,20,23 and 27 in TAU-2 and band number 11 weak, 18 weak and 28 weak were the characteristic band of TAU-2. TPU-4 showed band number 19 light and band number 12 (medium) were the characteristics bands. PU-9503-8 had very specific bands, band number 29 (weak), which was not present in other genotypes. Band number 16 (weak) is also specific band for PU-9503-8. PU-9205-11-3 was characterized by the presence of five dense bands (band number 12, 13, 14, 26 and 37). Band number 35 and 36 (weak) were also specific bands for genotype PU-9205-11-3 (Fig. 15).

The banding pattern of rice bean seed protein represented in Fig. 16 reveals variation in the number and staining intensity of bands among different rice bean genotypes. The number of bands in each genotype ranged form 24-31 and a total of 35 bands were recognized in the material studied. All the genotypes recorded 9 bands common, band number 10 (dense), band number 8 and 9 medium, band number 21 and 26 (light) and band number 1, 3, 12 and 24 (weak). Certain bands however differed were helpful in identifying the individuals. Every genotype exhibited almost a unique banding pattern. The qualitative polymorphism was sufficient to discriminate the genotype. RBL-50 was characterized by present of band number 30, 31 and 32 (medium), RBL-99 showed band number 11 (dense), as specific band not present in other genotype. LRB-199 has characterized by the presence of band number 31, 32 and 33 and each of them was medium. LRB-202 characterized by the presence of band number 7 and 20 and each of them was light. Presence of continuous weak band (i.e. band number 22, 23, 24 and 25) was the characteristic feature of this genotype.

5.3b Electrophoresis of peroxidase isozyme

The banding pattern of green gram peroxidase isozyme represented in Fig. 17 reveals variation in number and staining intensity of bands among different green gram genotypes. The number of bands in each genotype ranged from 5-8 and a total of 8 bands were recognized in the material studied. All the genotypes recorded two common bands, band number 1 (medium) and band number 8 (weak).

The genotype PM-2 was characterized by presence of band number 2 (light) and 7 (light). PM-9339 showed band number 2 (dense) the characteristic band of this genotype band number 3 (light) is also characteristic band. AKM-8802 was characterized by the presence of four continuous medium bands, band number 1, 2, 4, and 7. TARM-18 was characterised band number 4 (light).

Kopergaon was characterized by presence of band number 3, 5 and 6 each of them was light.

The banding pattern of black gram peroxidase isozyme represented in Fig. 18 reveals variation in number and staining intensity of bands among different black gram genotypes. The number of bands in each genotype ranged from 7-8 and a total of 8 bands were recognized in the material studied. All the genotypes recorded one common band i.e. band number 1 (medium) except PU-9205-11-3 (dense).

TAU-1 was characterized by presence of two continuous dense bands, band number 4 and 5 and three continuous medium bands number 1, 2, and 3. Band number 6 (medium) and 8 light are also the characteristic band of these genotypes. The genotype TAU-2 showed two continuous medium band (i.e. band number 4 and 5) and two continuous light band (i.e. band no. 2 and 3) were the characteristic bands of TAU-2 genotype. Four continuous light bands, (band no. 2,3,4 and 5) were the characteristic bands of TPU-4 genotype. PU-9503-8 showed four continuous medium bands, (band no. 1,2,3 and 4), which were the characteristic bands of PU-9503-8. PU-9205-11-3 was characterized by the presence of band number 1 and band number 4 each of them are dense. Band number 5 was also the characteristic band of PU-9205-11-3.

The banding pattern of rice bean peroxidase isozyme represented in Fig. 19 revealed variation in number and staining intensity of bands among different rice bean genotypes. The number of bands in each genotype ranged from 5-8 and total of 8 bands were recognized in the material studied. All the genotypes recorded 4 common bands, band number 2, 4, 6 and 7.

RBL-36 had one characteristic band, (band number 6 medium), RBL-50 showed the characteristic band 8 (light). RBL-99 was characterized by the presence of specific bands, band number

5 (light), band number 3 (light) and band number 8 (weak). LRB-199 showed characteristic band number 4 (light), LRB-202 had three characteristics bands, band number 1 (light), band number 3 (light) and band number 1 (light).

5.4 Seed mycoflora

Seed play a vital role for the healthy production of a crop. They are known to carry pathogen, which cause heavy yield losses. Green gram and black gram was the two most important pulse crop widely grown in Maharashtra state. The seeds of these pulses were known to harbour several species of fungi (Agrawal *et al.,* 1972; Singh and Chohan 1973, Suhag, 1978, Saxena and Sinha, 1979, Rath and Mishra, 1986).

The presently cultivated genotypes of green gram and black gram were shattering type, since the pods mature and shatter during rainy season. They are exposed to rain/high humidity providing opportunity for mycoflora invasion.

The seven fungi *viz., Alternaria alternate, Fusarium moniliforme, Aspergillus* sp., *Penicillium* sp., *Rhizopus stolonifer, Macrophomina phaseolina* and *Curvularia lunata* were detected on green gram genotypes. Maximum incidence of all these fungi were recorded on the genotype PM-2 followed by AKM-8802 and PM-9339 where as least incidence was observed on TARM-18. These results are more or less in agreement with Banghel *et al.,* (1985), Patil *et al.,* (1990), Teggi and Hiremath (1990), Ahmed (1993) and Raut and Ahire (1998). Benghel *et al.,* (1985) reported the highest count of *Fusarium monoliforme* to seeds of *Vigna radiata.* Patil *et al.,* (1990) observed the presence *Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Curvularia lunata, Fusarium monoliforme, Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia loataticola* on green gram seeds. Teggi and Hiremath (1990) reported

Alternaria alternata, Cladosporium fulvam, Fusarium monoliforme, Aspergillus flavus, Aspergillus niger and *Trichothecium* were predominant on green gram seed, Ahmed (1993) noticed association of *Alternaria* sp., *Cladosporium* sp, *Fusarium* sp., *Curvularia* sp. with mung bean seeds. Raut and Ahire (1998) reported *Aspergillus* sp., *Curvularia lunata, Fusarium* sp. and *Macrophomina phaseolina* on green gram seeds.

In the case of back gram, the seed borne fungi associated were *Alternaria alternate, Fusairum monoliforme, Aspergillus* sp., *Penicillum* sp., *Rhizopus stolonifer, Macrophomina phasecolina.* Maximum incidence of fungi was detected on the genotypes TAU-2 followed by PU-9503-8 on the other hand least incidence was observed in TPU-4. These results are more or less in agreement with Bilgrami *et al.,* (1976), Saxena and Sinha (1977), Singh *et al.,* (1977) and Thakur *et al.,* (1990). Bilgrami *et al.,* (1976) reported the highest frequency of *Aspergilllum flavus* associated with udid bean. Saxena and Sinha (1977) reported that *Alternaria alternata, Fusarium monoliforme, Macrophomina phaseolina, Aspergillus niger* were associated with black gram seed. Singh *et al.,* (1977) reported that *Aspergillus niger, Curvularia* sp., *Fusarium equiseti, Fusarium oxysporum, Penicillium* sp., and *Phoma glomerata* were associated with black gram seed. Thakur (1990) reported that *Aspergillus* sp., *Botrytis cinerea, Penicillium* sp., *Fusarium* sp., and *Macrophomina phaseolina* were predominant on black gram seed.

In rice bean only the fungi detected were *Alternaria alternate, Fusarium monoliformae, Aspergillus* sp., *Penicillium* sp. and *Rhizopus stolonifer.* Maximum incidence of fungi was recorded in genotypes RBL-36 and LRB-199. No one has reported the association of seed borne fungi in case of rice bean.

The seed borne fungi *viz., Alternaria alternate, Fusarium monoliforme, Aspergillus* sp., *Penicillium* sp. and *Rhizopus* *stolonifer* were common in green gram, black gram and rice bean. Genotype TARM-18 in green gram, TPU-4 in black gram and RBL-50 in rice bean were found to be the least attacked by the fungi.

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSIONS

Pulses during storage are mostly affected by bruchids, resulting in substantial economic and nutritional losses. The present investigation entitled "Biochemical basis of resistance to stored grain pest (*Callosobruchus chinensis*) of different *Vigna* species" was designed and executed to evaluate the level of bruchid resistance of different genotypes of green gram, black gram and rice bean and to know biochemical factors responsible for imparting resistance to bruchid with the following objectives

1. To evaluate the level of resistance against pulse beetle (*Callosobruchus chinensis*) in seeds of green gram (*Vigna radiata*), black gram (*Vigna mungo*) and rice bean (Vigna *umbellata*).
2. To study ∝-amylase and trypsin inhibitor activities.
3. To study the protein banding of green gram, black gram and rice bean genotypes
4. To study the seed mycoflora associated with green gram, black gram and rice bean.

For the above studies three species of *Vigna radiata* (PM-2, PM-9339, TARM-18 and Kopergaon), *Vigna mungo* (TAU-1, TAU-2, TPU-4, PU-9503-8 and PU-9205-11-3) and *Vigna umbellata* (RBL-36, RBL-50, RBL-99, LRB-199 and LRB-202) were taken.

The research findings of the present investigation are summarized as under.

6.1 Evaluation of levels of resistance to the bruchid

1. Green gram was found to be most preferred host for oviposition followed by black gram and rice bean. The green gram genotypes, Kopergaon and AKM-8802 having lustrous green colour and bold seeds that favoured an insect for oviposition. The black gram genotypes, TAU-1 and TAU-2 recorded the highest oviposition. In rice bean, maximum oviposition was observed in a genotype LRB-202 followed by RBL-36 and RBL-99.
2. Highest number of adult emergence, damaged seeds and loss in seed weight were recorded in genotypes of green gram as compared to black gram. However no adult emergence, damaged seeds and loss in seed weight were found in case of rice bean. The green gram and black gram genotypes, TARM-18 and PU-9205-11-3 had recorded minimum number of damaged seeds, loss in seed weight thus found to be relatively resistant to bruchid than rest of the genotypes tested.
3. Highest seed hardness was recorded in rice bean followed by black gram and the least was in green gram. However in green gram, the highest seed hardness was recorded for the genotype, TARM-18 and in black gram the genotype, PU-9205-11-3. No significant differences were recorded in seed hardness of rice bean genotypes.
4. Maximum reduction in germination percentage was noticed in green gram and black gram, whereas it was negligible in rice bean. This may be due to destruction of embryo as evident by adult emergence from respective species.
5. At species level, rice bean recorded the highest seed weight (5.35 g) followed by black gram (5.13 g) and green gram (3.79 g). In

green gram and black gram, more seed weight is related to more susceptibility to bruchid in all genotypes.

6. In green gram and black gram, most of the related parameters to bruchid resistance were found to be strongly and negatively associated with resistant seed except seed hardness.

10. Maximum amount of positive direct effect was shown by the parameter seed hardness with seed resistance to bruchid, thus indicating its major role in conferring reistance.

6.2 Biochemical parameters

1. ∝-Amylase inhibitor and trypsin inhibitor activities were found to be the highest in rice bean followed by black gram and green gram. In green gram and black gram, the ∝- amylase inhibitor and trypsin inhibitor activity were higher in the resistant genotypes TARM-18 and PU-9503-8 respectively.
2. The average protein content was high in green gram followed by rice bean and black gram. The protein content was positively associated with the susceptibility to bruchid in green gram and black gram.
3. Highest content of phenol and tannic acid were recorded in rice bean followed by black gram and green gram and the lowest were in the highly susceptible genotype to bruchid.
4. The peroxidase activities in rice bean genotypes were significantly higher than black gram and green gram. The polyphenol oxidase activity was comparatively lower in rice bean than green gram and black gram.
5. Most of the biochemical parameters like ∝-amylase inhibitor potential, trypsin inhibitor potential, tannic acid and total phenol except protein content and peroxidase activity were

strong and positively associated with the seed resistant to bruchid in green gram and black gram.

6. ∝- amylase inhibitor potential, trypsin inhibitor potential, tannic acid and total phenol had maximum positive direct effect, indicating that their important role in seed resistance to bruchid.

6.3 Electrophoresis studies

6.3.1 Electrophoresis of total seed protein

Electrophoresis studies revealed the total number of bands for total seed protein 30, 37 and 37. Whereas range for them in different genotypes of green gram, black gram and rice bean were 22-28, 23-31 and 26-31 respectively.

In green gram, PM-2 showed two specific but weak bands, 9 and 22; where as PM-9339 had one specific band number 11 (medium). AKM-8802 was characterized by absence of band numbers, 13, 20 and 21. Genotype, TARM-18 was characterized by four continuous dense band 7, 8, 9 and 11, and band number 16 a specific but weak band. Kopergaon showed 6 continuous but characteristic light bands, 12, 13, 14, 15, 17 and 18.

In black gram, TAU-1 was characterized by the presence of band number, 1 (weak), 3 (light), 27 (weak) and 5 (medium). TAU-2 showed three specific but weak bands 11, 18 and 28. TPU-4 showed 2 characteristic bands, 12 (medium) and 19 (light). PU-9503-8 showed 2 specific but weak bands, 16 and 29. PU-9205-11-3 was characterized by the presence of band numbers 12, 13, 14, 26 and 37 all dense and 35 and 36 both specific bands.

In rice bean, RBL-50 was characterized by the presence of band numbers 30, 31 and 32 all medium. RBL-99 showed one specific but dense band 11. LRB-199 had 3 specific bands, 31, 32 and 33 all medium. LRB-202 was characterized by 6 specific but weak bands, 7, 20, 22, 23, 24 and 25.

6.3.2 Electrophoresis of peroxidase isozyme

Electrophoresis of peroxidase isozyme revealed the banding pattern in different genotypes that ranged between 5-8, 7-8 and 7-8 and a total of eight bands each respectively were recognized.

In green gram, PM-2 was characterized by presence of band number, 2 and 7 (each light), while PM-9339 had two characterized bands, 2 (dense) and 3 (light). AKM-8802 showed 4 specific bands, 1, 2, 4 and 7 (each medium). The genotype TARM-18 was characterized by band number 4 (light). Kopergaon showed presence of 3 specific bands, band number 5, 6 and 3 (each light).

In black gram, TAU-1 was characterized by band number 4 and 5 (each dense) and band numbers 1,2 and 3 (each medium). TAU-2 showed four specific bands, 2 and 3 (light) and 4 and 5 (medium). TPU-4 was characterized by presence of 4 continuous light bands 2,3,4 and 5. Genotype PU-9503-8 showed four continuous bands, 1,2,3 and 4 as characteristic band. PU-9205-11-3 showed 3 specific bands, 1 and 4 (Dense) and 5 (medium).

In rice bean, RBL-36 showed band number 8 (medium), where as, RBL-50 showed band number 8 (light) as characteristics band. RBL-99 showed 3 specific bands, number 5 and 3 (each light) and number 8 (weak). Band number 4 (light) was specific band of LRB-199. Genotype, LRB-202 was characterized by presence of band number 1 and 8 (light).

6.4 Seed mycoflora study

The seed borne fungi like *Alternaria alternata, Fusarium monoliforme, Aspergillus* sp., *Penicillium* sp. and *Rhizopus stolonifer* were common in green gram, black gram and rice bean.

In green gram, the lowest incidence of seed borne fungi was observed in TARM-18. Whereas highest was recorded in PM-2.

In black gram, genotype TPU-4 showed the minimum incidence of seed borne fungi as against TAU-2, which showed the highest in incidence.

In rice bean, the lowest incidence of seed borne fungi was observed in RBL-50 where as it was highest in RBL-36.

Conclusions

1. The green gram (TARM-18) and black gram (PU 9205-11-3) genotypes were found to be the least susceptible to bruchid.
2. All the five genotypes of rice bean were found to be resistant to bruchid.
3. Seed weight and seed hardness were played an important role in bruchid resistant. Seed weight governed by seed size, might have influenced the amount of surface available to bruchid for Oviposition. The seed hardness led to the failure of bruchid larvae to enter the seed.
4. Biochemical parameters, α-amylase inhibitor, trypsin inhibitor, total phenol and tannic acid had positive correlations with percent resistant seed, indicating that their role in the resistance to bruchid.
5. Electrophoresis studies for seed protein and peroxidase isozyme in green gram, black gram and rice bean genotypes under study were unique. Genotypic differences were

evidenced by the presence or absence of particular band in electrophoregrams. The qualitative and quantitative variations were observed in the banding pattern in green gram, black gram and rice bean. Genetic variation for seed protein and peroxidase isozyme was noticed in different genotype and might be useful as probe to mark genotype. TARM-18 in green gram, PU-9205-11-3 in black gram and RBL-99 in rice bean due to different banding patterns from other four genotypes.

7. The seed borne fungi like *Alternaria alternara, Fusarium monoliforme, Aspergilus* sp., *Penicillium* sp. and *Rhizopus stolonifer* were common in green gram, black gram and rice bean. The lowest incidence of seed borne fungi was recorded in green gram (TARM 18), black gram (TPU 4) and rice bean (RBL 50).

Table 40 : Evaluation of various genotypes of green gram against the parameters responsible for resistance to pest and diseases.

Sr. No.	Parameters For Resistance	Green gram Genotypes				
		PM-2	PM-9339	AKM-8802	TARM-18	Koperga
1.	**Oviposition**	40.75	47.00	52.75	37.50	56.50
2.	**Number of adult emerged**	261.75	281.75	300.00	**87.00**	429.5
3.	**Loss in seed weight**	22.00	23.68	25.10	**11.26**	28.15
4.	**Reduction in germination**	29.15 (31.83)	39.36 (38.83)	43.92 (41.48)	**7.92 (16.26)**	56.88 (48.96
5.	**100 seed weight**	3.30	3.66	4.15	**3.19**	4.66
6.	**Seed Hardness**	4.22	4.30	3.82	**4.80**	3.40
7.	**∝-Amylase inhibitor**	15.50	13.45	13.15	16.00	12.67
8.	**Trypsin inhibitor**	74.00	72.00	56.50	**93.20**	53.00
9.	**Protein content**	20.77	21.32	26.13	19.79	28.54
10.	**Total phenol**	32.50	37.35	25.00	**57.50**	22.50
11.	**Tannic acid**	1.10	1.06	0.96	**1.28**	0.74
12.	**Incidence of seed borne fungi**	19	15	18	**9**	10

Table 41 : Evaluation of various genotypes of black gram against the parameters responsible for resistance to pest and diseases.

Sr. No.	Parameters For Resistance	Black gram Genotypes				
		TAU-1	TAU-2	TPU-4	PU-9503-8	PU-92 11-
1.	**Oviposition**	42.50	50.25	41.75	40.00	38.
2.	**Number of adult emerged**	304.75	395.00	239.75	194.50	**154.**
3.	**Loss in seed weight**	19.20	27.00	18.20	12.10	**11.**
4.	**Reduction in germination**	28.57 (32.25)	35.96 (35.92)	26.52 (30.94)	19.84 (26.38)	**16. (24.**
5.	**100 seed weight**	5.13	5.76	5.50	4.72	4.5
6.	**Seed Hardness**	5.10	5.02	5.32	5.62	**5.7**
7.	**∝-Amylase inhibitor**	24.52	22.60	25.62	27.17	26.
8.	**Trypsin inhibitor**	64.50	59.50	67.85	68.75	**70.**
9.	**Protein content**	21.54	24.93	19.79	17.38	16.
10.	**Total phenol**	35.00	32.56	42.50	47.50	**55.**
11.	**Tannic acid**	1.21	1.02	1.11	1.45	1.3
12.	**Incidence of seed borne fungi**	18	24	16	22	1

Table 42 : Evaluation of various genotypes of rice bean against the parameters responsible for resistance to pest and diseases.

Sr. No.	Parameters For Resistance	Rice bean Genotypes				
		RBL-36	RBL-50	RBL-99	LRB-199	LRB-20
1.	Oviposition	38.00	35	37	32	42
2.	Number of adult emerged	--	--	---	--	--
3.	Loss in seed weight	--	--	--	--	--
4.	Reduction in germination	1.33 (6.70)	0.80 (5.47)	0.79 (5.59)	0.53 (4.99)	1.06 (6.05)
5.	100 seed weight					
6.	Seed Hardness	6.30	6.50	6.25	6.12	6.47
7.	α-Amylase inhibitor	28.37	25.00	25.05	25.02	24.51
8.	Trypsin inhibitor	71.50	78.25	78.50	82.50	79.75
9.	Protein content	21.43	22.09	20.34	21.65	23.07
10.	Total phenol	57.50	65.00	60.00	52.50	50.00
11.	Tannic acid	1.70	1.75	1.98	1.90	1.95
12.	Incidence of seed borne fungi	26	12	19	21	19

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