

कैलोसोब्रुकस प्रजातियों के विरुद्ध मूँगबीन, *वीग्ना रेडिएटा* में प्रतिरोधिता
— कारक

**“Factors for resistance in mung bean, *Vigna radiata*
against *Callosobruchus* spp.”**

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**Division of Entomology
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New Delhi-110012
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**Factors for resistance in mung bean, *Vigna radiata* against
Callosobruchus spp.**

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This is to certify that the thesis entitled “**Factors for resistance in mung bean, *Vigna radiata* against *Callosobruchus* spp.**” submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in ENTOMOLOGY** is a faithful record of *bona fide* research work carried out by **Ms. SOUMIA P S**, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

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“When a person is devoted to something with complete faith, I unify his faith in that. Then, when his faith is completely unified, he gains the object of his devotion.”

-Bhagavad Gita

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Grain legumes/ pulses are the second most important group of crops after cereals, particularly in developing countries where the availability and consumption of animal protein is rather low due to various socio-economic constraints. Pulses are excellent and cheapest source of dietary proteins (25-40%), carbohydrates (50-60%), fat, minerals, vitamins and certain amino acids like lysine and tryptophan (Lambrides and Godwin, 2007). This makes them a good supplement to the cereal based diets; which are usually rich in carbohydrates but low in proteins. According to recommendation of the World Health Organization, a person must consume about 80 g of pulses per day in India. Pulses are grown on 68.3 million hectares and furnish 57.5 million tonnes to the world's food basket. The major pulse crops grown in India are chickpea, pigeonpea, mung bean, urdbean, cowpea, lathyrus, horsegram, lentil and peas. India is the world's largest producer of pulses with a total production of about 14.7 million tonnes from about 23.4 million hectares, but the average productivity (550-625 Kg/ha) is quite low (Economic Survey, 2011). Unless the country witnesses a major technological advance in pulse cultivation to meet the domestic demand, imports of pulses seem inevitable. The slow growth in pulse production compared to burgeoning population had led to progressive decline in per capita availability of pulses from 60 gm/day/person in 1951 to 31 gm/day/person in 2008.

Among the various pulses, mung bean *Vigna radiata* (L.) Wilczek also known as green gram, moong dal, chickasono pea, golden gram, belonging to the genus *Vigna*, sub genus *Ceratotropis* is one of the important short duration grain legumes grown widely in South and South East Asia. Globally, mung bean is grown in 5.5 million hectares with 2.5 to 3.0 million metric tonnes of production. Of these, about 1.75 million tonnes of pulses are produced in Southeast Asia from 5.5 million hectares. In India, green gram stands third and contributes eleven per cent of the total pulse production from an area of about 3.42 million hectares with a production of 1.61 million tonnes and with an average productivity of 512 kg/ha (<http://www.indiastat.com>).

Mung bean plants are erect with branches carrying pods in clusters near the top of the plant. Pods contain 8 - 15 seed grains. The grains are green or brown coloured and globose in shape with a flat hilum. Young pods, seeds and sprouts are considered as a nutritional and healthy food. Pulses are rich source of protein and amino acids thus, it serve as a valuable and healthy food source for human consumption. It can be used in many forms from salads to soup or just as a vegetable. Sprouted seeds of mung bean contain pure vitamins A, B, C and E, and minerals such as iron, calcium and phosphorus. Green gram contains approximately 23.86-27% protein, 1.0-1.5% fat, 60-62% carbohydrates, 16.3% fiber, 6.60% total sugars, 4.5- 5.5% ash and 9.05% water (El-Adawy, 2003). The sprouts are free of cholesterol but rich in fibers that are easily digestible. The biological value improves to a great extent, when cereals are combined with green gram as there is a complementary relationship of essential amino acids. Thus the food from the legumes is considered as an alternative for tackling malnutrition.

Besides its nutritive value, the crop is highly valued as a warm season annual legume. Due to short duration (75 - 90 days) and low water requirement, it fits easily into crop rotations with cereals like wheat and rice. It grows well under most adverse arid and semiarid conditions. Moreover, it can improve soil physical properties by fixing atmospheric nitrogen (222 kg/ha) (Murakami *et al.*, 1991) through symbiotic relationship with *Rhizobium* in the root nodule of the crop. Although India's share is 35.2% of the area and 27.6% of the global production, full yield potential of the crop is not realized due to number of constraints. Among which, post harvest losses especially due to insect-pests encountered during the production, storage and marketing are more crucial.

In mung bean, storage insects alone cause post-harvest losses of 6 to 7.5% per annum (Chelladuari *et al.*, 2012). In storage, totally 25 species of the insects have been recorded on pulses (Prabhakar, 1979). Of these, coleopteran pest cause major damage to stored grains and its products. In India, 117 species of bruchids belonging to 11 genera have been recorded infesting different pulses (Arora, 1977). Among them, the

genus *Callosobruchus* contains several cosmopolitan, tropical and subtropical pests which attack grain legumes during both pre and post harvest stages.

In India *Callosobruchus chinensis* (Linnaeus, 1758), *Callosobruchus maculatus* (Fabricius, 1775) and *Callosobruchus analis* (Fabricius, 1775) belonging to family Bruchidae are predominant pest species of the genera (Dias, 1986). These are most devastating stored pests that infest mung bean and have great economic importance (Southgate, 1979).

The primary infestation occurs in the field, where female bruchid lays eggs on mature pods in close proximity with the seeds. Later eggs hatch into larvae and subsequently penetrate the seed testa and feed on internal content. In field, pods and grains are damaged by *Callosobruchus* spp. as reported by Raina (1971) and by Gujar and Yadav (1978). Though, the field infestation by bruchids to pods and grains is only a minor problem, but when such infested seeds are harvested and stored, the developing larvae continue to feed and eventually emerge from the seeds as adults, and cause secondary infestation. These secondary infestations are more damaging and usually result in total destruction of a seed lot within few months rendering unfit for human consumption (Mahendaran and Mohan, 2002 and Bhalla *et al.*, 2006). Due to the secondary infestation the seed lots become warm causing quality loss by the mould growth (Rees, 2004), lost in seed weight and seed viability (Sarwar *et al.* 2012). Bruchid damage in general inflicts both quantitative and qualitative losses that result in overall weight loss, altered nutritional quality (Oke and Akintunde, 2013) and loss of seed viability. When left unattended they can cause up to 100 % loss (Dongre *et al.*, 1996; Akinkurolere *et al.*, 2006 and Sharma *et al.*, 2011).

In the current situation, prevention of losses in stored products due to insects is of paramount importance. Although agricultural chemicals stand as “Miracle Weapons” in the front line of defence, stored grains are not free from problems. Chemicals can control bruchids, but sole reliance on an array of chemicals has led to several problems associated with pesticide residues (Somta *et al.*, 2008). The complexities of chemical pest control involving pest resistance to insecticides, environmental residues, environmental pollution etc. are also to be taken into account

while planning pest management strategies. Due to the limited availability of other control methods, use of bruchid-resistant cultivars has considerable potential for minimizing the losses in storage. To offer an alternative to plant breeders and entomologists, sources of resistance must be identified. The detection of the varietal susceptibility of seeds could allow the selection of natural resources for the management of these pests through the genetic improvement of resistance in pulses (Tarver *et al.*, 2006 and Olakojo *et al.*, 2007).

Host plant resistance (HPR) is the key to integrated pest management as this approach is eco-friendly, provides cumulative protection against insect pest and is often compatible with other pest management methods (Kogan, 1998). Main stay of HPR is crop plants own inherent ability to restrict, retard or overcome pest infestations (Kumar, 1984), thereby improving the yield and/or quality of the harvestable crop or stored product. From the farmer's perspective, the use of resistant cultivars may represent one of the simplest and most convenient methods of bruchid control. Three approaches (Painter, 1951) viz., antibiosis, antixenosis (non-preference) and tolerance are pursued by plant breeders to develop resistant cultivars. Among these three, the approach of tolerance possibly will not be applicable to storage pests because once insect pests attack the stored grains, it is irreversible. So, both antibiosis and antixenosis are main resistance source against storage insects. Host plant resistance can be categorised as either vertical or horizontal resistance. Vertical resistance is controlled by various combinations of major genes, which are highly heritable. On the other hand, horizontal resistance is polygenically inherited and does not involve a gene-for-gene relationship with the insect (Dent, 2000). Understanding the genetics of bruchid resistance and its mode of inheritance is necessary in order to develop an efficient and effective breeding programme.

Co-evolution between bruchids and pulses has rendered the former with enormous defence mechanisms so that any resistant variety released would become susceptible in a short span of time (Oghiakhe, 1995). So, there is a need to identify new resistant varieties that can effectively check the bruchid. A clear understanding of morphological and physiological causes of resistance will help in reducing the post

harvest losses (Somta *et al.*, 2006). Biochemical compounds in seeds confer resistance to bruchids in mung bean, but the basis of the resistance is complex and ambiguous. The resistance in *Vigna* species is either a result of a single component or a combination of chemicals. Some of the chemicals are novel and unique. Moreover, all of this information will help in identifying efficient management practices for reducing the pest infestation.

Plants contain certain anti-nutritional chemicals that may be in toxic levels to insects but are harmless to man as it gets degraded by cooking. Presence of such anti-nutritional chemicals are well known in legumes. According to Edwards and Singh (2006), legumes as a group employ an array of direct and indirect defenses including morphological barriers, secondary metabolites and anti-nutritional compounds. These chemicals cause adverse effect on larval growth. But such anti-nutritional compounds have already been eliminated or reduced in commercial varieties. So far proteins such as lectin, trypsin inhibitor, amylase inhibitor and high molecular weight heteropolysaccharides have been reported as bruchid resistance factor in food legumes.

Although mung beans are an important pulse crop cultivated by the Indian farmers, very little research work on breeding for resistance to storage pests has been done so far. Most studies on grain legume resistance to different insect pests have been undertaken at various international institutes *viz.*, International Institute for Tropical Agriculture (IITA), Nigeria; International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad; Asian Vegetable Research and Development Center (AVRDC), Taiwan; and International Center of Tropical Agriculture (CIAT), Columbia and International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria. However, concerted research efforts by the Indian Institute of Pulses Research (IIPR) and international collaborative partners have led to the development of superior varieties, which are currently under commercial production in India. A brief assessment of the impact of these new varieties has shown that household food security and income levels have improved to some extent. However, many small-hold farmers complain about storage pest, a main post-harvest problem which needs to be tackled.

To date, entomological studies conducted in India have concentrated more on field than storage pests and previous breeding programmes have not focused on developing bruchid resistant lines. This has resulted in the development of superior varieties with high levels of resistance or tolerance of other biotic and abiotic constraints, but is more vulnerable to bruchid invasion in storage. Genetic diversity is an important factor for crop improvement programme and its nature and degree of variability would be useful for selecting desirable parents from available germplasm for a successful breeding programme. Despite the fact that many workers have conducted varietal screening for bruchid resistance, there is further need to identify resistant varieties, as bruchids have inherent ability to tackle the resistance mechanisms.

Through this research, it was hoped that mung bean cultivars with improved levels of resistance to bruchids would be identified for use in breeding resistance and the genetic inheritance of this resistance would be determined to enable the development of an effective breeding strategy. Subsequently, resistant varieties, incorporating traits preferred by farmers, could be developed and deployed to farmers. This will contribute significantly to the national goal of improving food and nutritional security in India.

With these observations in view, investigations were carried out with the following objectives:

- 1) Evaluation of selected mung bean, *V. radiata* genotypes for resistance against three species of pulse beetles viz., *C. maculatus*, *C. chinensis* and *C. analis*
- 2) Identification of physical and biochemical factors in susceptible and resistant varieties of *V. radiata* and to study their heritability
- 3) Confirmation of bruchid resistance in mung bean genotypes using artificial seed system
- 4) Finger printing of bruchid resistant mung bean genotypes



Plate 1.1 Damaged seeds of green gram by pulse beetle

Legumes are severely affected by storage insect pests predominantly in the Tropics and Sub-tropics where temperatures and relative humidity are high that cause both qualitative and quantitative loss. Unlike field pests, stored grain pests are more serious not only due to the magnitude of loss they cause but also by the irreversibility of the damage they cause (Keneni *et al.*, 2011).

The literature relevant to the present study has been reviewed under the following headings:

- Role of pulses in food security and associated constraints in pulse production
- Post harvest losses in pulses
- Pulse beetle – *Callosobruchus Spp.* biology
- Different tactics to manage pulse beetle
- Mechanism of resistance against pulse beetles
- Bruchid resistance testing methods
- Screening of mung bean against pulse beetle
- Resistance factors to storage pests
- Artificial seed system
- Genetic diversity analysis in green gram

2.1 Role of pulses in food security and associated constraints in pulse production

Pulses containing 25 per cent protein, which is approximately thrice that of cereals, play a fundamental role in the food security of most developing countries. Pulses are also a low-fat, fibre rich source of protein and an indispensable part of traditional food baskets. It is included in all dietary guidelines especially in the World Food Programme (WFP) to eliminate ‘protein calorie’ malnutrition (Kakati *et al.*, 2010). Hence, a significant part of human population relies on pulses that can be complemented with cereals. Moreover, of the total pulse production, about 60% is deployed mainly for human consumption with more utilization in low income countries.

On the other hand, the world has entered an era of “peak food” production, with almost stagnant production of 11 and 14 million tonnes over the last two decades. One third of world’s population that resides in India will face serious food shortage as the burgeoning population would reach 1.68 billion by 2030. Although India is the world’s largest producer of pulses, yet 3.5 million tonnes of pulses were being imported during 2009-10. Consequently, it has been projected that India may require 32 million tonnes by 2030 to meet its domestic demand (<http://www.icar.org.in/files/ICAR-Vision-2030.pdf>).

Most pulses producing countries are still under developed. It is very difficult to transfer new technologies to these areas, which become a major constraint on pulses production. Leading pulse producing countries such as Bangladesh, India, Iran, Myanmar, Malaysia, Mexico, Turkey and Syria also face serious abiotic (soil fertility, drought, hurricanes, flood incidence, and unpredictable weather) and biotic problems (pests and diseases). Besides, it is evident that in near future, pulse cultivation in these areas would face more problems thereby leading to inconsistency in production, import and export. Even with the best efforts, pulses production is stagnant as pulses are grown mainly as residual crops on marginal lands. Furthermore, under changing climatic condition yield loss by diseases and pests outbreaks in pulses will increase (Srivastava, 2002). Among the various biotic problem that invade pulse production, pests and diseases alone might be responsible for multi-million rupees economic losses. It has been predicted that 2-2.4 million tonnes of pulses are ravaged by pests with monetary loss of Rs. 6,000 crores annually (Reddy, 2010). In India, over 200 insect pests have been reported to attack various pulses both in the field and storage (CABI, 2007). However, the present study exclusively focuses on *Callosobruchus* spp. which is responsible for serious post-harvest loss in pulses in during storage.

2.2 Post harvest losses in pulses

Post-harvest losses have posed a serious question to food security as it causes 9.5% loss in total pulse production. Among the different losses in post-harvest operation, storage holds a major share (7.5%) followed by processing (1%), transport (0.5%) and threshing (0.5%). Deshpande and Singh (2001) reported that pulses (5%)

were most vulnerable to storage insect pests in comparison to cereals like maize (3.5%), wheat (2.5%) and paddy (2%). Insect pests readily attack pulses due to the presence of high protein content. Controlling these post-harvest losses is obligatory to reduce the import quantity of our nation. According to FAO's prediction approximately 70% of the farm products are stockpiled by farmers for various purposes like their own consumption, animal feed, seed and other purposes. Indian situation, farmers generally use locally designed storage structures for bulk store of their commodities which in most cases are not insect or moisture proof (Das *et al.*, 2013). Pulse beetle, being cosmopolitan can thrive well in a variety of pulses and hence it is very difficult to control this obnoxious pest. It has been documented that pulse beetle can cause up to 30% loss within a short period of time (Raja and John, 2008). They inflict both qualitative and quantitative loss to legumes, making them unfit for sowing (loss viability) or for food or feed (due to spoilage and bad smell) (Aslam *et al.*, 2006; Haile, *et al.*, 2006).

2.3. Pulse beetle – *Callosobruchus* spp. biology

The systematic placement of pulse beetles (Kergoat *et al.*, 2007):

Family : Chrysomeloidae

Subfamily : Bruchinae

Genera : *Callosobruchus*

Three important species: *Callosobruchus maculatus*, *Callosobruchus analis* and *Callosobruchus chinensis* (Bushara *et al.*, 1988; Kashiwaba, *et al.*, 2003)

Callosobruchus spp. is the most important field carry over storage pest of pulses such as chickpeas, lentils, green gram, broad beans, soybean (Desroches *et al.*, 1995; Bushara, 1988; Talekar, 1988; Kashiwaba, *et al.*, 2003) adzuki bean and cowpeas (Udayagiri and Wadhi 1989). Primary infestation starts in the field, where adult beetles deposit their eggs on matured pods which are later carried over to the storage where substantial losses may occur within three months (Singh and Jackai, 1985). Damage made by this pest mainly due to the feeding of the larvae inside the seed, which is important because of overall weight loss; altered nutritional, aesthetic quality and germination potency of the seed (Talekar, 1988). Bruchids in stored seed

are a major problem because of their ability to re-infest stored seed. A single beetle is able to cause 3.5% weight loss in cowpea seeds (Brooker, 1967).

Callosobruchus spp. originated in Africa and is distributed throughout the tropics and sub-tropics region of Asian and African countries (<http://www.cabi.org>). The spread of this species occurred with the movement of harvested legumes, either from the paddock into storage, or in contaminated seed within and between storage facilities (<http://www.padil.gov.au/pbt>).

There are number of *Callosobruchus* spp. found to be a pest for pulses. *C. maculatus*, *C. analis* and *C. chinensis* are the most dominant pest species to the pulses found in tropical and sub-tropical countries of Asia and Africa (<http://www.cabi.org>). Male and female beetles of *Callosobruchus* spp. are easily distinguished by their general appearance (Plate 2.6). Generally, females are stout and larger than males, but there is much variation. The most unique characteristic is the cuticular colour of the abdominal plate (pygidium) covering at the rear end. In some strains, females are black in colorations and males are in brown, but in others both sexes are brown (Southgate, 1958).

Adults are small grey, brown and reddish-brown beetles with distinctive patterns like spots near the mid-dorsal side of the elytra. Abdomen is exposed as the elytra do not cover the abdomen entirely, called as pygidium (Southgate, 1958). Adult beetles are short-lived which do not feed on stored produce. During this time the female lay egg and glued firmly to the seed coat. Eggs are small (0.75mm long) oval or spindle shaped glued either singly on to the grains or often inserted into the pod in close proximity to seeds. Fresh eggs are translucent smooth and shining which hatch into fleshy, curved, creamy white coloured grubs which penetrate into the seed and feeds internally. The egg period ranges from 8-9 days. The hatching occurs on 5-9 days after the oviposition (Howe and Currie, 1964); freshly emerged larva penetrates into the cotyledons and were completes their entire larval stage and pupate (Ahmed *et al.*, 1989). During the emergence the adults leaves the seed through a circular flaps on seed coat called operculum (Beck and Blumer, 2011).

In general *Callosobruchus* spp has an optimum developmental conditions about 23-35°C and 70 per cent RH, but may vary depending upon species and weather parameters (<http://www.cabi.org>). Of the suitable host and optimum development conditions (30° C and 70% RH), the minimum development period for *C. maculatus* is about 24 days and 22.3 days for *C. chinensis* (Raina, 1970).

2.3.2. a. Description of *Callosobruchus maculatus*

Callosobruchus maculatus is largest among the three species of pulse beetle. Both sexes have slightly serrate antennae (Mbata *et al.*, 1997). Female beetle has enlarged pygidium and is darkly colored on both sides whereas, in male beetle pygidium is smaller and lacks stripes.

Female beetles have a distinct large spot on each elytron whilst male is without distinct spots (Southgate, 1958). A pair of distinct inner and outer ridges is present on the ventral side of hind femur and at the apical end, each ridge bears a tooth. The inner tooth is triangular, and equal to or slightly longer than the outer tooth.

2.3.2.b. Description of *Callosobruchus analis*

Callosobruchus analis is intermediate in size and both sexes are reddish brown in colour and with lateral black spots on elytra surrounded by white setae. Female pygidium has a wide median testaceous (*ie.*, brownish yellow) strip or ‘V’ shaped area, whilst male with a narrow median testaceous strip (Southgate, 1958).

2.3.2.c. Description of *Callosobruchus chinensis*:

Callosobruchus chinensis is smallest of all three species. Female and male beetles exhibit antennal dimorphism. Female has pectinate antennae whereas; male has serrate type of antennae (Southgate *et al.*, 1958).

2.4 Different tactics to manage pulse beetle

In general, farmers are forced to sell their produce shortly after harvest due to inevitable losses. Therefore, there is a need to develop sustainable ways to manage this belligerent pest. Several options are available for controlling stored grain pests. Control can be at two levels *i.e.*, domestic / small scale and large commercial level.

Although various control measures (chemicals, dusts, oils, fumigants, irradiation *etc.*,) are available, it is often not ideal to add any foreign substance to the

produce in close proximity to the time of consumption. This may invite problems related to persistent toxic residues, insect resistance and adverse environmental impact.

2.4. a. Host plant resistance

Host plant resistance, where bruchid resistant plants would be a better option and can be integrated with other control measures, provided a promising impact on bruchid management in stored pluses. Thus, development of resistant cultivars to bruchids is a major target in mung bean breeding projects. Fujii and Miyazaki (1987) identified a wild mung bean (*V. radiata* var. *sublobata* (Roxb.) Verdc.). That had been exhibiting complete resistance to both *C. chinensis* and *C. maculatus* and was widely used in mung bean breeding programme (Kitamura *et al.*, 1988).

During the course of evolution, both insects and plants continuously interact with each other in a complex way. During the mutual relationship insects provide pollination and defense whereas, plants offer food, shelter and oviposition sites for insects (Panda and Khush, 1995). Conversely, insect pest might be harmful to plants based on the intensity of attack leading to their death. Plants overcome insect attack through various defense mechanisms viz., physical and biochemical barriers such as trichome density, waxy coating on the leaves, seed coat hardness, stimulation of defensive proteins, volatiles and secondary metabolites (Fordyce and Agrawal, 2001; Birkett *et al.*, 2000; Kliebenstein *et al.*, 2001). This phenomenon is commonly called as co-evolution or reciprocal selection.

Painter (1951) described host plant resistance (HPR) as “those characters that enable a plant to avoid, tolerate or recover from attacks of insects under conditions that would cause greater injury to other plants of the same species”. HPR is eco-friendly as well as compatible with other control methods and might synergise their effects (Eigenbrode and Trumble, 1994). The innate protection of resistant plants disrupts the normal association of the pest with its host plant (Dicke, 2000). The processes of resistance involve morphological, physiological and/or biochemical mechanisms which range from simply minimizing the effect of insect attack to adversely affecting the insects cellular processes, growth and development

(Gatehouse, 2002). Varietal resistance to control storage pests could provide a base for developing an integrated pest management (IPM) programme (Semple, 1992; Dobie, 1984). Edwards and Singh (2006) proposed that legumes employ a range of direct (morphological barriers) and indirect (secondary metabolites and anti-nutritional compounds) protection. Seed of a resistant host plant produce adverse effects on stored pest larvae during feeding may also have combination of morphological and biochemical features of the host plant (Panda and Khush, 1995).

2.5 Mechanism of resistance against pulse beetles

The ecological relationship between plants and insects is a complex one with physical as well as chemical interactions. It is argued that plant resistance is not dependent on a single mechanism, but there are overlaps between the morphological and biochemical bases of resistance. The relationship between bruchids and their host plant is complex; therefore, the adaptation mechanism to these pests is difficult (Taylor, 1981). In this situation, through knowledge of the underlying mechanisms of bruchid resistance is vital for developing suitable breeding strategies. As far as the mechanism of seed resistance against bruchids is concerned, many strategies are used by seeds to protect themselves against insects: i) seed may be too hard for newly hatched larvae to penetrate; ii) seed may be physically too small or with an inconvenient shape for larvae to reach full size; iii) seed may contain too little food to support the larvae; iv) seed may contain toxins or other substances in the cotyledon or its enveloping seed coat that inhibit the larval development (Kashiwaba *et al.*, 2003).

Paniter (1951) proposed three mechanisms of HPR viz., antixenosis (non-preference), antibiosis and tolerance which are worldwide accepted. In general, Non-preference type is refractory to the pest thereby avoiding the insect from using the plants for feeding, egg laying (oviposition) and shelter or combination of these three processes. Non preference type of resistance has been reported in maize varieties against weevils (Kang *et al.*, 1995; Derera *et al.*, 2001). Antibiosis is differed from antixenosis by allowing the insect to colonize the host plants, but provide resistance after ingestion of host tissues by producing adverse effect on insect's growth, reproduction and survival (Dent, 2000). Phytotoxins (primary metabolites) and

secondary metabolites are associated with antibiosis. Earlier workers have reported that phenolics in grains (hydroxycinnamic acids) act as an important resistance factor to storage pests (Classen *et al.*, 1990; Arnasson *et al.*, 1992; Sen *et al.*, 1994) which clearly indicated that biochemical analysis needs to be considered for screening bruchid resistance genotypes. Tolerance is the ability of the host to rapidly recover, repair or withstand infestation. However, tolerance is not applicable in storage condition because the damage caused to stored grains is irreversible (Horber, 1989). Therefore, antixenosis and antibiosis are highly important in this study.

2.6. Bruchid resistance testing methods

McCain *et al.*, (1964) claimed that the quantity of damage is to be taken as a measure of resistance. Therefore, scientists have exploited different screening methods to test resistance. Under storage condition, basically two methods are used to check resistance against stored pests that are free-choice and no-choice (force) tests. In free-choice test, the insects can infest the seed samples of their choice (Giga, 1995). The ability of a specific variety to repel insects (antixenosis) is usually measured with this method. This method is mostly used when a greater number of germplasm to be screened. Horber and Mills (1976) suggested that free-choice test method is suitable to eliminate most susceptible genotypes. Next method, no-choice test, where the insects are restricted in their choice of seed sample (Nwanze and Horber, 1975; Giga, 1995). This method is widely used in laboratory screening of genotypes for resistance against storage pests. Here, antibiosis plays a major role in resistance, which is measured in terms of the percent weight loss, mean development period and number of insect emergence. Further, all these parameters are used for calculating growth index. Both these methods have been previously used to screen germplasms against bruchid resistance (Tipping *et al.*, 1989; Cardona *et al.*, 1990; Derera *et al.*, 2001; Eddie and Amatobi, 2003; Cardona *et al.*, 2005).

2.7 Screening of mung bean against pulse beetle

Few accession of mung bean has showed Bruchid resistance. Fujii and Miyazaki (1987) and Fujii *et al.*, 1987 reported complete resistance to *C. chinensis*, *C.*

maculatus, *C. phaseoli* (Gyllenhal) in wild mung bean [*V. radiata* var. *sublobata* (Roxb.) Verdc.] (accession TC196).

In AVRDC study seeds of 500 mung bean accessions were screened for resistance to *C. chinensis*. Ten accessions were classified as highly resistant. Two entries, V-2802-G and V1128B-B2 were found free from bruchid infestation (Talekar and Lin, 1992).

Sixteen mung bean genotypes were evaluated for their resistance to *C. maculatus* on the basis of three parameters viz., number of healthy seeds (resistance), number of eggs oviposited (ovipositional preference), and total emergence holes (adult survival) for 50 seeds. Number of eggs per 50 seeds had slightly lower heritability estimate. The resistance was found to be negative function of adults emerged, indicating the importance of this parameter while selecting bruchid resistant mung bean varieties (Sarkar *et al.*, 1990). Laboratory study conducted at AVRDC also showed that *C. chinensis* resistant mung bean accession was VM2164 (Talekar and Lin, 1992).

Talekar and Lin (1992) investigated characteristics of resistance to *C. chinensis* in two mung bean and one black gram accessions; and the result concluded that smaller seed size of the accessions was not responsible for the resistance. A study indicating that the cultivars with hard seed coat showed non-preference by pulse beetle (Shaheen, *et al.*, 2006).

Erler *et al.* (2009) screened several mung bean varieties for resistance to *C. chinensis* under both choice and no choice tests and reported that, among the tested chickpea genotypes, only ICC 4969 can be used as a source of *C. maculatus* resistance in breeding programmes that could then be grown in organic cultivation free from pesticides. Eight different varieties of *V. radiata* were evaluated for their susceptibility to *C. chinensis* on the basis of number of eggs laid, duration of development of the immature stages, percentage adult emergence and weight loss due to damage by the pest. The varieties MB-2456 and Kanti were found to be highly susceptible, with 13.6 and 13.0% loss in seed weight, respectively (Hussain *et al.*, 2010).

Liu-xuming *et al.* (1998) studied both artificial and natural infestation of *C. chinensis* and identified resistance source of mung bean from different land races. Among the tested germplasm, 17 lines from AVRDC showed moderate resistance to immunity, while only three land races from Gangxi showed moderate resistance. The extent of damage inflicted by beetle was taken as an evaluation index.

Khattak *et al.* (1987) and Shafique and Ahmad (2002) revealed that oviposition, adult progeny development and grain weight loss varied significantly among promising cultivars of various pulses and correlations study indicated significant relationship between the variables.

Lambrides *et al.* (2000) screened twenty six mung bean varieties and found three accessions (TC 1966, ACC23 and ACC41) were resistant to bruchid and possessed characteristics like small seed size and the presence of well-formed texture layer on the seeds.

Raghvani *et al.* (2001) conducted varietal screening of different mung bean accessions against *C. analis* and observed significantly low (4.70%) seed damage when one pair of adult was released whereas, was highest (28.14%) damage was recorded with eight pairs of pulse beetle.

Susceptibility of five mung bean accessions against pulse beetle, *C. analis* was conducted under force choice test. None of the mung bean accession was found to be immune. Depending on the mean pest population and seed weight loss, accession AEM-6/20 was more tolerant/ resistant to bruchids infestation in comparison to genotype number No. 25/20 (Deeba *et al.*, 2006).

The study was undertaken to investigate the characteristics of resistance to *C. chinensis* and *C. maculatus* in two new resistant mung bean accessions V1128 and V2817, and two previously reported resistant accessions V2709 and V2802, compared to the susceptible mung bean cultivar, KPS1. The expression of resistance was found to be different for different maturity stages, whole seeds and decorticated seeds between old and new resistant cultivars (Somta *et al.*, 2008)

The study was undertaken to investigate the characteristics of resistance to *C. chinensis* and *C. maculatus* in two new resistant mung bean accessions V1128 and

V2817 and two previously reported resistant accessions V2709 and V2802, compared to the susceptible mung bean cultivar KPS1. The expression of resistance was found to be different for different maturity stages, whole seeds and decorticated seeds between old and new resistant cultivars (Somta *et al.*, 2008).

In investigating the feasibility of breeding large-seeded cultivars with high levels of bruchid resistance, revealed a complex relationship between seed mass and bruchid resistance (Mei *et al.*, 2009).

An experiment was conducted using free choice and forced choice test to screen varietal resistance against three species of pulse beetle in mung bean. Different parameters like per cent emergence, number of eggs on grain surface, mean development period and growth index of all the species was recorded. Out of eight cultivars, Pusa Baisakhi was found to be resistant for *C. maculatus* under both conditions (Jha *et al.*, 2011).

Duraimurugan *et al.* (2014) also found a similar kind of result in *C. chinensis* against 335 accessions of green gram where four accessions moderately resistant with minimum growth index ranged 0.051 to 0.055 and longest grub developmental period ranged 30.7 to 31.5 days.

2.7.1 Screening of different pulses against pulse beetles

Many varieties of cowpea screened for resistance against the cowpea bruchid, *C. maculatus* and other *Callosobruchus* spp. showed that some varieties possess a very high level of resistance (Jackai and Daoust, 1986). Ahmad *et al.* (1993) reported that coefficients of phenotypic and genotypic variations were positively correlated with damaged seeds and emergence holes.

Ashraf *et al.* (1985) tested the cultivars of chickpea (86208, CM-72, 86221, 86037 and C-44) for their relative resistance against this beetle and found that C-44 was relatively susceptible whereas 86037 appeared to be resistant against the beetle. Edward and Gunathilagaraj, (1994)) screened 26 accessions of chickpea for their resistance against pulse beetle and concluded that resistance was due to antibiosis as reflected in lower survival, prolonged development period and adults with reduced longevity.

Singh and Sharma (2001) evaluated thirteen varieties of chickpea for oviposition preference and subsequent larval development of the pulse beetle *C. chinensis*. PG-5 was the most resistant variety with minimum GI of 1.358 and longest grub development period of 28.33 days. On the other hand, GNG-663 was found to be the most susceptible with maximum GI of 2.211 and minimum grub development period of 28 days.

Singh and Sharma (2003) evaluated seven varieties viz., L-4076, DPL-61, K-75, K-303, Sehore-747, DPL-59 and L-414-7 of lentil. The pulse beetle showed high ovipositional preference for all the varieties, as grain with eggs in the varieties ranged from 97.0 to 99.6 per cent. Of the varieties evaluated, DEP-3 was observed to be the most preferred one with GI of 1.376; the grub developmental period in the variety was 33.66 days. The variety RPG-3 had minimum GI of 0.671 (developmental period, 38.00 days). The pest GI ranged from 1.051 (BR-12) to 0.805 (L-116) in the other varieties.

Kananji (2007) evaluated forty-two genotypes of Malawian dry bean germplasm for resistance to *Z. subfasciatus*. Significant differences were observed in grain weight loss (%), number of adult bruchids emerged and Dobie susceptibility index among the genotypes. The relationship between grain weight loss (per cent) and adult bruchid emergence was highly significant ($P < 0.001$) and positively correlated ($r = 0.803$). There was also a highly significant ($P < 0.001$) and positive correlation ($r = 0.734$) between weight loss (per cent) and Dobie's susceptibility index (DSI).

Obiadalla-Ali *et al.* (2007) screened 21 cultivars of cowpea for resistance to weevil pest based on pest development assessment including number of adults emerged, percentage of damaged seeds, developmental period (days), number of eggs laid on seeds, mean of eggs laid per female, average lifespan of female (days), per cent adult emergence and per cent loss in seed weight. The assessed cowpea cultivars were classified into three groups, sensitive (Dokki 331 and Creamy 7), moderate tolerant (Black eye and IT 90 K 2840-2), high tolerant (IT 93 K 12904 and IT 81 D-1064).

Laserna-Ruiz *et al.* (2012) screened a lentil germplasm collection of 571 accessions from 27 different countries, and including wild species for susceptibility to

seed bruchids under natural field conditions in Central Spain. A total of 32 accessions, including *L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, *L. nigricans*, and *L. lamottei* showed lower infestation rates than the check and were selected as potential sources of resistance to this pest.

Relative resistance of 12 chickpea genotypes against of *C. maculatus* was carried out. The genotypes CH-52/02 and B-8/03 were found to be least susceptible to bruchids attack. The tolerant genotypes were dark brown coloured, hard seeds with wrinkled seed coat and small sized grains Sarwar (2012).

Sarwar (2013) studied the relative susceptibility among eight lentil genotypes against *C. analis*. Feeding preference, seed weight loss, frass weight, reproduction and developmental period of the pest, seed coat thickness and germination percentage was estimated to determine the differential response of lentil genotypes. Genotype AEL-49/20 displayed relatively tolerant response than AEL-23/40.

2.8 Resistance factors to storage pests

The present knowledge about crop resistance factors against stored pests had mainly focused to establish the factors which confer resistance. Understanding the mechanism will help plant breeders to effectively incorporate these resistance factors as an integral part along with other desirable traits for developing new varieties which inherently express resistance against storage pests. In general, for storage pests, only physical and biochemical factors are more influential that governs resistance (Semple, 1992).

2.8.1 Physical factors

The physical factor like seed coat roughness and seed coat hardness confer resistance to pulse beetle (Van Huis and De Rooy, 1998). Seed with hard coat may prevent the larvae from effectively piercing the seed, while a rough seed coat makes oviposition difficult (Nwanze and Horber, 1976; Messina and Renwick, 1985). Similar trend of results obtained in cowpea against *C. maculatus* (Shade *et al.*, 1996) in chickpea and brad bean against *C. chinensis* (Brewer and Horber, 1983).

Kapila and Pajni (1989) screened 17 cultivars of French beans for resistance against *Z. subfasciatus* and concluded that physical parameters such as seed size as well as colour did not have any role in bruchids resistance.

Talekar and Lin (1992) investigated characteristics of resistance to *C. chinensis* in two mung bean and one black gram accessions and concluded that smaller seed size of the accessions was not responsible for the resistance. The results indicated the presence of anti-nutritional factors in the resistance accessions. Cultivars with hard seed coat showed nonpreference by pulse beetle (Ahmed *et al.*, 1993).

Modi *et al.* (1994) studied the relative susceptibility for 44 Red gram cultivars against *C. chinensis* and reported that resistance was attributed to the seed size and shape, but not due to the seed colour. Conversely Hussain *et al.* (1997) size colour and protein content of the seeds had no influence on the susceptibility of mung bean seeds to *C. chinensis*. Shade *et al.* (1996) reported that seed with tough seed coat may hinder the larval penetration into the seeds to an extent, though primary feeders are well adapted to chew through.

Twenty-six mung bean (*V. radiata*) varieties and accessions were screened for resistance to four bruchid species (Coleoptera: Chrysomelidae). On the basis of percentage of seeds damaged, commercial mung bean varieties tested were highly susceptible to *C. maculatus* and *C. chinensis*. Three accessions (TC1966, ACC23 and ACC41) showed bruchid resistance due to small seed size and the presence of a well-formed texture layer on the seed. The results revealed that small seed size in mung bean may act as an oviposition deterrent to bruchids (Lambrides *et al.*, 2000).

The presence of anti-nutritional factors in the cotyledons of resistant accessions of *V. umbellata* confirmed that the bruchid larvae were able to penetrate through the seed coat into cotyledons but, failed to support a normal adult emergence. This was due to the mortality of first and second instars larva inside cotyledons of the seed. The result suggested that the seed coat of all resistant accessions had no protective role against the bruchids (Kashiwaba *et al.*, 2003).

Landerito, *et al.* (1993) studies the resistant and susceptible accession of mung bean by seed hardness. It was found that resistant seed varieties found to be harder

than susceptible variety. Mei *et al.* (2009) reported a strong association between small seed size and bruchid resistance in mung bean, whereas seed mass showed a complex relationship.

2.8.2 Biochemical factors

In many legumes, the chemical defense system is mediated mainly by several chemicals of one class or compounds of several classes to increase the defense levels. This indicate that a number of specific insect resistance mechanisms have been identified in these plant groups although they have not yet been widely integrated into mainstream breeding programs (Edwards and Singh, 2006). High resistance of some of the cultivated or wild *Vigna* spp. to *C. maculatus* may be due to the presence of multiple chemical factors with additive action to protect seeds from predation.

Trypsin inhibitors (Savelkoul *et al.*, 1992) and tannins present in the seed coat (Deshpande, 1992) of legumes confer resistance against bruchids. Lattanzio *et al.*, (2005) reported that the resistance against *C. maculatus* in cowpea is contributed by condensed tannins (Proanthocyanidins). Lale and Kolo (1998) documented that some resistant cowpea genotypes have some certain biochemical factors in the testa/seed coat, oviposition and survival of pulse beetle eggs on some resistant cowpea genotypes irrespective of seed coat texture. Biochemical factors in the seed coat might influence the egg hatching (Lale, and Makoshi, 2000). However trypsin inhibitor, tannin and starch content present in the seeds found to have significant positive correlation with bruchid resistance (Marconi, 1997). Ali and Smith (2000) reported that in the decorticated faba seeds allowed for the higher percentage of larval penetration and subsequent growth and emergence than the whole seeds. It is indicating that a characteristic of seed coat and biochemical antibiosis provides resistance in faba bean varieties. However, Edde and Amatobi (2003) observed that bruchids preferred whole cowpea seeds for oviposition compare to decorticated seeds. Therefore, in the cowpea seeds, testa/seed coat may not be a valuable aspect to confer bruchid resistance.

Cardona *et al.* (1989) further confirmed that the seed coat may act as physical barrier; the actual factors that are responsible for bruchid resistance were present in the cotyledon and are chemical in nature. Thus, it is evident that bruchid resistance under

storage condition is due to the interaction of antibiosis and non-preference. *Phaseolus vulgaris* differing in their resistance reaction to *A. obtectus* and *C. chinensis* due to differential absorption of soluble heteropolysaccharides like arabinose, galactose, glucose, raffinose and xylose (Suzuki *et al.*, 1995). Bhattacharya and Banerjee (2001) reported that the penetration capacity of the neonate larvae of bruchid beetle was inversely related to the phenol contents of the seed. According to Horber (1983), breeding with genotypes containing more concentrations of heteropolysaccharide may confer resistance against bruchid beetles. Other biochemical factors present in the seed like tannins (seed coat), arcelin and phytohemagglutinin (PHA) (cotyledons) including α -amylase inhibitors also conferred resistance in dry beans (Schoonhoven *et al.*, 1983; Osborn *et al.*, 1986; Ishimoto and Kitamura, 1989; Gatehouse, 1990). All of these studies demonstrated the importance of antibiosis as main source of resistance that affects the growth and development of larvae (Gatehouse *et al.*, 1979; Baker *et al.*, 1989).

Susceptible and resistant accessions of mung bean and black gram were screened against bruchid resistance. Factors like crude fibre, silica and lignin contents were same in both resistant and susceptible varieties. Saponins were not detected while alkaloids were present in both the susceptible and resistant accessions. Bruchid resistant varieties had higher trypsin inhibitor activities than susceptible varieties. Moreover, polyphenol contents in the seeds were not correlated with bruchid resistance. Albumin and globulin fractions of protein adversely affected egg deposition of bruchid beetles (Landerito., 1993). Cowpea weevil found difficulty to developing even with the low concentration (1%) of bean α amylase inhibitor (Pedra *et al.*, 2003). Similarly, Ishimota and Kitamura (1989) and Shade *et al.* (1994) reported the detrimental effect of α amylase inhibitor in the growth and development of *C. maculatus* and *C. chinensis*.

There is sufficient body of literature explaining the bean resistance to mechanism of bruchids resistance under controlled laboratory conditions (Hartweeck *et al.*, 1991; Fory *et al.*, 1996). The presence of the Arcelin, seed harness, seed coat thickness, tannins, lectins and trypsin inhibitors have been used to explain antibiosis or

no preference resistance mechanisms to bruchids infestation. Bhattacharya and Banerjee (2001) studied the effects of physical parameters in large garden pea, chickpea, and the texture and thickness of the host preference, shape, colour and size of pulse seeds and the texture and thickness of the seed coat affected egg laying. Moisture contents in lentil seeds differed significantly ($P < 0.05$), and these affected ($P < 0.05$) egg laying and fecundity of the female.

2.8.3 Heritability of the physical parameters

Saeed *et al.* (2007) studied for the association of seed yield with some important morphological characters in forty three mung bean accessions. High heritability was observed for seed yield, node of the first peduncle, pod clusters on branches as well as per plant.

Fifty six mung bean accessions were evaluated to determine the variability, heritability between the agronomic and physiological characters associated with yield. Heritability was found to be high for days to flowering and pod length (Khajudparn, and Tantasawat, 2013).

Rahim *et al.* (2010) evaluated heritability, variability and genetic advance in 26 mung bean genotypes. High broad sense heritability and genetic advance was observed for some plant traits like plant height, number of pod, number of seeds per pod, 1000-seed weight and grain yield.

Twenty mung bean genotypes were evaluated for genetic variability and correlation among different yield contributing traits. Heritability was high for pod length and plant height, while pods per plant and seed per pod had low heritability (Zaid *et al.*, 2012).

Pure line of mung bean seeds was treated with four doses of ethyl methane sulphonate to analyze the genetic advance and heritability of some major agro-metrical traits in second generation mutant. PCV was found to be higher than GCV for all the traits studied. Low heritability (38.43) and genetic advance (5.37) were noticed in control for the total seed yield per plant (Roychowdhury *et al.*, 2012).

Eighty-eight finger millet germplasm were tested to find out the heritability, variance components and genetic advance for some yield related agronomic traits. The

variance components like the phenotypic and genotypic variance were found to be high. Heritability coupled with expected genetic advance was recorded high for number of tillers per plant, number of ears per plant, number of finger per plant, finger length, grain yield and thousand seed weight (Wolie *et al.*, 2013).

Genetic variability was studied in forty mung bean genotypes by Narasimhulu *et al.* (2013). High GCV and PCV were found for number of branches per plant, pods per plant, and yield per plant and harvest index. High estimates of genetic advance as percent of mean were observed for 100 seed weight and harvest index.

2.9. Artificial seed system:

An artificial seed system was used to evaluate the effect of allelochemicals and other plant fractions on growth and development of bruchid, *C. maculatus*. Artificial seeds were prepared by using different proportions of various inhibitors of mammalian digestive proteases with the flour of susceptible cowpea cultivar. This experiment demonstrated the applicability of the technique to evaluate the effect of different chemicals on bruchid growth and development (Shade *et al.*, 1986).

Evaluation of resistance using artificial seeds showed that increasing the percentage of resistant seed powder adversely affected bruchid growth and development. Both resistant groups varied in resistance when compared to susceptible cultivar. The results suggest that biochemical(s) in cotyledon tissue are responsible for the resistance and the seed coat had no protective role against the bruchids (Somta *et al.*, 2008).

When artificial seeds made of *V. umbellate* flour were used, decreased adult emergence and prolonged mean developmental period of *C. maculatus* was observed. The biochemical constituents present in the cotyledon tissue had a negative correlation with that of index of suitability. The anti-nutritional factors present in the cotyledon tissues affected the adult emergence in *C. maculatus* (Kashiwaba *et al.*, 2003)

A polysaccharide from seeds of common bean, *P. vulgaris*, was found to be toxic to *C. chinensis* as evident by increasing larval mortality, reduced growth and development and fewer eggs (Applebaum *et al.*, 1970).

More or less same number of eggs was laid on the artificial seeds (control) in comparison to black-eyed beans, yet per cent survival from artificial seeds was abridged. Hardness of the artificial seeds prejudiced oviposition behavior of the beetle with more eggs laid on the harder seeds (Hudaib *et al.*, 2013).

2.10. Genetic diversity of *Vigna* spp

Characterization and assessment of qualitative and quantitative traits among *Vigna* spp. have great significance in designing breeding strategies (Li *et al.*, 2001). Usually the morphological traits, pedigrees, geographic origins, isozymes and DNA markers are used for the assessments of the genetic diversity *Vigna* spp. (Fatokun *et al.*, 1992; Kaga and Ishimoto, 1998; Bisht *et al.*, 2005). However, the morphological characters are limited in number and are often subjected to constraints from environmental and epistatic interactions (Tantasawat, *et al.*, 2010). To overcome these environmental discrepancy, the genetic markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites or simple sequence repeats (SSRs) and inter-SSR (ISSR) are used to the characterization of genotypes in *Vigna* spp. (Tantasawat, *et al.*, 2010; Sarwat, 2012). In addition, they also allow variety identification in the early stages of development (Tan *et al.*, 2012).

RAPD, AFLP, SSR and ISSR used widely for estimation of genetic variability in *Vigna* spp. (Kaga and Ishimoto, 1998; Lakhanpaul *et al.*, 2000; Chattopadhyay *et al.*, 2005). The SSR markers consist of randomly repeated units of short nucleotide motifs with 1 to 6 bp long. Although SSRs are highly polymorphic and their single-locus nature still limit their uses for genetic diversity study in several plant species. But several reports had been found the SSR markers were successfully yielding the reproducible pattern of genomic fragments in cowpea (Li *et al.*, 2001), mung bean (Kumar, 1999; Tan *et al.*, 2012) and azuki bean (Wang *et al.*, 2004).

A Comparative study of marker systems in *Vigna* spp. showed that SSR marker was more efficient in detecting genetic variability when comparing with other marker in mung bean and cowpea (Dikshit *et al.*, 2007). Bhuyan *et al.*, (2014) analyze the diversity of in Indian mung bean germplasm with SSR, RAPD, ISSR markers and

the result suggested that ISSR and RAPD were quite effective in resolving the genetic variability in the studied genotypes.

As well the single locus (SSRs) and multilocus analysis (RAPD and ISSR) of DNA not only useful for genotyping, but could also be used for mapping, gene tagging and marker-assisted selection of the superior traits. Genetic linkage maps mung bean have been constructed using RFLP, RAPD and SSR markers (Fatokun *et al.*, 1992; Menancio-Hautea *et al.*, 1993; Lambrides *et al.*, 2000; Humphry *et al.*, 2002; Humphry *et al.*, 2003; Humphry *et al.*, 2005; Chankaew *et al.*, 2011; Young *et al.*, 1993; Kaga and Ishimoto, 1998; Mei *et al.*, 2009) have been used to map genes for azuki bean weevil resistance (Young *et al.*, 1993; Kaga and Ishimoto, 1998; Mei *et al.*, 2009), seed color (Lambrides *et al.*, 2004), to identify QTLs for seed weight (Fatokun *et al.*, 1992; Humphry *et al.*, 2005; Mei *et al.*, 2009) and hardness of seed (Fatokun *et al.*, 1992).

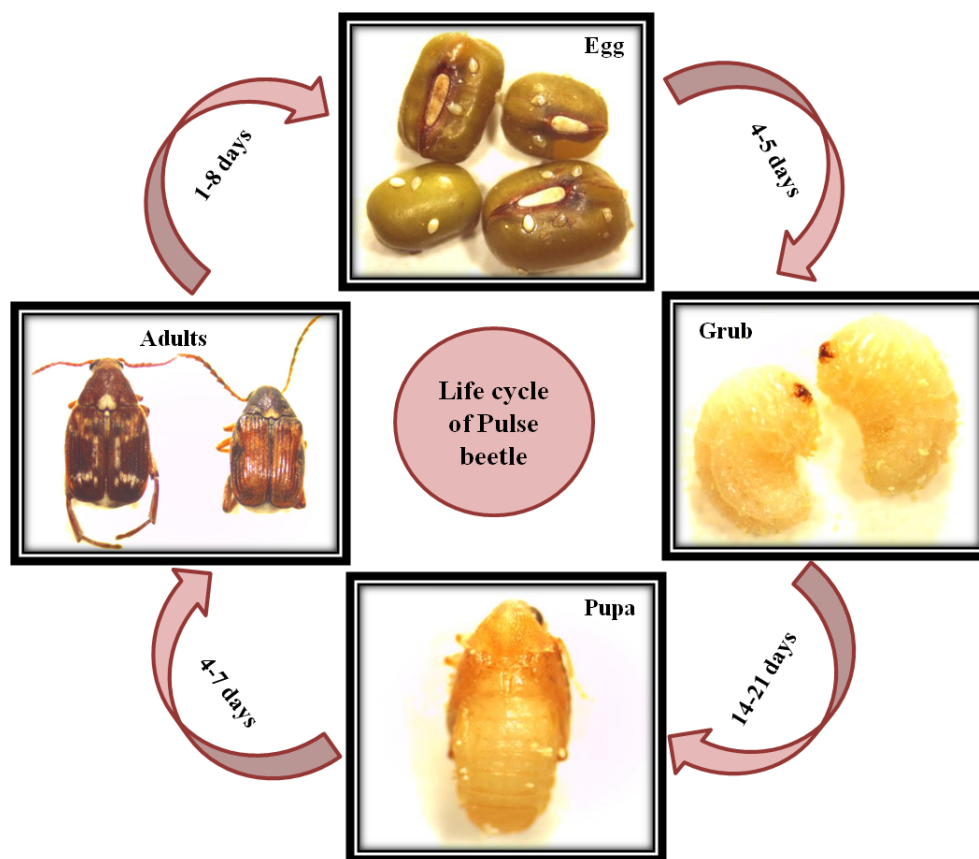


Plate 3.1 General lifecycle of pulse beetle



Female



Male

Callosobruchus analis



Female



Male

Callosobruchus chinensis



Female



Male

Callosobruchus maculatus

Plate 3.2 Different species of pulse beetle

The materials and methods used in the study on the Factors for resistance in mung bean, *V. radiata* against *Callosobruchus* spp. are presented in this chapter under following headings:

- Rearing of bruchid beetle - *Callosobruchus* spp.
- Evaluation of mung bean, *V. radiata* genotypes for resistance against three species of pulse beetles viz., *C. maculatus*, *C. chinensis* and *C. analis*
- Evaluation of physical and biochemical factors in susceptible and resistant varieties of *V. radiata* and to study their heritability
- Confirmation of bruchid resistance in mung bean genotypes using artificial seed system
- Finger printing of bruchid resistant mung bean genotypes

3.1 Rearing of the test insect - *Callosobruchus* spp.

The nucleus culture of the three species of bruchids namely *C. maculatus*, *C. chinensis* and *C. analis* were obtained from the Storage Laboratory, Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi. They were reared and bred on local susceptible mung bean variety (Plate 3.1) as per the procedure described by **Swella and Mushobozy (2007)** throughout the study period. These seeds were kept in glass jar (15.5 x 10.5cm) inside the Biological Oxygen Demand (B. O. D.) incubator at $27 \pm 2^{\circ}\text{C}$ temperature and $65 \pm 5\%$ relative humidity (RH). The mung bean seeds for insect culture were cleaned properly and conditioned under the culture conditions. Initially the adults were identified as male (σ) and female (ϕ) (Plate 3.2) and paired using the key characters (**Arora, 1977**). Later on, paired adults were released at the rate of 20 pairs for 150 seeds contained in glass jars. These jars were covered with perforated lids to allow for aeration, and a maximum of 3 days were allowed for mating and oviposition. The parent insects were removed afterwards and the seeds containing the eggs were transferred to fresh seeds in rearing jars which were covered with pieces of muslin cloth, fastened with rubber bands to prevent the contamination of the seeds and escape of the beetles. The jars were labeled with date of release of adults and species name. The insects were raised for about 4-5

generations before starting the experiments. Sub-culturing was done at regular intervals for the continuous supply of insects. Subsequent progenies emerging from the sub-culture were then used as parental generation for the experiments.

3.1.1 Handling of the culture

Although sedentary, pulse beetles are capable of flying though they do it rarely. Freshly emerged beetles could be handled either using sorting brushes or forceps or aspirators while removing or shifting the insects from one culture jar to another. *C. analis* was more motile and smaller in size compared to *C. maculatus* and *C. chinensis*. Freshly emerged adults were more sedentary than aged adults and were easy to handle. As the population builds up in the culture, adults become very much motile and start flying rigorously. Males are aggressively motile in comparison to females. The adults were collected using empty insect culture jar kept inverted (mouth to mouth) on the culture jar and insects were transferred to the new clean jar with the fresh host seeds for a new culture. Freshly emerged adult insects were collected using vacuum pump.

3.2 Evaluation of mung bean, *V. radiata* genotypes for resistance against three species of pulse beetles viz., *C. maculatus*, *C. chinensis* and *C. analis*

3.2.1 Mung bean, *V. radiata* germplasm

The samples of mung bean germplasm required for the screening were procured from the Pulse Research Laboratory, Division of Genetics, IARI, New Delhi. 100 accessions were taken for the study. The collected samples were first cleaned and further examined to make sure that these grains are undamaged and eggs have not been laid or there are no pest exit holes on them. After the preliminary screening it was found that 85 out of 100 accessions met the requirements for further study.

3.2.2 Evaluation of bruchid resistance using mature seeds

For the purpose of screening of genotypes to test the bruchid resistance, both free choice and forced choice test were conducted.

3.2.2.1 Free choice test

Eighty five accessions of green gram were taken for free choice test. The present study was conducted with the help of olfactory chamber (13 x 13 x 4 cm)

containing 24 separate circular disks like craters. Pre weighed ten grains of each accession were kept in a single hole (diameter 1.2 and depth- 2.2 cm) and each cultivar being replicated thrice in the chamber. Counted numbers (five pairs) of 1-2 days old adults of *C. chinensis* were transferred into a central hole (diameter 5.5 and depth- 2.2 cm) of the chamber (Plate 3.3). Similar procedures were repeated with *C. maculatus*, and *C. analis*.

3.2.2.2 Force choice test

This experiment was carried out to with the both resistant and susceptible to *C. chinensis* through free choice condition as described by (Nwanze, and Horber, 1975). Under forced choice test, insects were restricted in choice of grain from a sample. For this purpose, counted numbers (ten) of grains from selected accession were taken in separate homeopathic vials and five pairs of adult insects of *C. chinensis* (1-2 days old) were transferred into these vials and allowed to oviposition (Plate 3.4). After 24 hours of release, the test insects were removed from the vials. This experimentation was replicated five times for each cultivar. Later on, these vials were kept for incubation in a BOD chamber at $27 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ RH. Similar procedures were repeated in case of *C. maculatus* and *C. analis* (Jha *et al.*, 2011).

3.2.3 Evaluation of bruchid resistance using decorticated seeds

Resistant seeds from the above experiment were evaluated for the effect of their seed coat for bruchid resistance. In this treatment, the decorticated seeds were used. At first the seeds were soaked in water for 10–12 hr, and then the seed coat was removed. Later on, the seeds were subsequently dried at 60°C in a hot air oven for 24 hrs (Somta *et al.*, 2008). The dried decorticated seeds were kept in a moisture chamber until their moisture content reached 12–15% in order to provide optimum conditions for growth and development of bruchids. Three replications, each with counted number (ten) of decorticated seeds of selected accession were set up separately as described in the above experiment (Plate 3.5).

3.2.4 Observations

The cultivars were examined on daily basis to record the various parameters like number of eggs laid per seed, development period, number of adults emergence,

percentage, seed damage and weight loss. About 25 days after infestation (DAI), as adult emergence was initiated, observations for emergence were recorded at a regular interval of 24 hr and development period was calculated. The adult emergence observations in each accession were made until 45 DAI. Based on these observations various parameters were calculated as follows

3.2.4.a. Percent adult emergence

Percentage of adult emergence was calculated using following formulae (Howe, 1971).

$$\text{Percentage of adult emergence} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

3.2.4.b. Mean development Period

Mean development Period (days) is the time taken for 50 per cent of the adults to emerge since oviposition period. It was estimated by using the following formula (Howe, 1971).

$$\text{Mean development Period} = \frac{D_1A_1 + D_2A_2 + D_3A_3 + \dots + D_nA_n}{\text{Total number of adults emerged}}$$

Where,

D_1 -Day at which adults started emerging (first day)

A_1 - Number of adults emerged on D_1^{th} day

3.2.4.c. Growth Index

Growth Index (*i.e.*, percentage of the natural logarithm of the total number of emerged adults on the MDP) and was calculated using the formula (Howe, 1971; Jackai and Singh, 1988)

$$\text{Growth Index} = \log S/T$$

Where,

S = Per cent Adult emergence

T = Mean development time (Days)

3.2.4.d. Per cent weight loss

Per cent weight loss was calculated using the following formulae,

$$\text{Per cent weight loss} = \frac{\text{Initial weight of grains} - \text{Final weight of grains}}{\text{Initial weight of grains}} \times 100$$

3.3 Evaluation of physical and biochemical parameters in susceptible and resistant varieties of *V. radiata* and to study their heritability

3.3.1 Evaluation of physical parameters of seed

Different accessions of mung bean were evaluated for their physical parameters of seed viz., colour, size, hardness, seed coat hardness, seed coat percentage, seed volume and 100 seed weight

3.3.1.a. Seed colour was determined by comparing the colour of seed coat with different shades of various colours in the Royal Horticultural Society (RHS) colour chart (Plate 3.6).

3.3.1.b. Seed size was determined by measuring the length and width of the seeds by using digital Vernier caliper expressed in mm. L:B ratio also determined by using this data (Plate 3.7).

3.3.1.c. Seed hardness was determined by using Texture Analyzer (Model: TA+Di, Scientific Microsystem, UK), available at Division of Post Harvest Technology, IARI. The hardness of grains was measured with comparison test. Pressure was exerted on an individual grain until it cracked and the cracking point was recorded and expressed in Newton (**Mohsenin, 1980**) (Plate 3.8).

3.3.1.d. Seed coat hardness was determined by water absorption method and expressed as percentage increase in seed weight. For this purpose, pre weighed 20 seeds were soaked in 25ml of distilled water and incubated at 25°C for overnight. Later on, water decanted and seeds rubbed over blotters and weighed again. Lower the water absorption indicates the greater hardness of seed coat.

3.3.1.e. Seed coat percentage was determined by the procedure described by Giami and Okwechime, (1993) with slight modification. Twenty seeds of each mung bean accession were weighed and soaked in 40 ml distilled water for 1 hr at room temperature. The seed coat was removed manually and weighed immediately for determining the wet of the seed. The dry reweight of the were taken after 24 hr incubation in hot air oven (**AOAC, 2000**).

3.3.1.f. Seed volume was determined by the method described by **Phirke *et al.* (1982)**. In a graduated cylinder containing water, 20 pre weighed seeds of mung bean were immersed, and the amount of water displaced was measured and calculated as follows:

Seed Volume/Seed = Volume Increased/Number of seeds

3.3.1.g. 100 seed weight was determined by weighing 100 uniformly sized seeds in an analytical balance and expressed in grams.

3.3.2 Evaluation of biochemical parameters of seed

Biochemical parameters namely sugars, starch, soluble protein, crude protein, polyphenols, tannin, phytic acid content and α -amylase inhibitor activity were studied.

3.3.2.a.1. Extraction of starch

The sample (0.1g) was homogenized in hot 80% ethanol to remove sugars. For this purpose the samples were centrifuged at 10000 rpm for 15 min and the residue was retained. Again the residue was repeatedly washed with hot 80% ethanol till the washing does not give colour with anthrone reagent. After that the residue was well dried over a water bath, and then 5.0mL of water and 6.5mL of 52% perchloric acid were added. The tubes were kept at 0°C for 20min and then centrifuged at 10000rpm for 15 min and the supernatant was saved. The extraction was repeated by using fresh perchloric acid. Again the residue was centrifuged and the supernatant was pooled. Further the volume was made up to 100mL.

3.3.2.a.2. Estimation of starch

Total starch contents were estimated spectrophotometrically by anthrone reagent method (**McCready *et al.*, 1950**). 0.2 μ L of the supernatant was pipetted out and the volume was made upto 1mL with water. 4mL of anthrone reagent was added into each tube. Further, the contents were heated for eight minutes in a boiling water bath. The samples were cooled rapidly and then measured the intensity of green to dark green color at 630nm against a reagent blank. Standard curve was prepared with different concentrations of glucose. The results were expressed as mg of glucose per gram of the sample which was calculated from the standard graph.

3.3.2.b.1. Extraction of total soluble sugar

The sample (0.1g) was homogenized in hot 80% ethanol to remove sugars. For this purpose the samples were centrifuged at 10000rpm for 15 min and the supernatant was retained. Again the residue was repeatedly washed with hot 80% ethanol and the supernatant was collected then pooled with previous one.

3.3.2.b.2. Estimation of total soluble sugar

Total soluble sugar contents were estimated spectrophotometrically by anthrone reagent method (**Somogyi, 1952**). 0.1μL of the supernatant of different samples was pipetted out and then 4mL of anthrone reagent was added into each tube. Further, the mixture were heated for eight minutes in a boiling water bath. The samples were cooled rapidly and then measured the intensity of green to dark green color at 630nm against a reagent blank. Standard curve was prepared with different concentrations of Glucose. The results were expressed as mg of glucose per gram of the sample which was calculated from the standard graph.

3.3.2.c.1. Extraction of soluble protein

One gram of the decoated seeds were crushed and extracted with 5ml of phosphate buffer (7.5pH). The crushed material was centrifuged at 10000 rpm for 10 min and the supernatant was retained for further estimation.

3.3.2.c.2. Estimation of soluble protein

The protein contents were estimated spectrophotometrically by Bradford reagent method (**Bradford, 1976**). 0.5μL of the supernatant of different samples was pipetted out and then the volume was made up to 500μl using distilled water. 1.5 ml of Bradford reagent was added in to each tube and vortexed. The contents were allowed to stand for 2 min at room temperature and subsequently the absorbance were taken at 595 nm against a reagent blank. Standard curve was prepared with different concentrations of Bovine Serum Albumin (BSA). The results were expressed as mg of BSA per gram of the sample which was calculated from the standard graph.

3.3.2.d. Determination of crude protein

The nitrogen concentration in mung beanseeds were determined by modified Kjeldahl method. The method has two main steps:

(i) Digestion of the sample to convert the N compound in sample to NH_4^+ form and

(ii) Determination of NH_4^+ in digested samples.

3.3.2.d.1. Digestion

The digestion of sample was done according to modified version of Kjeldahl procedure. Accurately weighed 0.5 g of finely ground samples was placed in digestion flask of 100 ml capacity. In each flask, 3-4 g of catalyst mixture (anhydrous sodium sulphate and copper sulphate pentahydrate in 10:1 ratio) and 10 ml concentrated H_2SO_4 were added. Samples were digested and clean aliquot of the samples were collected in a digestion unit.

3.3.2.d.2. Distillation

The digested material was transferred into vacuum jacket of Micro Kjeldahl distillation apparatus. Twenty ml of 4 % boric acid solution was taken in a conical flask containing bromocresol green and methyl red indicator to which the condenser outlet of the flask was dipped. In the Kjeldahl flask, 100 ml of 40% NaOH solution was added. After completion of distillation, the boric acid was titrated against 0.02 N H_2SO_4 . A blank was also carried out and titration was done to the same end point of sample. The nitrogen content of the sample was estimated as:

$$\text{Amount of N in samples (S)} = \frac{(\text{ml of acid used for sample} - \text{ml of acid used for blank})}{10 \times 14 \times \text{Normality of acid}}$$

$$\text{N (\%)} \text{ in samples} = S \times \frac{100}{\text{Weight of sample (g)}} \times 100$$

3.3.2.e.1. Extraction of phenol

For extraction of total phenol, 0.5 g of mung beanseed powder was ground with 5 ml of 99% methanol with a pestle and mortar. Then the homogenate was centrifuged at 10000rpm for 20 min. The supernatants were saved and the residue was allowed to re-extracted with same extraction described earlier. The supernatants were pooled together in a test tube. Later on, evaporate the contents to dryness in hot water

bath. The residue was dissolved in 5 ml of distilled water. This extracts was used as a source of total phenol (**Malik and Singh, 1980**).

3.3.2.e.2. Estimation of phenol

Total phenolic contents were estimated spectrophotometrically by Folin-Ciocalteu reagent method with slight modification (**Malik and Singh, 1980**). Different aliquots of samples (0.5 mL) were pipetted out into test tubes. In the test tube, 0.5mL of Folin-Ciocalteu reagent was added. After 3 min, 2mL of 20% Na₂CO₃ solution was added to each tube. The contents in the tube were mixed thoroughly. Later on, the tubes were kept in boiling water for exactly one min, then cooled and measured the absorbance at 765nm against a reagent blank. Standard curve was prepared with different concentrations of Gallic acid. The results were expressed as mg of gallic acid per gram of the sample which was calculated from the standard graph.

3.3.2.f.1. Extraction of Tannin

For extraction of tannin, 100mg seed powder was extracted with 5ml of acetone: water (70:30 v/v) mixture in a water bath at 30⁰C for 30min. and vortexed at frequent intervals. The sample was centrifuged at 10,000 rpm for 15 min. and the supernatant was collected. Extraction was repeated twice and the combined supernatants evaporated to dryness at 80⁰C and taken up in 2 ml of water. For the estimation of tannin, the procedure used by **Lattanzio *et al.*(2005)** was followed.

3.3.2.f.2. Extraction of Tannin content

Tannin was estimated by Folin Ciocalteu method and results are expressed as tannic acid equivalents from a standard graph prepared from various quantities of tannic acid. To 0.5 ml of extract, 0.5 ml of Folin Ciocalteu reagent (1:1 diluted with distilled water) was added followed by the addition of 1 ml of 20% sodium carbonate. This solution was made upto 10 ml with distilled water and incubated at 25- 30⁰C for 40min. and absorbance of the blue colour developed was read at 725nm in a BioTek ELISA plate reader.

3.3.2.g.1. Extraction of Alpha amylase

For extraction of α - Amylase inhibitor (AI), 100mg fine seed powder was dissolved in distilled water, stirred for one hour and filtered. The residue was washed in water, stirred for thirty minutes and filtered. Distilled water (1:10 w/v) was added to the residue and centrifuged at 8000×g for 10 min. Crystalline Procine pancreatic amylase (Sigma) (1mg/ml) in 0.05 M Tris- HCl buffer (pH- 6.9 containing 0.01 M CaCl_2) was used for the assay (Sen Gupta and Pandey, 2009)

3.3.2.g.2. Estimation of Alpha amylase inhibitor

Amylase activity was assayed using the method of **Bernfeld (1955)**. 1.5 ml of AI was incubated with 0.1 ml of amylase enzyme for 30 min. at 30°C. One ml of 1% starch was added to the AI and allowed to incubate at 37°C for 15 min. The reaction was terminated with Dinitro Salicylic Acid (DNSA) reagent, heated in a boiling water bath for 10 min and cooled. After the cooling and 10 ml with distilled water and made the reaction mixture up to 10 ml. The brown colour developed was read at 540nm in a BioTek ELISA plate reader with DNSA reagent as blank. Amylase enzyme was also incubated with starch solution without AI. The inhibitor activity was expressed as percentage of inhibition of α - amylase. The reducing sugar released from the starch was estimated as maltose equivalents from a standard graph.

3.3.3 Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV)

The phenotypic and genotypic coefficient of variation was worked out as per the methods suggested by **Burton (1952)**. The six parents were replicated four times. The highly heterozygous F2 progenies were not replicated. The genotypic coefficient of variation of the progenies was calculated by deducting the environmental variance of the parents from the phenotypic variance of the progenies.

$$\text{Genotypic Coefficient of Variation} = \frac{\sqrt{\sigma^2_g}}{\text{General mean}} \times 100$$

$$\text{Phenotypic Coefficient of Variation} = \frac{\sqrt{\sigma^2_p}}{\text{General mean}} \times 100$$

$$\text{Environmental Coefficient of Variation} = \frac{\sqrt{\sigma^2_e}}{\text{General mean}} \times 100$$

Where,

σ^2_p = Phenotypic Variance (Sample variance of the population)

σ^2_e = Environmental Variance (Computed by finding the error mean)

σ^2_g = ($\sigma^2_p - \sigma^2_e$)

The PCV and GCV are classified as follows as suggested by Sivasubramanian and Madhavamenon (1973).

Low	:	Less than 10 %
Moderate	:	10 – 20 %
High	:	More than 20 %

3.3.3a. Heritability and genetic advance as percentage of mean

Heritability in the broad sense (h^2) was derived based on the formula proposed by Lush (1949) and expressed in per cent.

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

$$h^2 = \frac{(\sigma^2_g)}{(\sigma^2_p)} \times 100$$

Where,

(σ^2_g) - Genotypic Variance

(σ^2_p) - Phenotypic Variance

As suggested by Johnson *et al.* (1955), heritability values are categorized as follows:

Low	:	Less than 30 %
Moderate	:	30 – 60 %
High	:	More than 60 %

Genetic advance was estimated by the following formula as per the method of Johnson *et al.* (1955).

$$\text{Genetic advance} = \sqrt{\sigma^2_p} \times h^2 \times k$$

Where

σ^2_p - Phenotypic Variance

h^2 - Heritability

k - Selection differential, the value of which is 2.06 at 5% selection intensity

The genetic advance as a percentage of mean was estimated by adopting the following formula.

$$\text{Genetic advance as \% mean} = \frac{GA}{\text{General mean}} \times 100$$

The range of Genetic advance as per cent of mean was classified below as suggested by Johnson *et.al.* (1955).

Low	:	Less than 10 %
Moderate	:	10 – 20 %
High	:	More than 20 %

3.4. Preparation of artificial seeds

Modified method of Shade *et al.* (1986) was followed for preparation of artificial seeds (AS) by soaking the mung bean seeds namely, PS-16, KM-12-10, KM-12-5, KM-11-10 and M1319B in distilled water for about 1 hr. Seed coat was removed manually and the seeds were dried at 27°C for 48 h. The de-coated seeds were then ground into fine powder using pestle and mortar. Susceptible accession M1319B was mixed in different proportion with resistant cultivars to create different percentage of artificial seed. In the present study above mentioned flour of resistant genotypes were thoroughly mixed with susceptible accession M1319B in 25%, 50%, 75% and 100% combinations, according to Kashiwaba *et al.*, 2003. One gram of decoated mung bean flour was mixed with one percent Tween 80 (0.40 – 0.44 ml) and made into a fine paste. Later, the paste was converted into similar sized balls as that of mung bean seeds and kept in deep freezer for 12hrs. Further, AS was dried at 27°C temperature and 35 % RH for 1 week to equilibrate moisture content of AS with atmospheric levels (Hudaib *et al.*, 2013). The average weight of the AS was 0.23 g (n = 10) in comparison with mung bean that weighed 0.25 g (n = 10). AS were then taken in separate homeopathic vials and subjected to force choice test against three species of bruchid as performed earlier to evaluate the potency of resistance factor present in the seeds (Plate 3.9).

3.5. Statistical analysis

Statistical analysis was performed using Statistical Analysis Software, Version 9.2 (SAS, 2009). One way analysis of variance (ANOVA) was carried out to assess the difference in susceptibility of various treatments in which means were compared based on the least significant difference (LSD) at $P=0.05$. A one-tailed Pearson's correlation coefficient (r) analysis was performed to indicate the relationship between GI and other growth parameters of pulse beetle. Based on the infestation and susceptibility the accessions were ranked across the germplasm, wherein least preferred accession was ranked as one and most preferred accession with highest value to calculate the mean rank for better understanding of their growth index (Badii *et al.*, 2013).

3.6. Molecular methods

3.6. 1. DNA Extraction

Extraction of DNA from the test material is the first step for any molecular analysis. DNA isolation and its purification are of crucial importance since pure and high molecular weight DNA is an absolute requirement for DNA analysis and other molecular techniques. The approach to the preparation of the DNA from plants is determined by the species, type of tissue or sample available and the analysis required on DNA (Henry, 2001). For the extraction of plant genomic DNA, various types of tissues can be used like whole seedling, leaves, cotyledon, seeds/grain, endosperm, tissue culture callus etc. The process of DNA extraction may be considered to start when the plants are sampled. Samples are often frozen into liquid nitrogen for storage. The sample must remain frozen until DNA extraction as thawing of frozen tissue often results in degradation of the DNA. Many protocols are available in literature for extracting plant genomic DNA. DNA extraction protocols that are being used routinely world-wide in molecular laboratories were initially given by **Murray and Thompson (1980)** and **Delloporta *et al.* (1983)**.

3.6. 1.a. Procedure

The genomic DNA in the present sample of green gram was extracted by modified CTAB method (Doyle and Doyle, 1990). The steps for extraction are described below:

- Freshly harvested leaf samples (10-13 gm/accession) were finely ground by adding 4-5mL of pre-warmed CTAB buffer and 10 μ L β -Mercaptoethanol using pre-cooled mortar and pestle. (Before the start of the experiment, CTAB buffer was kept in a water bath at 60⁰C for 15-20 minutes).
- The finely ground tissue (1.0mL) was then transferred into a 2.0mL eppendorf tube and incubated at 60⁰C for 1 hour in a water bath.
- Later on, equal volume of Chloroform: Isoamyl alcohol (24:1) was added to the tube and mixed thoroughly by periodic inversions.
- Then these tubes were centrifuged for 20 minutes at 12,000 rpm at 4⁰C and the upper phase (supernatant) was carefully removed and transferred to the clean 2.0mL micro-centrifuge tube.
- To the supernatant, 0.6 volume of ice cold Isopropanol (100%) was added and mixed by inverting the tubes at least 4-5 times.
- Then 0.1 volume of 1M Sodium acetate, pH 5.2 was added to the tube and mixed thoroughly by inverting and the tubes were kept at 4⁰C for overnight.
- On the successive day, eppendorf tubes were centrifuged for 20 minutes at 12,000 rpm at 4⁰C to precipitate the DNA and then supernatant was discarded.
- The DNA pellet was washed by adding 100 μ L of 70% ethanol to each tube and centrifugation for 5 minutes at 10,000 rpm at 4⁰C. This step is repeated 2-3 times.
- The supernatant was carefully discarded and pellet was dried by keeping at 37⁰C for 30 minutes to 1 hour.
- Then 50-100 μ L of TE buffer was added to the pellet and incubated at room temperature for 15 minutes to rehydrate and dissolve the pellet.
- DNA was stored at -20⁰C for long-term use.

3.6. 1.b. DNA purification

- Volume of the DNA was made up to 100µL by adding TE buffer. To this, 2µL of RNase A (to a final concentration of 10mg/mL) and 1µL of Proteinase-K was added and it was kept at 37°C for 1 hour.
- Now equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added and mixed by inverting at least 15-20 times. Then centrifuged for 20 minutes at 12,000 rpm at 4°C.
- Supernatant was carefully transferred into a new 2.0 mL eppendorf tube and 0.6 volume of 100% ice cold Isopropanol was added and the same steps were followed as mentioned in DNA extraction procedure until storage of DNA.

3.6. 1.c. DNA Quantification

DNA concentration was estimated by using Spectrophotometer (Thermo Scientific NanoDropTM 1000). The NanoDrop was calibrated with 1 µl TE buffer. One µl of each sample was used for DNA concentration reading. For each sample three readings were taken and average was used for dilution. The sensor surface was cleaned with Milli-Q water to remove any dried sample that might be present. The following steps were followed sequentially.

- The NanoDrop program and the nucleic acid module were opened.
- The top and bottom sensors of the instrument were wiped off with Kimwipe tissues.
- One µl of TE buffer was pipetted onto the sensor to initialize. The lever arm was brought down.
- Onscreen prompts were followed to calibrate.
- The sensors were cleaned and 1 µl of TE buffer was pipetted as blank.
- The lever arm was brought down.
- The sensors were again wiped and 1µl of each DNA samples was pipetted. This was followed by recording the concentration. For good quality DNA the 260/280 ratio should be between 1.8 and 2.0 and A230/A280 value also should be between 2.0 to 2.2.

3.6.2. Dilution of DNA Samples

Small quantity of each DNA sample was diluted with Tris-EDTA buffer to yield a working concentration of 50ng/μl. The diluted samples were stored at 4°C for immediate use, while the original undiluted samples were kept for long term storage at -20°C. The diluted samples were checked for quality in 0.8 per cent agarose gel in 1X TAE buffer.

3.6.3. Selection of SSR primers

For SSR primers screening, one accession per species (i.e. total eighteen accessions) was selected. On these eighteen samples 20 SSR primers were screened (Table). After initial screening, 7 primers (Table 3.3) were selected for in depth molecular analysis of mung beangenotypes.

3.6.4. Gel electrophoresis

Amplified PCR products were resolved on 3% agarose (metaphor) gel through the following steps sequentially:

- At the end of PCR reaction, PCR tubes were taken out and the amplified products were separated by electrophoresis in 3 per cent agarose (metaphor) gel pre-stained in Ethidium Bromide (EtBr).
- For preparation of gel, 7.0 g (Biomatik, A2127) was weighed, dissolved in 1X Tris-Boric acid-EDTA (TBE) to get final concentration of 3 per cent.
- It was melted in a microwave oven. Molten agarose was cooled down to 50°C and 10 μl EtBr (10mg/ml stock solution, 5 μl EtBr/100 ml agarose) was added.
- It was then poured into a gel tray having 26 well-combs in place. After polymerization, the comb was removed carefully.
- The gel was transferred to an electrophoresis apparatus (Scie-plus HU-25 Unit) having two liters of 1X TBE buffer (pH 8.0).
- The PCR amplicons were then mixed with 2 μl of 6X loading dye and separation of amplicons was carried out by applying constant voltage of 100 V for 3 hrs.
- The sizes of the amplified products were determined by using a 50 bp DNA ladder (Fermentas Cat. SM0323).
- DNA fragments were visualized under UV light and photographed using Syngene gel documentation system for gel analysis and data recording.

3.6.5. Data analysis

The gel photographs were visually inspected and converted into binary data. Homology of bands was based on the distance of migration in the gel. The presence of bands was scored as '1' and absence as '0' while the missed samples were designated as '9'.

		j	
		+	-
i	+	a	b
	-	c	d

Where,

$$m = a + d \text{ (number of matches)}$$

$$u = b + c \text{ (number of unmatches)}$$

$$n = m + u \text{ (total sample size)}$$

Then Jaccard's similarity coefficient matrix (Jaccard, 1908) calculated between pairs of accessions as follows:

$$J_{ij} = a / (n - d)$$

The similarity matrix was subjected to cluster analysis by Unweighted Pair Group Method for Arithmetic Average (UPGMA) and a dendrogram was generated. The cluster analysis was performed using SAHN clustering algorithm. All analysis was performed using NTSYSpc software version 2.02 (Rohlf, 2000).

3.6.5. a. Principal coordinate analysis (PCA)

Principal coordinate analysis (PCA) was carried out with NTSYSpc software using Dcenter and Eigen procedures. The jaccard's similarity matrix of genetic similarity was also used to generate a 3D plot of principal coordinate to resolve the pattern of variation among the genotypes. This coordination method makes use of multidimensional solution of the observed relationships. PCA resolves complex

relationships into a function of fewer and simpler factors. In this technique, the data matrix is derived from the distances (similarities) between the operational taxonomic units. To simplify the description of these ‘clouds’ of points, the (principal) axes through the hyper-ellipsoid are calculated. The successive principal axes, representing the first major axis, the second axis accounts for the greatest, the second greatest, amount of variation.

3.6.5. b. Polymorphism Information Content (PIC)

Polymorphism Information Content (PIC) value described by **Botstein *et al.* (1980)** and modified by Anderson *et al.* (1993) for self-pollinated species was described below:

$$PIC = 1 - \sum_{j=1}^n p_{ij}^2$$

Where, p_{ij} is the frequency of the j^{th} allele for the i^{th} marker, and summed over n alleles. PIC is also an estimate of the discriminatory power of ISSR marker locus.



Plate 3.4 Free choice test setup (a,b)

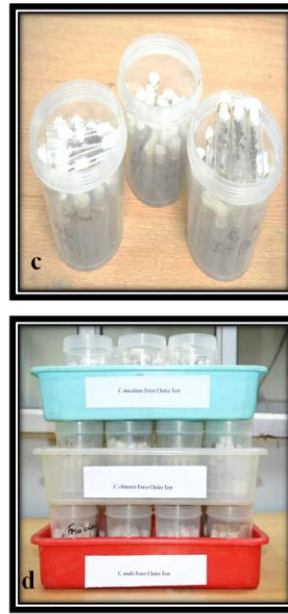


Plate 3.5 Force choice test setup (c,d)



Plate 3.6 Force choice bioassay using decorticated seeds

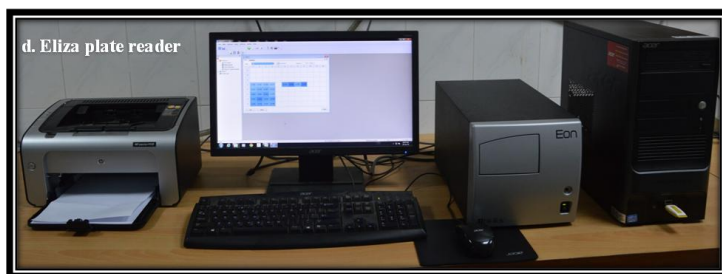


Plate 3.7 Instruments used during the study

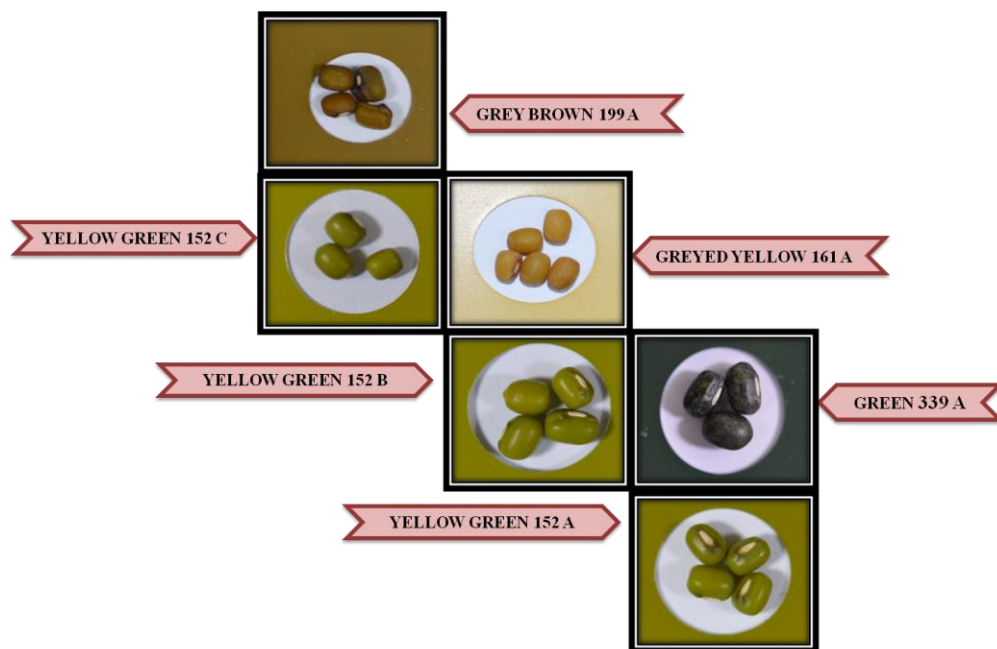


Plate 3.8 Variability of green gram accessions colour chart (RHS, 5th edition)

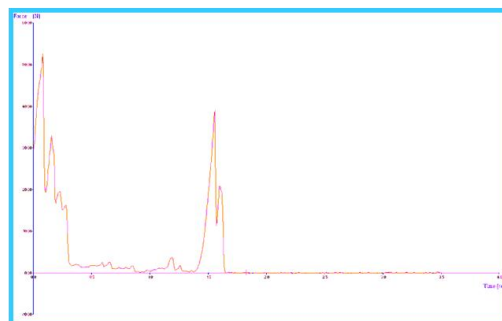
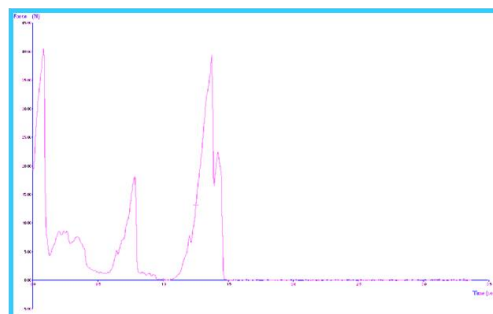


Plate 3.9 Texture analyzer to estimate seed hardness



Plate 3.4 Rearing of pulse beetles under BOD



Plate 3.9 Bioassay with artificial seeds

The result of the present study has been listed under the following headings:

- Evaluation of green gram genotypes for resistance against three species of pulse beetles under free choice test
- Evaluation of selected green gram genotypes for resistance against three species of pulse beetles under force choice test
- Physical and biochemical factors responsible for bruchid resistance and their heritability
- Evaluation of bruchid resistance using artificial seeds under ‘force choice’ test
- DNA finger printing of the selected green gram accession

4.1. Evaluation of bruchid resistance using whole seeds under ‘free choice’ test

In the free-choice test, insects can infest the seed samples of their choice, where antixenosis is usually measured with this method. Eighty five green gram accessions were screened for their reaction to pulse beetles viz, *C. maculatus*, *C. analis* and *C. chinensis* under free choice test based on various growth parameters (Table 4.1, 4.2, 4.3) viz., oviposition, adult emergence, maximum development period and growth index.

4.1.1 Screening of green gram accessions against *C. maculatus* under free choice test.

Egg laying pattern of *C. maculatus* that showed higher variation among the evaluated green gram accessions of *V. radiata* that ranged from 2.33 to 18.33 eggs/10 seeds. Minimum number of eggs (< 2.5 eggs/ 10seeds) were observed on genotypes such as KM-12-6 and KM-12-49 each, while PUSA 1171, KM-12-7 and KM-12-22 accessions had higher number of eggs (> 16 eggs/ 10seeds). Rest of the accessions shared eggs deposition within these range. No accessions were found to be free from oviposition.

Maximum development period of *C. maculatus* (Table 4.1) on different green gram accessions varied significantly from each other ($P=0.05$). The duration of development ranged between 19.67 and 33.00 days. KM-12-2 and KM-12-21 took

shorter period for grub development as 19.67 days, while KM-12-10, KM-11-10 and KM-11-32 required longer time (>30 days). Adult emergence also exhibited variation amongst the green gram accessions with more number of adults emerged from IPM-02-19 (91.5%), whereas 19.2% of adult emerged from PUSA 9971.

Growth Index (GI) (Table 4.1) was calculated based on per cent adult emergence and MDP. It ranged from 0.043 to 0.093. Out of 85 germplasm, 12 were found to have low growth index (GI <0.06). The accessions with lower growth index are considered to have higher resistance against *C. maculatus*.

4.1.2 Screening of green gram accessions against *C. analis* under free choice test.

When moongbean accessions were screened against *C. analis* through free choice test higher variation among the screened green gram accessions was recorded (Table 4.2). The oviposition results varied between 2.33 to 16.33 eggs/10 seeds. Accessions such as KM-12-6, KM-12-7, KM-12-13, KM-12-19, PS-16, KM-12-5 and KM-11-16 recorded with less number of eggs (< 5.0 eggs/ 10seeds), whereas PDM 139 and KM-12-25 were recorded with higher number of eggs (> 15 eggs/ 10seeds). Regarding eggs deposition, remaining accessions were found in between this range. No accessions were found to be devoid of oviposition.

Significant variation were observed regarding the developmental period of *C. analis* among the different green gram accessions (P=0.05) (Table 4.1). MDP values ranged between 20 to 26 days. Accessions like KM-12-6, KM-11-30, KM-12-14, KM-12-7 and AKM 9904 shared shorter development period (< 21 days), while in Ganga 1, KM-12-5, MH-96-1, PUSA 1031 and KM-11-20 accessions longer duration (26 days) was recorded. Adult emergence from these tested accessions also exhibited disparity with more emergence of adults from KM-12-3 and KM-12-21 (> 90%), whereas < 15 % of adult emerged from accessions like KM-12-5 and PS-16.

Results showed that in screened greengram accessions growth index of *C. maculatus* (Table 4.2) ranged from 0.041 to 0.095. Out of 85 germplasm, 8 were found to have low growth index (GI <0.06). it was observed that accessions KM-12-5, P-S-16, PDM 91-269, PUSA 1031, KM-12-9, KM-12-32, Ganga 8 and MH-96-1,

were found to be the least preferred by *C. maculatus* with GI ranging from 0.041 to 0.060.

4.1.3 Screening of green gram accessions against *C. chinensis* under free choice test.

In the present study the oviposition pattern of *C. chinensis* showed significant variations ($P=0.05$) (Table 4.3). Ovipositional behavior of the adult beetles varied from 6.00 eggs/10 seeds on KM-12-20 to 16.33 eggs/10 seeds on PDM 139. Thirty three out of 85 accessions shared less number of eggs (< 10 eggs/10 seeds), while four accessions were found have higher number of eggs (> 15 eggs/10 seeds).

Developmental period with various accessions ranged between 20 to 31 days for *C. chinensis* (Table 4.3) which showed significant variations ($P=0.05$). Fifteen out of 85 accessions had shorter development period (< 21 days), while only KM-11-10 took longer duration of 31 days for completion of grub period. Adult emergence from these tested accessions also exhibited disparity with more emergence of adults from KM-12-14 and KM-12-21 ($> 90\%$), whereas $< 15\%$ of adult emerged from accessions like KM-11-11, KM-11-10, KM-7-134 and PUSA Baisakhi.

It was observed that GI (Table 4.3) of *C. chinensis* in different cultivars of mung bean ranged from 0.032 to 0.090. Ten out of 85 germplasms (KM-11-11, KM 11-10, P-S-16, KM 12 -32, KM-7-134, PUSA Baisakhi, MUM-16, KM-12-19, GANGA -8 and PUSA 0831) were found to have low growth index ($GI < 0.06$); whereas accessions KM-12-21, KM-12-6, MH-318 and KM-11-75 showed high growth index (< 0.09).

Free choice test provided preliminary result of each accession with respect to bruchid biological parameters like oviposition, developmental period and adult emergence. On the basis of their growth index (GI), accessions were categorized and grouped as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible. Those accessions which showed growth index less than 0.06 was further subjected to rigorous screening under forced choice.

4.2 Evaluation of bruchid resistance using whole seeds under ‘force choice’ test

Differential reactions of selected green gram accessions under force choice test against *C. maculatus*, *C. analis* and *C. chinensis* are summarized in Table 4.2, 4.3 and 4.4 respectively. Based on GI the green gram accessions were categorized as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible against all three bruchid species studied.

4.2.1 Evaluation of green gram accessions to *C. maculatus* with ‘force choice’ test

Ovipositional behavior significantly showed variations among the evaluated green gram accessions for *C. maculatus* and ranged from 6.67 to 13.00 eggs/10 seeds. Genotypes significantly influenced the oviposition of bruchid as lowest number of eggs were observed to be eggs laid on the seeds of KM-11-10 (6.67 ± 0.33) and KM-12-32 (7.67 ± 1.67) whereas highest number of eggs with PUSA 9971 (13.00 ± 0.58), M1319B (12.67 ± 0.88), KM-12-23 (9.67 ± 0.67) and Ganga 1 (9.33 ± 0.88) (Table 4.2). The ovipositional performance of bruchid on certain accessions like KM-12-10, KM-12-7 and MUM-2 was at par (8.33 eggs per 10 seeds) with each other.

Results showed that the mean developmental period (MDP) (days) of *C. maculatus* varied significantly between the insects reared on various green gram accessions and ranged between 23.75 to 32.21 days ($F = 14.28$, $P < 0.05$) (Table 4.2). Maximum MDP period (32.21 ± 0.78 days) for development of *C. maculatus* was recorded in KM-12-10 accession, whereas M1319B showed the least (23.75 ± 1.15 days) development period. In addition, other accessions like KM-11-32 and KM-11-10 recorded 31.82 ± 0.51 and 31.59 ± 0.49 days for grub development which was on par with KM-12-10 (Table 4.2).

A wide range of adult emergence was observed when insects were reared on different cultivars and ranged from 21.34 to 84.05% ($F = 8.60$, $P < 0.05$). High percentage of adults >70 percentage could be recorded from accessions like M1319B ($84.05 \pm 1.16\%$), PUSA 9971 ($77.02 \pm 3.94\%$) and KM-12-23 ($72.39 \pm 3.21\%$). Accession KM-12-10 showed lowest adult emergence of $21.34 \pm 6.22\%$.

In the present study, the weight loss in seeds caused by pulse beetle infestation varied significantly among different accessions ($F = 91.49$, $p < 0.0001$). Higher weight loss (86.61%) occurred in M1319B, whereas KM-12-10 showed the least (9.40%),

minimum weight loss of other genotypes ranged in between 85.88 to 11.66 per cent. with the rest of the genotypes ranged in-between. Overall, ten accessions suffered more than 20 per cent weight loss while two accessions sustained less damage.

Pulse beetle *C. maculatus* displayed highest GI in M1319B (0.081), whereas low GI was identified in KM-12-10 (0.041). In general, genotypes like KM-12-10, KM-11-32 and KM-11-10 were found to be least preferred accessions, with GI (0.041 to 0.049) (Table 4.2, Fig 4.). On the contrary, MUM-2, KM 12 -23, PUSA 9971 and M 1319B had GI ranging from 0.074 to 0.081 were most preferred. Based on the GI, accessions were categorized into resistant (3accessions), moderately resistant (2accessions), moderately susceptible (3accessions), susceptible (3accessions) and highly susceptible (1accessions).

GI of *C. maculatus* had a significant positive relation with number of eggs ($P < 0.05$, $r = 0.688$), number of adult emergence ($P < 0.01$, $r = 0.985$) and per cent weight loss ($P < 0.01$, $r = 0.959$) while developmental period ($P < 0.01$, $r = -0.987$) was negatively correlated. Per cent adult emergence had positive correlation with seed weight loss ($P < 0.01$, $r = 0.963$), whereas negatively correlated with MDP ($P < 0.01$, $r = -0.947$) (Table 4.8).

4.2.2 Reaction of green gram accessions to *C. analis* with 'force choice' test

C. analis showed a differential behavior in oviposition preference among the evaluated green gram accessions. The number of eggs laid on these accessions by adult females is presented in Table 4.3. Oviposition ranged from 6.67 to 15.67 eggs/10 seeds. Maximum number of eggs (15.67 ± 1.86 , 13.00 ± 1.5 , 12.67 ± 0.88 , 12.00 ± 0.58 and 11.67 ± 1.20) was laid on Ganga 8, KM-12-32, PDM-91-269, MH-96-1 and KM-12-9 respectively, whereas KM-12-5, P-S-16 and PUSA 1031 recorded the least number viz., 6.67 ± 1.20 , 9.00 ± 2.52 and 9.00 ± 1.16 respectively.

Similarly, the developmental period (days) of *C. analis* grub on different greengram accessions showed a significant difference among them ($F = 4.67$, $p < 0.005$). Accession P-S-16 recorded the highest number of days (26 days) while Ganga

8 recorded the least MDP (20.67 days). Also PDM 91-269 and KM-12-32 recorded 21 days and 21.33 days, respectively (Table 4.3).

There were substantial variations among the accessions with respect to percentage of adult emergence ($F = 20.26$, $p < 0.0001$). Table 4.3 showed per-cent adult emergence was ranged from 12.22 to 81.77 per cent across the different accessions. Significantly more adults emerged from PUSA 1031, Ganga 8, KM-12-32 and PDM 91-269 while KM-12-5, P-S-16 and MH-96-1 recorded the least adult emergence.

The loss in seed weight of greengram accessions due to infestation by *C. analis* is presented in Table 4.3. Weight loss was found to be significantly different among the genotypes ($F = 5.76$, $p < 0.001$). the observed seed weight loss data showed that genotype Ganga 8 had highest percentage weight loss (46.46%), whereas Km-12-5 recorded the least (5.61%). Seed weight loss of other genotypes ranged in between these two values. It was recorded that with six accessions more than 10 per cent weight loss occurred whilst two accessions sustained less than 10 per cent weight loss.

The growth index (GI) of *C. analis* in eight genotypes ranged from 0.046 to 0.09 but there were no significant differences among them (Fig. 2). Accession Ganga 8 recorded the highest GI value of 0.09, whereas KM-12-5 recorded the least (0.046). Overall, KM-12-5, P-S-16, and MH-96-1, were found to be the least preferred by *C. analis*, with growth index ranging from 0.046 to 0.058. On the other hand, PDM 91-269, PUSA 1031, KM-12-32 and Ganga 8 appeared to be the most preferred, with GI ranging from 0.08 to 0.09. Based on the GI values accessions were classified into resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

Results showed (Table 4.9) that GI of *C. analis* had significant positive correlation with oviposition ($P < 0.05$, $r = 0.734$), per cent weight loss ($P < 0.01$, $r = 0.952$) and adult emergence ($P < 0.01$, $r = 0.931$) whereas negatively correlated with

MDP ($P < 0.01$, $r = -0.924$). Adult emergence was not significantly influenced by the number of egg laid by females.

4.2.3 Reaction of green gram accessions to *C. chinensis* with ‘force choice’ test

Oviposition preference of *C. chinensis* was found to be non- significant for the tested accessions. The mean number of eggs varied from 7.33 to 12.67 eggs/10 seeds. Relatively fewer eggs were deposited on the seeds of KM-11-10 (7.33 ± 2.33); while PUSA Baisakhi, KM-12-32 and PUSA 0831 were recorded with higher oviposition approximately 12eggs/10 seed (Table 4.4).

As far as MDP values of *C. chinensis* through forced choice method evaluated, it was found to be significantly different among the tested accessions ($F = 4.02$, $p < 0.005$) with longer developmental being recorded on accessions like KM-11-11 and KM-11-10 (31.77 ± 2.00 and 30.30 ± 1.51 days respectively). Shortest MDP value was observed (23.33 ± 0.75 days) in PUSA0831 (Table 4.4).

Pulse beetle *C. chinensis* also revealed similar trend of per cent adult emergence with germplasm Ganga 8 detected highest adult emergence (81.82%) while minimum adults emerged from KM-11-11 (12.33 %) ($F = 8.85$, $P < 0.0001$). Two accessions viz., PUSA 0831 and MUM 16 were found to be at par with Ganga 8 with 76% emergence (Table 4.4).

Significant difference was recorded in seed weight loss of evaluated green gram genotypes due to *C. chinensis* infestation ($F = 4.11$, $p < 0.005$) (Table 4.4). Damage as seed weight loss in these accessions ranged between 7.30 to 61.40%. Eight out of 10 germplasm suffered more than 20% weight loss.

The GI of *C. chinensis* on different accessions is presented in Table 4.4. GI varied between 0.03-0.081. Growth indices of two accessions viz. KM-11-11 and KM-11-10 were found to be low (< 0.05) while on accessions like PS-16, KM 12 -32, KM-7-134, PUSA Baisakhi, MUM-16, KM 12-19, GANGA -8, PUSA 0831 recorded $GI > 0.05$. Based on GI values of *C. chinensis* these accessions are grouped into resistant (2 accessions), moderately resistant (2 accessions), moderately susceptible (2 accessions), susceptible (3 accessions) and highly susceptible (1 accessions).

Moreover, GI of *C. chinensis* had a significant positive relation with adult emergence ($P < 0.01$, $r = 0.974$) and Weight loss ($P < 0.01$, $r = 0.954$) meanwhile MDP ($P < 0.01$, $r = -0.982$) was negatively correlated. But oviposition was found to have non-significant positive relation with GI ($r = 0.332$)(Table 4.10).

4.3 Evaluation of bruchid resistance using decorticated seeds under ‘force choice’ test

As per the categorization of accessions based on GI values, two resistant and one highly susceptible accession against each bruchids species from force choice test of whole seeds were further screened with decorticated seeds to identify the effect of seed coat on egg laying and adult emergence of all three species of bruchids under study.

For *C. maculatus*, oviposition was comparable for both resistant accessions as well as for the susceptible check (table 4.11). On the other hand, adult emergence was recorded from resistant accessions was less than the susceptible one ($P < 0.001$). Resistant accessions (KM-12-10 and KM-11-10) exhibited 10.67 ± 1.67 and 9.67 ± 0.88 eggs per 10 seeds respectively, whereas the susceptible cultivar (M1319B) had 15.33 ± 1.45 eggs per 10 seeds. Adult emergence was significantly low ($0.33 \pm 0.33\%$ and $0.67 \pm 0.33\%$) on the resistant genotypes (KM-12-10 and KM-11-10 respectively) than their counterpart M1319B ($13.33 \pm 1.20\%$).

Results for oviposition and adult emergence with decorticated seeds and whole seeds for *C. analis* ($P < 0.01$) are presented in Table 4.12. Resistant accessions (KM-12-5 and PS-16) exhibited 5.00 ± 1.00 and 10.67 ± 1.20 eggs per 10 seeds respectively and the susceptible cultivar Ganga 8 recorded 19.33 ± 1.20 eggs per 10 seeds. Adult emergence from PS-16 and Ganga 8 was observed as ($1.00 \pm 0.58\%$) and ($16.00 \pm 0.56\%$), respectively. Nil adult emergence was recorded in KM-12-5 accession.

Similarly, for *C. chinensis* decorticated seeds showed significant difference in egg laying ($P < 0.05$) and adult emergence ($P < 0.001$)(Table 4.13). The two resistant accessions (KM-11-10 and PS-16) displayed 5.67 ± 1.45 and 11.33 ± 1.86 eggs per 10 seeds respectively while PUSA 0831, the susceptible check was found to have maximum number of eggs (16.33 ± 1.45 eggs per 10 seeds). Reduced adult emergence

1.67± 0.67 % was observed with PS-16 while PUSA 0831 sowed higher adult emergence (13.33± 1.20%). KM-11-10 was completely devoid of adult emergence.

4.4 Physical and biochemical factors responsible for bruchid resistance and their heritability

Both physical and biochemical parameters were evaluated for 24 green gram accessions which were previously being selected from the force choice test against three species of bruchids.

4.4.1 Evaluation of physical basis of resistance

The physical parameters viz., seed colour, seed luster, seed stature, seed length, seed width, seed volume, seed hardness, seed coat hardness, seed coat percentage and hundred seed weight were estimated for 24 green gram accessions (Table 4.14, 4.15, 4.16).

The physical (qualitative) parameters viz., seed colour, lustre, stature of 24 accessions are presented in table 4.14. Seed coat colour varied among the accessions and were categorised under five groups (yellow green-152-A, yellow green-152-B, yellow green-152-C, Green-339-A and Grey brown-119-A), with an exception being greyed yellow 161-A for Sonamoong, an exotic variety. Eight accessions had yellow green-152-B followed by 7 apiece in yellow green-152-A and yellow green-152-C, while 1 each in Green-339-A and Grey brown-119-A (Plate 4.1 and Figure 4.5). Based on the seed luster, accessions were classified into dull and shiny. Observations showed that maximum accessions (18) had shiny seed surface while eight accessions were found with dull seed coat (Table 4.14, Figure 4.5). Seed stature of the accessions grouped into small, medium and bold. Eight green gram accessions falls under small and medium category each while six accessions were found as bold (Figure 4.5).

The physical (quantitative) parameters viz length, breadth, volume, seed hardness, seed coat hardness, seed coat percentage and 100 seed weight varied significantly among the green gram genotypes. Seed length ranged from 3.77 to 5.33 mm with the maximum in M1319B (5.33 ± 0.20 mm) followed by Ganga 1 (5.16 ± 0.11 mm) and the minimum in KM-12-5 (3.77 ± 0.10 mm) (Table 4.15). Similarly seed breadth differed significantly among the green gram accessions that ranged

between 2.83 to 3.60 mm with the maximum breadth in KM-12-10 (3.61 ± 0.04 mm) followed by Ganga 1 (3.60 ± 0.04 mm) and least in KM-12-9 (2.83 ± 0.07 mm). Correspondingly the length $\times$ breadth (L: B) ratio also showed a noticeable variation among the tested accessions with M1319B having bigger seeds (1.52 ± 0.05 mm) whereas MH 96-1 had smaller seeds (1.23 ± 0.04 mm). Seed density and seed volume significantly differed in the tested green gram accessions. Higher seed volume was spotted in MUM-16 (0.80 ml/20seed), while KM-11-10 (0.39 ml/20seed) had lowest. In general, seed density was found to vary between 1.12-1.71 g/ml.

The frequency distribution revealed that 7 accessions with low seed harness in the range of 25-40 Newtons, 6 had high seed hardness of > 55 Newtons while the remaining 12 came in the category of medium seed hardness in the range of 40.1-55 Newtons (Table 4.16). Based on the percentage weight increase due to water absorption, seed coat hardness ranged from 40.8 to 58.5 % in the different green gram accessions. Lower the water absorption higher is the seed coat hardness. Among the accessions 7 fell to low category with the range between 40-45%, 1 accession comes under high seed coat hardness of $> 55\%$ whereas remaining 18 genotypes in the medium category (45.1-55%). Seed coat percentage considerably varied among the germplasm with higher per cent in M1319B (19.38 %) and lowest with KM-12-5 (10.4 %).

100 seed weight maximum for MUM-16 (4.84 g) followed by PUSA 9971 (4.8 g), 13 accessions had low seed weight group ranging from 2.5-3.5 g while the left over 9 germplasm were in the medium group (3.5-4.5 g).

4.4.1.a Heritability of physical traits of green gram seed

The mean of different physical parameters such as length, breath, hundred seed weight, seed volume, seed density, seed hardness, seed coat hardness and seed coat percentage were 4.3, 3.2, 3.6, 0.5, 1.4, 48.1, 48.0 and 14.9 respectively are enlisted (Table 4.10). The corresponding traits ranged between 3.8-5.3, 2.8-3.6, 2.7-4.8, 0.8-0.4, 1.1-1.7, 29.4-88.5, 40.8-58.5 and 19.4-10.4 respectively. The phenotypic and genotypic variance of length (0.48 and 0.40), breath (0.19 and 0.16), hundred seed weight (0.87 and 0.85), seed volume (0.02 and 0.03), seed density (0.07 and 0.06),

seed hardness (731.47 and 737.47), seed coat hardness (84.77 and 47.91) and seed coat percentage (22.16 and 14.54) were highly significant among the 24 green gram accessions. All the observed seed parameters exhibited >50 % heritability. Besides the genetic advance for the plant traits were 117.1, 75.4, 187.3, 31.9, 50.4, 5571.4, 1071.9 and 636.1% respectively for length, breadth, hundred seed weight, seed volume, seed density, seed hardness, seed coat hardness and seed coat percentage. The effectiveness of selection depends upon genetic advance of the character selected along with heritability.

4.4.2 Evaluation of biochemical basis of resistance

To understand the biochemical basis of resistance to bruchids, parameters such as soluble protein, crude proteins, starch, total soluble sugars (TSS), phenol, tannin and α amylase inhibitor (AI) content were studied for the twenty four accessions.

The soluble protein content of the green gram accessions varied significantly among the green gram accessions that ranged from 173.67 ± 3.53 mg / g (KM-11-10) to 282.67 ± 5.46 mg / g (PUSA 1031) of the seed on fresh weight basis (Table 4.18). Frequency distribution table 4.19 revealed that twenty accessions were categorized as having low protein content (170- 220 mg / g), nine accessions had medium (220.1 – 270 mg /g) and remaining three accessions had high protein content (> 270 mg / g).

Similarly the crude protein content of these tested accessions varied significantly among them (Table 4.18). Crude protein ranged between 10.06 ± 0.05 % to 25.93 ± 0.08 % in M1319B and PUSA 1031 respectively. Of the 24 accessions, four accessions had low crude protein content (< 16%), while fifteen accessions had high (> 22.1 %) and the remaining five accessions had medium range (16.1- 22%) (Table 4.19).

Apparent difference was observed in the starch content of these 24 green gram accessions with lowest content in KM-11-11 (172.47 ± 1.54 mg / g) and the maximum of 555.33 ± 4.34 mg / g in PUSA 1031 followed by Ganga 8 (378.65 ± 4.85 mg / g)(Table 4.18). Frequency distribution table 4.19 indicated that nineteen, three and two accessions were classified in low (< 272 mg / g), medium (272.1- 372 mg / g) and high (>372.1mg / g) range respectively(Table 4.19).

Correspondingly, TSS content of these 24 green gram accessions significantly varied from 0.081 ± 0.01 mg / g to 4.25 ± 0.13 mg / g with lowest in KM-7-134 and maximum in MUM-16 respectively (Table 4.18). Table 4.19 showed that the eighteen, four and two accessions were classified in low (<1.4 mg / g), medium (1.41 - 2.4 mg / g) and high (>2.41 mg / g) range respectively.

Phenol content ranged from 2.11 ± 0.02 mg / g to 4.03 ± 0.28 mg / g on the fresh weight basis (Table 4.18). The lowest phenol content was recorded in KM-12-5 (2.11 ± 0.02 mg / g) and maximum in KM-11-11 with 4.03 ± 0.28 mg / g followed by KM-7-134 (3.90 ± 0.16 mg / g). Frequency distribution table 4.19 indicated that two accessions were classified in high (> 3.71 mg / g) while eleven accessions were grouped in low (< 3.0 mg / g) and medium (3.1 - 3.7 mg / g) range apiece (Table 4.19).

Results (Table 4.18) revealed that Tannin content of different of different green gram accessions ranged between 2.20 ± 0.09 mg / g and 5.95 ± 0.09 mg / g on fresh weight basis with maximum in KM-11-32 and minimum in PUSA 1031 correspondingly. Of the 24 accessions, thirteen accessions had low tannin content (<3.2 mg / g), while six accessions had high (>4.21 mg / g) and the remaining five accessions had medium range (3.21 - 4.2 mg / g) (Table 4.19).

The α -AI activity recorded in the seeds of different green gram accessions ranged from 5.02 ± 0.14 - $65.72 \pm 0.60\%$ inhibition (Table 4.18). Table 4.19 indicated that Nine accessions categorized as having low α -AI activity ($< 25\%$), twelve accessions had medium range (25.1 - 45%), while remaining three accessions had very high α -AI activity ($> 45.1\%$)

4.6 Evaluation of bruchid resistance using artificial seeds under ‘force choice’ test

Artificial seeds were prepared by mixing the resistant and susceptible flour of the accessions in different proportions to study the oviposition preference and adult emergence of the bruchids. Difference in the constituents of resistant and susceptible accession in artificial seeds did not influence the number of eggs laid in all the three species of bruchids. Nevertheless, adult emergence was affected by the resistant flour in different proportions with susceptible flour for the respective species.

For *C. maculatus* except two all other combination found to inhibit adult emergence. Ratio 1:3 of (KM-12-10+M1319B) and (KM-11-10 +M1319B) recorded adult emergence of 0.67 ± 0.67 % and 1.00 ± 0.58 % correspondingly. However, 1:1 of KM-12-10 with susceptible check (M1319B) also recorded meager adult emergence (0.67 ± 0.67 %) which was on par with the 1:3 ratio of (KM-12-10+M1319B). However, more than 50% adults emerged from seeds made with 100% M1319B (Table 4.26).

In case of *C. analis* adult emergence of *C. analis* was inhibited by all the combination of KM-12-5 with Ganga 8 viz., 25:75, 50:50, 75:25 ratios of resistant (KM-12-5) and susceptible (Ganga 8) and 100% of the susceptible alone. Other resistant genotype P-S-16 also exhibited same kind of inhibition in 1:1 ratio as well as in 100% of the resistant flour (Table 4.27).

Similar trend of adult emergence was observed in *C. chinensis* with the combination of resistant genotypes (KM-11-10 and P-S-16) with susceptible accession (PUSA 0831) in both 1:3 and 1:2 proportions (Table 4.28). 25% KM-11-10, with 75 % PUSA 0831 exhibited 0.67 ± 0.67 % of adult emergence, while 50% KM-11-10+50 % PUSA 0831 was on par with 25% PS-16 +75 % PUSA 0831 (0.33 ± 0.33 %). Meanwhile, 1:1 proportion of PS-16 and PUSA 0831 showed higher adult emergence (1.33 ± 0.58 %).

4.7 DNA finger printing of the selected greengram accessions

Twenty SSRs are used for genetic diversity in of 18 green gram accessions (Table 4. 29). Eight SSRs were found polymorphic out of twenty namely; CEDG037, CEDG044, CEDG127, CEDG154, CEDG178, CEDG214 and CEDG305 were amplified with polymorphic alleles indicating their complete transferability. The total number of alleles detected ranged twenty. Two alleles were amplified by CEDG037, CEDG154 and CEDG214. Polymorphism information content (PIC) was computed for each primer. It is an indication of band informativeness $PIC = 1 - \sum P_i - \sum \sum P_i P_j$ where 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the 'i' allele in the set of 18 genotypes investigated and $j = i+1$. The PIC value of primers ranged from 0.32 to 0.49 with an average PIC value for the 8 markers

to be 0.42. The resolving power (Rp) of primer was calculated as $R_p = \sum I_b$, where I_b (band informativeness) takes the value: $1 - [2 \times (0.5 - p)]$, p being the proportion of genotype of different *Vigna* species containing that band. Resolving power ranged from 1.01-1.36.

The unweighted neighbour-joining (UNJ) dendrogram constructed on the basis of genetic similarity matrix grouped the 18 genotypes into two major clusters. Cluster 1 comprised of three genotypes such as KM-7-134, KM-11-32 and KM-12-5 and Cluster II comprised of remaining 15 germplasm which is further divided into Cluster II A (14 genotypes) and Cluster II B (M1319B) (Fig.).

The UPGMA based grouping was in conformity with the Principal Coordinate Analysis (PCoA) results of the dendrogram (Fig.). The two dimensional PCoA plot (Fig 2) separated all the accessions into two major clusters. The accessions like Pusa 1031, MH-96-1, MUM 16, KM-11-11, Pusa 0831, Ganga-8 and Pusa Baisakhi were appeared on the left top of the X-axis and some other accessions such as KM-12-10, PDM 91-269 and KM-11-10 were appeared right top of the X-axis. PS-16, M1319B, KM-12-32 and MUM 2 were present in the left bottom of the X axis, while KM-12-5, Km-11-32 and Pusa 9971 were seen at right bottom of X- axis.

Table 4.4 Reaction of green gram accessions to *C. maculatus* under ‘force choice’ test

Accession	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Weight loss	Growth Index	Category
KM 12 -10	8.33±1.45 ^{bc}	21.34±6.22 ^g	32.21±0.78 ^a	9.40±1.35 ^g	0.041	Resistant
KM 11-32	8.67±1.45 ^{bc}	30.64±4.30 ^{fg}	31.82±0.51 ^a	11.36±1.48 ^g	0.047	-do-
KM 11-10	6.67±0.33 ^c	34.92±4.20 ^{efg}	31.59±0.49 ^a	23.24±0.35 ^f	0.049	-do-
KM 12 -32	7.67±1.67 ^{bc}	48.48±1.52 ^{def}	30.18±0.15 ^a	32.48±0.56 ^e	0.056	Moderately Resistant
KM 12 -7	8.33±0.67 ^{bc}	52.38±9.91 ^{cde}	30.10±0.18 ^a	49.12±5.62 ^{cd}	0.057	-do-
MUM-16	8.00±0.58 ^{bc}	54.17±4.17 ^{cd}	26.49±0.22 ^{bc}	42.94±1.44 ^d	0.065	Moderately Susceptible
GANGA -1	9.33±0.88 ^{bc}	64.06±9.83 ^{bcd}	26.53±1.22 ^{bc}	44.97±3.03 ^d	0.068	-do-
KM 11-40	8.67±1.45 ^{bc}	68.02±7.61 ^{abc}	26.73±1.11 ^b	56.05±4.94 ^c	0.069	-do-
MUM-2	8.33±1.20 ^{bc}	66.30±8.61 ^{abcd}	24.64±0.61 ^{bcd}	65.05±1.98 ^b	0.074	Susceptible
KM 12 -23	9.67±0.67 ^b	72.39±3.21 ^{ab}	24.59±0.67 ^{bcd}	78.50±3.37 ^a	0.076	-do-
PUSA 9971	13.00±0.58 ^a	77.02±3.94 ^{ab}	24.31±1.07 ^{cd}	85.88±1.58 ^a	0.078	-do-
M 1319B	12.67±0.88 ^a	84.05±1.16 ^a	23.75±1.15 ^d	86.61±3.00 ^a	0.081	Highly Susceptible
LSD @ 5%	2.80	18.05	2.31	8.23	-	
SE(d)	1.50	8.65	1.10	4.05	-	-
SE(m)	1.06	6.11	0.78	2.86	-	-

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at *P* = 0.05

Table 4.5 Reaction of green gram accessions to *C. analis* under 'force choice' test

Accession	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Weight loss	Growth index	Category
KM-12-5	6.67±1.20 ^c	12.22±6.19 ^e	25.67±0.33 ^a	5.61±1.65 ^d	0.042	Resistant
P-S-16	9.00±2.52 ^{bc}	14.29±8.25 ^{de}	26.00±0.58 ^a	9.09±4.63 ^{cd}	0.044	-do-
MH 96-1	11.67±1.20 ^{ab}	25.22±6.37 ^{cde}	24.00±0.58 ^{ab}	11.53±2.30 ^{cd}	0.058	Moderately Resistant
KM-12-9	12.00±0.58 ^{ab}	30.04±4.24 ^{cd}	23.67±0.67 ^{abc}	18.73±5.20 ^{bcd}	0.062	Moderately Susceptible
PDM 91-269	12.67±0.88 ^{ab}	42.32±3.02 ^c	21.00±1.53 ^{cd}	24.20±5.78 ^{bc}	0.077	Susceptible
PUSA 1031	9.00±1.16 ^{bc}	81.77±2.29 ^a	23.33±0.88 ^{abcd}	31.45±9.14 ^{ab}	0.082	Highly Susceptible
KM-12-32	13.00±1.53 ^{ab}	59.90±9.41 ^b	21.33±1.45 ^{bcd}	31.40±6.74 ^{ab}	0.083	-do-
Ganga 8	15.67±1.86 ^a	71.67±2.54 ^{ab}	20.67±0.88 ^d	46.46±6.77 ^a	0.09	-do-
LSD @ 5%	4.43	17.56	2.85	17.24	-	-
SE(d)	2.08	8.28	1.85	8.13	-	-
SE(m)	1.5	5.9	1.0	5.8	-	-

All values represent mean of three replications, Values represent Mean ± Standard Error.

Means in a column followed by different letter(s) are significantly different at *P* = 0.05

Table 4.6 Reaction of green gram accessions to *C. chinensis* with ‘force choice’ test

Accession	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Weight loss	Growth index	Category
KM-11-11	11.00±1.16 ^{ab}	12.33±3.09 ^d	31.77±2.00 ^{ab}	7.30±1.15 ^d	0.034	Resistant
KM-11-10	7.33±2.33 ^b	25.56±7.29 ^{cd}	30.30±1.51 ^a	16.29±2.73 ^{cd}	0.046	-do-
PS-16	9.33±1.20 ^{ab}	34.07±15.73 ^{cd}	28.20±0.95 ^{abc}	23.86±13.11 ^{bcd}	0.054	Moderately Resistant
KM-12-32	12.33±0.67 ^{ab}	36.13±9.47 ^{cd}	27.70±0.63 ^{bc}	21.67±6.82 ^{bcd}	0.056	-do-
KM-7-134	11.00±1.73 ^{ab}	49.08±7.94 ^{bc}	27.63±1.61 ^{bc}	25.77±8.04 ^{bcd}	0.061	Moderately Susceptible
PUSABAISAKI	12.67±0.88 ^a	66.19±3.37 ^{ab}	26.77±1.54 ^{bcd}	43.27±3.42 ^{bd}	0.068	-do-
MUM-16	10.67±1.45 ^{ab}	76.95±7.88 ^a	26.40±1.55 ^{cd}	36.44±4.51 ^{abc}	0.071	Susceptible
KM-12-19	9.00±3.61 ^{ab}	71.73±1.81 ^{ab}	25.33±1.82 ^{cd}	41.94±17.37 ^{ab}	0.073	-do-
GANGA 8	10.67±0.33 ^{ab}	81.82±10.50 ^a	24.63±0.78 ^{cd}	54.66±2.36 ^a	0.078	-do-
PUSA 0831	12.67±0.88 ^a	76.54±3.77 ^a	23.33±0.75 ^d	61.40±4.62 ^a	0.081	Highly Susceptible
LSD @ 5%	5.02	24.78	3.99	25.26	-	-
SE(d)	2.39	11.52	2.06	11.421	-	-
SE(m)	1.69	8.15	1.45	8.08	-	-

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at *P* = 0.05

Table 4.7 Correlation coefficient (r) between different parameters of *C. maculatus*

Parameters	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Weight loss	Growth index
No. of eggs/10seed	--	0.699*	-0.674*	0.746**	0.688*
% Adult Emergence		--	-0.947**	0.963**	0.985**
Developmental Period (days)			--	-0.929**	-0.987**
% Weight loss				--	0.959**
Growth index					--

*- Correlation is significant at the 0.05 level; ** - Correlation is significant at the 0.01 level

Table 4.8 Correlation coefficient (r) between different parameters of *C. analis*

Parameters	No. of eggs/10seed	% Weight loss	Developmental Period (days)	% Adult Emergence	Growth index
No. of eggs/10seed	--	0.740*	-0.849**	0.487 NS	0.734*
% Weight loss		--	-0.854**	0.915**	0.952**
Developmental Period (days)			--	-0.727*	-0.924**
% Adult Emergence				--	0.931**
Growth index					--

^{NS} - Non significant; *- Correlation is significant at the 0.05 level; ** - Correlation is significant at the 0.01 level

Table 4.9 Correlation coefficient (r) between different parameters of *C. chinensis*

Parameters	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Weight loss	Growth index
No. of eggs/10seed	--	0.303	-0.374	0.377	0.332
% Adult Emergence		--	-0.927**	0.928**	0.974**
Developmental Period (days)			--	-0.957**	-0.982**
% Weight loss				--	0.954**
Growth index					--

*- Correlation is significant at the 0.05 level; ** - Correlation is significant at the 0.01 level

Table 4.10 Oviposition preference and adult emergence of *C. maculatus* from decorticated seeds of selected green gram accessions.

Accession	No. of eggs/10seed	Adult Emergence (%)
KM-12-10	10.67±1.67 ^{ab}	15.87±3.30 ^c
KM-11-10	9.67±0.88 ^b	30.45±3.29 ^b
M1319B	15.33±1.45 ^a	77.09±5.38 ^a
LSD @ 5%	5.4	12.6

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at $P = 0.05$

Table 4.11 Oviposition preference and adult emergence of *C. analis* from decorticated seeds of selected green gram accessions.

Accession	No. of eggs/10seed	Adult Emergence (%)
KM-12-5	5.00±1.00 ^b	10.32±5.20 ^a
PS-16	10.67±1.20 ^b	11.79±6.05 ^a
Ganga 8	19.33±1.20 ^a	65.38±3.32 ^a
LSD @ 5%	4.3	19.7

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at $P = 0.05$

Table 4.12 Oviposition preference and adult emergence of *C. chinensis* from decorticated seeds of selected green gram accessions.

Accession	No. of eggs/10seed	No. of Adult Emergence
KM-11-10	05.67±1.45 ^b	19.72±5.28 ^a
PS-16	11.33±1.86 ^b	26.30±2.25 ^b
PUSA 0831	16.33±1.45 ^a	68.70±8.61 ^b
LSD @ 5%	4.1	24.9

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at $P = 0.05$

Table 4.13 Physical parameters (Qualitative) of selected Green gram accessions

Greengram accessions	Seed colour	Seed luster	Seed stature
KM-11-11	Yellow green-152-C	dull	small
KM 12 -10	Yellow green-152-C	shiny	small
KM 11-32	Yellow green-152-C	shiny	medium
KM 11-10	Yellow green-152-B	dull	small
KM-12-5	Yellow green-152-A	shiny	small
P-S-16	Yellow green-152-A	shiny	medium
KM 12 -32	Yellow green-152-B	shiny	bold
KM 12 -7	Yellow green-152-C	shiny	medium
MH 96-1	Yellow green-152-A	shiny	small
KM-12-9	Yellow green-152-C	shiny	bold
KM-7-134	Yellow green-152-B	shiny	small
MUM-16	Yellow green-152-A	dull	medium
PUSABAIKAKHI	Yellow green-152-A	dull	bold
GANGA -1	Yellow green-152-A	shiny	medium
KM 11-40	Yellow green-152-C	shiny	medium
KM-12-19	Yellow green-152-B	shiny	small
MUM-2	Yellow green-152-C	dull	small
KM 12 -23	Yellow green-152-B	shiny	medium
PUSA 9971	Yellow green-152-B	dull	medium
PDM 91-269	Yellow green-152-B	shiny	small
PUSA 0831	Yellow green-152-A	dull	medium
M 1319B	Green-339-A	dull	bold
PUSA 1031	Grey brown-119-A	shiny	bold
GANGA -8	Yellow green-152-B	shiny	bold

Table 4.14 Physical parameters (Quantitative) of selected green gram accessions

Greengram accessions	Length (mm)	Breadth (mm)	L:B ratio	100 seed weight (gm)	Seed volume (ml 100seeds ⁻¹)	Seed density (g ml ⁻¹)	Seed hardness (Newton)	Seed coat hardness (%)	Seed coat (%)
KM-11-11	(4.47±0.16) ^{BC}	(3.47±0.17) ^{ABC}	(1.30±0.10) ^{CDEF}	(2.90±0.04) ^{LM}	(0.49±0.029) ^D	(1.17±0.03) ^{JKL}	(65.82±0.01) ^D	(58.48±3.49) ^A	(12.70±2.70) ^{EFG}
KM 12 -10	(4.44±0.02) ^{BC}	(3.61±0.04) ^A	(1.23±0.03) ^{EF}	(3.54±0.03) ^{EFG}	(0.49±0.007) ^D	(1.37±0.01) ^{DEF}	(64.54±0.06) ^E	(49.89±3.99) ^{ABCD}	(16.23±1.31) ^{ABCDE}
KM 11-32	(4.44±0.21) ^{BC}	(3.33±0.07) ^{ABCDE}	(1.34±0.10) ^{BCDEF}	(2.65±0.10) ^M	(0.51±0.007) ^D	(1.16±0.01) ^{KL}	(29.35±0.02) ^V	(53.41±4.36) ^{AB}	(12.60±2.71) ^{EFG}
KM 11-10	(3.89±0.22) ^{EFG}	(2.97±0.13) ^{FGHIJ}	(1.31±0.02) ^{CDEF}	(3.54±0.06) ^{EFG}	(0.39±0.007) ^E	(1.71±0.01) ^A	(42.76±0.01) ^M	(51.94±3.18) ^{AB}	(12.79±2.56) ^{CDEF}
KM-12-5	(3.77±0.10) ^G	(2.91±0.12) ^{HIJ}	(1.30±0.02) ^{CDEF}	(3.29±0.11) ^{GHIJ}	(0.51±0.007) ^D	(1.39±0.01) ^{DEF}	(44.05±0.02) ^K	(54.09±3.99) ^{AB}	(10.42±0.35) ^G
P-S-16	(4.41±0.14) ^{BCD}	(3.20±0.05) ^{CDEFG}	(1.38±0.02) ^{ABCDE}	(3.37±0.08) ^{FGHI}	(0.51±0.018) ^D	(1.25±0.03) ^{GHIJ}	(31.55±0.02) ^U	(48.61±3.97) ^{ABCD}	(17.35±2.12) ^{ABC}
KM 12 -32	(4.17±0.05) ^{BCDEFG}	(3.01±0.09) ^{FGHIJ}	(1.39±0.05) ^{ABCD}	(3.97±0.09) ^{BC}	(0.60±0.023) ^C	(1.37±0.01) ^{DEF}	(50.54±0.02) ^F	(44.72±5.64) ^{ABCD}	(13.77±1.09) ^{BCDEF}
KM 12 -7	(3.87±0.10) ^{FG}	(2.86±0.05) ^{IJ}	(1.35±0.03) ^{BCDEF}	(3.55±0.10) ^{EF}	(0.50±0.046) ^D	(1.43±0.10) ^{CDE}	(88.46±0.01) ^A	(50.41±2.86) ^{AB}	(16.16±1.07) ^{ABCDEF}
MH 96-1	(4.14±0.06) ^{BCDEFG}	(3.37±0.07) ^{ABCD}	(1.23±0.04) ^F	(3.07±0.03) ^{LKJ}	(0.50±0.000) ^D	(1.23±0.01) ^{IJK}	(43.64±0.02) ^L	(52.89±2.22) ^{ABCD}	(15.72±0.56) ^{ABCDEF}
KM-12-9	(4.06±0.04) ^{CDEFG}	(2.83±0.07) ^J	(1.43±0.05) ^{ABC}	(3.28±0.01) ^{IHJ}	(0.50±0.023) ^D	(1.34±0.04) ^{EFGH}	(84.45±0.01) ^B	(49.53±0.32) ^{ABCD}	(17.25±2.15) ^{ABCD}
KM-7-134	(4.05±0.33) ^{CDEFG}	(3.26±0.14) ^{BCDEF}	(1.24±0.08) ^{DEF}	(3.65±0.06) ^{DE}	(0.61±0.013) ^A	(1.24±0.01) ^{HIJK}	(68.82±0.02) ^C	(45.67±4.86) ^{ABCD}	(18.35±0.35) ^{AB}
MUM-16	(4.32±0.09) ^{BCDEF}	(3.05±0.07) ^{EF GHIJ}	(1.42±0.05) ^{ABC}	(4.84±0.09) ^A	(0.80±0.042) ^C	(1.22±0.05) ^{IJK}	(31.64±0.02) ^T	(48.90±4.91) ^{ABCD}	(15.05±1.36) ^{ABCDEF}
Pusabaisakhi	(4.46±0.05) ^{BC}	(3.33±0.05) ^{ABCDE}	(1.34±0.02) ^{BCDEF}	(3.99±0.04) ^{BC}	(0.41±0.007) ^E	(1.59±0.01) ^B	(50.54±0.02) ^F	(46.90±4.93) ^{CD}	(14.49±1.61) ^{ABCDEF}
GANGA -1	(5.16±0.11) ^A	(3.60±0.04) ^A	(1.44±0.03) ^{ABC}	(3.33±0.13) ^{FGHI}	(0.50±0.012) ^D	(1.49±0.02) ^C	(38.86±0.01) ^Q	(41.08±6.43) ^{ABCD}	(16.10±1.66) ^{ABCDEF}
KM 11-40	(4.31±0.10) ^{BCDEF}	(3.19±0.13) ^{CDEFGH}	(1.35±0.03) ^{BCDEF}	(3.57±0.41) ^{DEF}	(0.60±0.012) ^C	(1.12±0.01) ^L	(41.93±0.02) ^N	(45.36±4.35) ^{ABC}	(13.19±0.29) ^{BCDEF}
KM-12-19	(4.36±0.15) ^{BCDE}	(3.13±0.02) ^{DEFGHI}	(1.39±0.06) ^{ABCD}	(3.18±0.14) ^{IJK}	(0.40±0.000) ^E	(1.44±0.01) ^{CD}	(37.85±0.01) ^R	(50.91±6.07) ^{ABC}	(15.62±1.08) ^{ABCDEF}
MUM-2	(4.15±0.22) ^{BCDEFG}	(3.19±0.09) ^{CDEFGH}	(1.31±0.10) ^{CDEF}	(3.47±0.15) ^{EFGH}	(0.49±0.035) ^D	(1.37±0.07) ^{DEF}	(35.45±0.02) ^S	(41.50±7.90) ^{CD}	(13.91±0.81) ^{ABCDEF}
KM 12 -23	(4.46±0.10) ^{BC}	(3.37±0.11) ^{ABCD}	(1.33±0.03) ^{BCDEF}	(3.81±0.08) ^{CD}	(0.60±0.000) ^C	(1.31±0.01) ^{FGHI}	(44.67±0.01) ^I	(44.31±6.23) ^{BCD}	(15.81±2.08) ^{ABCDEF}
PUSA 9971	(4.62±0.32) ^B	(3.47±0.17) ^{ABC}	(1.33±0.07) ^{BCDEF}	(4.80±0.10) ^A	(0.70±0.023) ^B	(1.15±0.03) ^{KL}	(48.45±0.03) ^H	(46.08±5.63) ^{ABCD}	(16.62±1.27) ^{ABCDE}
PDM 91-269	(3.92±0.03) ^{DEFG}	(3.01±0.06) ^{FGHIJ}	(1.30±0.02) ^{CDEF}	(3.01±0.03) ^{LJ}	(0.51±0.007) ^D	(1.21±0.02) ^{IJKL}	(41.10±0.02) ^B	(50.14±3.02) ^{ABCD}	(11.65±1.67) ^G
PUSA 0831	(4.15±0.26) ^{BCDEFG}	(3.18±0.18) ^{FGHIJ}	(1.31±0.09) ^{CDEF}	(3.22±0.05) ^{IHJK}	(0.51±0.007) ^D	(1.18±0.01) ^{JKL}	(41.26±0.01) ^O	(48.44±5.32) ^{ABCD}	(12.63±1.06) ^{EFG}
M 1319B	(5.33±0.20) ^A	(3.50±0.11) ^{AB}	(1.52±0.05) ^A	(4.21±0.08) ^B	(0.59±0.029) ^C	(1.41±0.05) ^{CDE}	(48.66±0.02) ^G	(40.89±3.01) ^D	(19.38±0.97) ^A
PUSA 1031	(4.32±0.09) ^{BCDEF}	(3.05±0.07) ^{EF GHIJ}	(1.42±0.05) ^{ABC}	(4.00±0.10) ^{BC}	(0.60±0.012) ^C	(1.30±0.01) ^{FGHI}	(35.47±0.01) ^S	(47.17±0.82) ^{ABCD}	(16.34±1.86) ^{ABCDE}
GANGA -8	(4.33±0.09) ^{BCDEF}	(2.95±0.06) ^{GHIJ}	(1.47±0.04) ^{AB}	(3.46±0.02) ^{EFGH}	(0.50±0.031) ^D	(1.35±0.06) ^{DEFG}	(44.18±0.01) ^J	(44.78±5.02) ^{ABCD}	(14.39±1.08) ^{ABCDEF}
LSD @ 5%	0.49	0.29	0.15	0.25	0.06	0.10	0.06	9.98	4.54
SE(d)	0.25	0.14	0.08	0.12	0.03	0.05	0.03	6.51	2.23
SE(m)	0.18	0.10	0.06	0.09	0.02	0.04	0.02	4.60	1.57

All values represent mean of three replications, Values represent Mean ± Standard Error.

Means in a column followed by different letter(s) are significantly different at $P = 0.05$

Table 4.15 Frequency distribution of green gram accessions with respect to physical parameters

Seed Hardness (Newton)	Low(25-40)	Medium(40.1-55.0)	High (>55.1)
	KM 11-32, P-S-16, MUM-16, MUM-2, KM 12 -19, Ganga -1, PUSA 1031	PUSA 9971, M 1319B, PUSA Baisakhi, KM 12- 32, PUSA 0831, Ganga-8, PDM 91-269, KM 12 -23, KM 11-40, MH 96-1, KM 11-10, KM 12 -5	KM 11-11, KM 12 -7, KM 12 -9, KM 12 -10, KM 7-134
Seed coat percentage (%)	Low(10-14)	Medium (14.1-18.0)	High (>18.1)
	KM-12-5, PDM 91-269, KM-12-32, KM 11-32, KM 11-10, KM 12 -32, KM 11-40, MUM-2, KM-11-11, PUSA 0831	P-S-16, MH 96-1, KM-12-9, PUSA 1031, Ganga 8, KM 12 -10, KM 12 -7, MUM-16, Ganga -1, KM 12 -23, PUSA 9971, PUSA Baisakhi, KM-12-19	M1319B, KM-7-134
Seed coat hardness (%)	Low(40-45)	Medium (45.1-55.0)	High (>55.1)
	Ganga 8, KM-12-32, KM 12 -32, Ganga -1, MUM-2, KM 12 -23, M 1319B	KM-12-5, P-S-16, MH 96-1, KM-12-9, PDM 91-269, PUSA 1031, KM 12 -10, KM 11-32, KM 11-10, KM 12 -7, MUM-16, KM 11-40, PUSA 9971, KM-7-134, PUSA 0831, PUSA Baisakhi, MUM-16, KM-12-19	KM 11-11
100 seed weight (gm)	Low(2.3- 3.5)	Medium (3.51-4.50)	High (>4.51)
	KM-12-5, P-S-16, MH 96-1, KM-12-9, PDM 91-269, KM 11-32, Ganga -1, MUM-2, KM-11-11, PS-16, KM-12-19, Ganga 8, PUSA 0831	PUSA 1031, KM-12-32, KM 12 -10, KM 11-10, KM 12 -32, KM 12 -7, KM 11-40, KM 12 -23, KM-11-10, M 1319B, KM-12-32, KM-7-134, PUSA Baisakhi	PUSA 9971, MUM-16

Table 4.16 correlation matrix of growth index of *C. maculatus* and physical parameters of green gram accessions

Parameters	Growth index	L:B Ratio	Hundred Seed Weight	Seed volume	Seed density	Seed hardness	Seed coat hardness	Seed coat percentage
Growth index	1	0.525	0.507	0.478	-0.252	-0.225	-0.784**	0.486
L:BRatio		1	0.310	0.364	0.034	-0.204	-0.507	0.459
Hundred Seed Weight			1	0.794**	-0.202	0.019	-0.249	0.470
Seed volume				1	-0.670*	-0.228	-0.178	0.263
Seed density					1	0.233	-0.015	0.056
Seed hardness						1	0.178	0.391
Seed coat hardness							1	-0.460
Seed coat percentage								1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Table 4.17 correlation matrix of growth index of *C. analis* and physical parameters of green gram accessions

Parameters	Growth index	L:B Ratio	Hundred Seed Weight	Seed volume	Seed density	Seed hardness	Seed coat hardness	Seed coat percentage
Growth index	1	-0.032	0.518	0.767*	-0.130	0.015	-0.765*	-0.011
L:BRatio		1	0.164	-0.089	0.421	0.243	-0.172	0.532
Hundred Seed Weight			1	0.901**	0.141	-0.132	-0.732*	0.204
Seed volume				1	0.058	-0.188	-0.837**	-0.003
Seed density					1	0.284	0.050	-0.129
Seed hardness						1	0.023	0.207
Seed coat hardness							1	-0.305
Seed coat percentage								1

** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed).

Table 4.18 Correlation matrix of growth index of *C. chinensis* and physical parameters of green gram accessions

Parameters	Growth index	L:B Ratio	Hundred Seed Weight	Seed volume	Seed density	Seed hardness	Seed coat hardness	Seed coat percentage
Growth index	1	0.501	0.284	0.148	-0.082	-0.438	-0.555	0.134
L:BRatio		1	0.193	0.016	0.029	-0.605	0.014	-0.002
Hundred Seed Weight			1	0.688*	0.095	-0.303	-0.382	0.178
Seed volume				1	-0.600	-0.076	-0.312	0.264
Seed density					1	-0.137	0.034	-0.204
Seed hardness						1	-0.005	0.019
Seed coat hardness							1	-0.375
Seed coat percentage								1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Table 4.19 Mean, range, coefficients of variation and heritability of eight physical characters among twenty four accessions of mung bean.

Traits	Mean ± SE	Range (Min-max)	σ^2_p	σ^2_g	PCV (%)	GCV (%)	h^2	GA
Length	4.3±0.05	3.77 - 5.33	0.48	0.40	11.2	9.1	81.8	117.1
Breath	3.2±0.03	2.83 - 3.60	0.19	0.16	5.9	5.0	84.0	75.4
Hundred seed weight	3.6±0.06	2.65 - 4.84	0.87	0.85	24.5	23.8	97.3	187.3
Seed volume	0.5±0.02	0.80 - 0.39	0.02	0.03	4.9	4.7	95.1	31.9
Seed density	1.4±0.04	1.12 - 1.71	0.07	0.06	4.9	4.6	94.5	50.4
Seed hardness	48.1±1.81	29.4 - 88.5	731.47	731.47	1521.2	1521.2	100	5571.4
Seed coat hardness	48.0±0.90	40.8 - 58.5	84.77	47.91	176.6	99.80	56.6	1071.9
Seed coat percentage	14.9±0.37	19.4 - 10.4	22.16	14.54	148.4	97.3	65.6	636.1

Table 4.20 Biochemical parameters of selected Green gram accessions

Green gram accessions	Soluble protein (mg/g seed)	Crude protein (%)	Starch (mg/g seed)	TSS (mg/ g seed)	Phenol (mg/ g seed)	Tannin (mg/g seed)	AI (%)
KM-11-11	186.00±1.16 ^{hi}	14.99±0.06 ⁱ	172.47±1.54 ^f	1.06±0.03 ^{ij}	4.03±0.28 ^a	4.66±0.10 ^b	43.61±0.33 ^c
KM 12 -10	175.33±5.81 ^{jk}	22.84±0.05 ^d	176.94±0.93 ^f	1.12±0.01 ^{hij}	2.19±0.03 ^g	5.82±0.05 ^a	65.72±0.60 ^a
KM 11-32	193.33±1.76 ^h	22.93±0.13 ^d	180.41±1.32 ^f	0.85±0.03 ^k	2.28±0.03 ^{efg}	5.95±0.09 ^a	43.69±0.53 ^c
KM 11-10	173.67±3.53 ^k	21.29±0.11 ^f	178.37±2.78 ^f	0.45±0.06 ^{lm}	3.24±0.42 ^{abcde}	5.64±0.18 ^a	23.54±0.45 ^{ij}
KM-12-5	176.33±1.45 ^{ijk}	14.99±0.06 ⁱ	179.80±2.71 ^f	1.06±0.03 ^{ij}	2.11±.002 ^g	4.66±0.10 ^b	48.87±0.47 ^b
P-S-16	204.33±3.76 ^g	23.04±0.03 ^d	184.46±3.15 ^f	1.30±0.02 ^{fg}	2.94±0.18 ^{bcdefg}	4.10±0.19 ^{cd}	31.95±0.55 ^{ef}
KM 12 -32	210.67±0.67 ^{efg}	21.47±0.11 ^f	182.13±1.87 ^f	1.09±0.04 ^{ij}	3.12±0.31 ^{abcdefg}	3.60±0.43 ^{de}	30.30±0.60 ^{fg}
KM 12 -7	206.67±2.91 ^{fg}	20.77±0.06 ^g	181.24±3.89 ^f	1.48±0.01 ^{de}	3.36±.010 ^{abcd}	4.19±0.05 ^{bc}	23.97±0.27 ⁱ
MH 96-1	246.67±4.06 ^c	24.30±0.56 ^b	181.59±3.79 ^f	1.52±0.06 ^d	3.48±0.63 ^{abc}	3.09±0.45 ^{ef}	33.54±0.98 ^e
KM-12-9	215.33±2.40 ^{ef}	21.53±0.05 ^f	181.59±2.02 ^f	1.02±0.07 ^j	3.24±0.26 ^{abcde}	2.99±0.19 ^{fg}	27.65±0.70 ^h
KM-7-134	184.67±1.76 ^{hij}	13.01±0.06 ^j	301.13±1.12 ^c	0.08±0.01 ⁿ	3.90±0.16 ^{ab}	5.54±0.12 ^a	37.94±0.77 ^d
MUM-16	218.67±1.76 ^e	24.56±0.06 ^b	181.95±1.87 ^f	4.25±0.13 ^a	3.30±0.48 ^{abcd}	3.91±0.13 ^{cd}	23.64±0.61 ⁱ
PUSABAIKAKHI	264.67±2.40 ^b	23.66±0.03 ^c	185.18±4.91 ^f	0.53±0.03 ^l	2.82±0.49 ^{cdefg}	2.84±0.18 ^{fg}	31.79±0.83 ^{ef}
GANGA -1	250.67±2.40 ^c	22.84±0.09 ^d	185.00±2.85 ^f	1.99±0.03 ^c	3.18±0.22 ^{abcdef}	3.08±0.05 ^{ef}	13.46±0.79 ^k
KM 11-40	228.67±0.67 ^d	20.21±0.05 ^h	281.42±0.78 ^{cd}	0.36±0.02 ^m	3.24±0.22 ^{abcde}	3.97±0.08 ^{cd}	20.97±0.25 ^j
KM-12-19	278.67±0.67 ^a	22.29±0.06 ^e	187.51±1.71 ^f	1.37±0.01 ^{ef}	3.48±0.18 ^{abc}	2.51±0.04 ^{ghi}	48.61±0.42 ^b
MUM-2	268.00±4.16 ^b	23.86±0.13 ^c	275.32±3.28 ^d	2.67±0.03 ^b	2.88±0.81 ^{cdefg}	2.73±0.03 ^{fgh}	24.56±0.31 ⁱ
KM 12 -23	264.00±5.03 ^b	22.23±0.10 ^e	182.49±4.05 ^f	0.56±0.02 ^l	2.76±0.21 ^{cdefg}	2.68±.06 ^{fghi}	41.54±0.75 ^c
PUSA 9971	264.00±6.43 ^b	22.90±0.11 ^d	192.35±1.40 ^f	1.48±0.02 ^{de}	2.82±0.25 ^{cdefg}	2.73±0.16 ^{fgh}	5.22±0.49 ^l
PDM 91-269	205.67±5.24 ^{fg}	23.66±0.03 ^c	236.43±2.41 ^e	0.53±0.03 ^l	3.17±0.16 ^{abcdef}	2.95±0.27 ^{fg}	14.10±0.57 ^k
PUSA 0831	266.67±1.77 ^b	22.26±0.08 ^e	193.42±0.65 ^f	1.03±0.03 ^j	2.82±0.26 ^{cdefg}	2.62±0.02 ^{fghi}	44.00±0.48 ^c
M 1319B	279.33±3.71 ^a	10.06±0.05 ^k	183.42±3.40 ^f	0.78±0.04 ^k	2.70±0.3 ^{cdefg}	2.66±0.29 ^{fghi}	5.20±0.14 ^l
PUSA 1031	282.67±5.46 ^a	25.93±0.08 ^a	555.33±4.34 ^a	1.16±0.01 ^{hi}	2.64±0.33 ^{cdefg}	2.20±0.09 ⁱ	28.56±0.57 ^{gh}
GANGA -8	260.67±3.53 ^b	23.80±0.10 ^c	378.65±4.85 ^b	1.21±0.08 ^{gh}	2.39±0.69 ^{defg}	2.25±0.09 ⁱ	43.95±0.75 ^c
LSD @ 5%	9.87	0.40	21.19	0.12	1.02	0.53	2.58
SE(d)	4.89	0.20	10.59	0.06	0.50	0.26	1.32
SE(m)	3.45	0.14	7.49	0.04	0.36	0.18	0.93

All values represent mean of three replications, Values represent Mean ± Standard Error.

Table 4.21 Frequency distribution of green gram accessions with respect to biochemical parameters

Protein	Low(170-220)	Medium(220.1-270)	High (>270)
(mg/g seed)	KM-11-11, KM 12 -10, KM 11-32, KM 11-10, KM-12-5, P-S-16, KM 12 -32, KM 12 -7, KM-12-9, KM-7-134, MUM-16, PDM 91-269	MH 96-1, PUSA Baisakhi, Ganga -1, KM 11-40, MUM-2, KM 12 -23, PUSA 9971, PUSA 0831, Ganga -8	KM 12 -9, M1319B, PUSA 1031
Crude protein	Low(10-16)	Medium (16.1-22)	High (>22.1)
(%)	M 1319B, KM-7-134, KM-12-5, KM-11-11	KM 11-40, KM-12-9, KM 12 -7, KM 12 -32, KM 11-10	PUSA 1031, Ganga -8, KM-12-19, MUM-2, KM 12 -23, PUSA 9971, PDM 91-269, PUSA 0831, Ganga -1, PUSA Baisakhi, MUM-16, MH 96-1, P-S-16, KM 11-32, KM 12 -10
Starch	Low(172-272)	Medium (272.1-372)	High (>372.1)
(mg/g seed)	KM-11-11, KM 12 -10, KM 11-32, KM 11-10, KM-12-5, P-S-16, KM 12 -32, KM 12 -7, MH 96-1, KM-12-9, MUM-16, PUSA Baisakhi, Ganga -1, KM-12-19, KM 12 -23, PUSA 9971, PDM 91-269, PUSA 0831, Ganga -8	KM-7-134, KM-11-40, MUM-2	M1319B, PUSA 1031
TSS	Low(0.4-1.4)	Medium (1.41-2.4)	High (>2.41)
(mg/g seed)	KM-11-11, KM 12 -10, KM 11-32, KM 11-10, KM-12-5, P-S-16, KM 12 -32, KM-7-134, KM-12-9, PUSA Baisakhi, KM 11-40, KM-12-19, KM 12 -23, PDM 91-269, PUSA 0831, M 1319B, PUSA 1031, Ganga -8	KM-12-7, MH-96-1, Ganga-1, PUSA 9971	MUM-2, MUM-16
Phenol	Low(2.3- 3.0)	Medium (3.1-3.7)	High (>3.71)
(mg/g seed)	KM 12 -10, KM 11-32, KM-12-5, PUSA Baisakhi, MUM-2, KM 12 -23, PUSA 9971, PUSA 0831, M 1319B, PUSA 1031, Ganga -8	KM 11-10, P-S-16, KM 12 -32, KM 12 -7, MH 96-1, KM-12-9, MUM-16, Ganga -1, KM 11-40, KM-12-19, PDM 91-269	KM-11-11, KM-7-134
Tannin	Low(2.2- 3.2)	Medium (3.21-4.2)	High (>4.21)
(mg/g seed)	MH 96-1, KM-12-9, PUSA Baisakhi, Ganga -1, KM-12-19, MUM-2, KM 12 -23, PUSA 9971, PDM 91-269, PUSA 0831, M 1319B, PUSA 1031, Ganga -8	P-S-16, KM 12 -32, KM 12 -7 MUM-16, KM 11-40	KM-11-11, KM 12 -10, KM 11-32 KM 11-10, KM-12-5, KM-7-134
α amylase inhibitor (%)	Low(5-25)	Medium (25.1-45)	High (>45)
	KM- 11-10, KM-12-7, MUN-16, Ganga-1, KM-11-40, MUN-2, PUSA 9971, PDM 91-269, M1319B	KN-11-11, KM-11-32, P-S-16, KM-12-32, MH-96-1, KM-12-9, KM-7-134, Pusa baisakhi, KM-12-23, PUSA 0831, Ganga 8,	KM-12-5, KM-12-10, KM-12-19

Table 4.22 Correlation matrix of growth index of *C. maculatus* and physical parameters of green gram accessions

Parameters	Growth index	Protein	Crude protein	Starch	TSS	Phenol	Tanin	α amylase inhibitor
Growth index	1	0.964**	-0.344	0.580*	0.206	0.242	0.933**	0.701*
Protein		1	0.331	0.577*	0.199	0.067	-0.939**	-0.600*
Crude protein			1	0.788**	0.322	0.061	0.243	0.398
Starch				1	-0.049	-0.048	-0.437	-0.460
TSS					1	0.264	-0.266	-0.204
Phenol						1	-0.303	-0.582*
Tannin							1	0.628*
α amylase inhibitor								1

** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed).

Table 4.23 Correlation matrix of growth index of *C. analis* and physical parameters of green gram accessions

Parameters	Growth index	Protein	Crude protein	Starch	TSS	Phenol	Tanin	α amylase inhibitor
Growth index	1	0.624	0.565	0.362	-0.493	0.072	-0.801*	-0.291
Protein		1	0.791*	0.640	0.149	0.031	-0.877**	-0.057
Crude protein			1	0.474	0.047	0.460	-0.799*	-0.550
Starch				1	0.034	-0.188	-0.515	-0.253
TSS					1	0.244	0.268	0.296
Phenol						1	-0.154	-0.694
Tannin							1	0.334
α amylase inhibitor								1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Table 4.24 Correlation matrix of growth index of *C. chiensis* and physical parameters of green gram accessions

Parameters	Growth index	Protein	Crude protein	Starch	TSS	Phenol	Tanin	α amylase inhibitor
Growth index	1	0.823**	0.545	0.095	0.210	-0.598	-0.742*	0.277
Protein		1	0.581	-0.250	0.073	-0.570	-0.949**	0.516
Crude protein			1	-0.655**	0.441	-0.808**	-0.643*	-0.263
Starch				1	-0.340	0.408	0.411	0.119
TSS					1	0.010	-0.141	-0.336
Phenol						1	0.656*	0.077
Tannin							1	-0.488
α amylase inhibitor								1

** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed).

Table 4.25 Oviposition preference and adult emergence of *C. maculatus* from various combinations of artificial seeds made from resistant and susceptible green gram accession.

Accession	No. of eggs/10seed	No. of Adult Emergence
25% KM-12-10 + 75% M1319B	12.67±0.88 ^a	0.67±0.67 ^b
50% KM-12-10 + 50% M1319B	11.33±0.67 ^a	0.00±0.00 ^b
75% KM-12-10 + 25% M1319B	11.67±1.45 ^a	0.00±0.00 ^b
100% KM-12-10	10.67±1.67 ^a	0.00±0.00 ^b
25% KM-11-10 + 75% M1319B	12.67±0.67 ^a	1.00±0.58 ^b
50% KM-11-10 + 50% M1319B	10.67±1.20 ^a	0.67±0.67 ^b
75% KM-11-10 + 25% M1319B	12.00±0.58 ^a	0.00±0.00 ^b
100% KM-11-10	12.33±0.88 ^a	0.00±0.00 ^b
100% M1319B	12.00±0.58 ^a	6.33±0.33 ^a
LSD @ 5%	3.21	1.22
SE(d)	1.45	0.54
SE(m)	1.02	0.39

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at $P = 0.05$

Table 4.26 Oviposition preference and adult emergence of *C. analis* from various combinations of artificial seeds made from resistant and susceptible green gram accession.

Accession	No. of eggs/10seed	No. of Adult Emergence
25% KM-12-5+ 75% Ganga 8	10.67±0.33 ^{bc}	0.00±0.00 ^b
50% KM-12-5+ 50% Ganga 8	11.00±0.58 ^{bc}	0.00±0.00 ^b
75% KM-12-5+ 25% Ganga 8	10.33±1.20 ^{bc}	0.00±0.00 ^b
100% KM-12-5	9.67±0.67 ^c	0.00±0.00 ^b
25% PS-16+ 75% Ganga 8	12.67±0.67 ^{ab}	1.00±0.58 ^b
50% PS-16+ 50% Ganga 8	11.67±1.20 ^{abc}	0.00±0.00 ^b
75% PS-16+ 25% Ganga 8	11.33±1.20 ^{bc}	0.33±0.33 ^b
100% PS-16	12.33±0.88 ^{abc}	0.00±0.00 ^b
100% Ganga 8	14.33±1.86 ^a	5.67±1.76 ^a
LSD @ 5%	2.96	1.90
SE(d)	1.48	0.89
SE(m)	1.05	0.63

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at $P = 0.05$

Table 4.27 Oviposition preference and adult emergence of *C. chinensis* from various combinations of artificial seeds made from resistant and susceptible green gram accession.

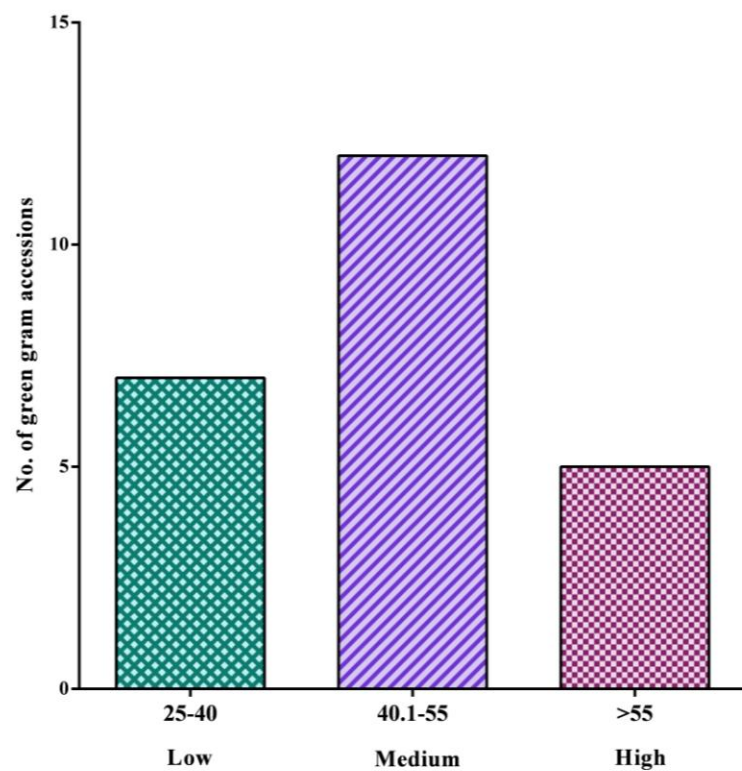
Accession	No. of eggs/10seed	No. of Adult Emergence
25% KM-11-10+ 75% PUSA 0831	11.67±0.33 ^a	0.67±0.67 ^b
50% KM-11-10+ 50% PUSA 0831	11.33±0.67 ^a	0.33±0.33 ^b
75% KM-11-10+ 25% PUSA 0831	10.33±1.20 ^a	0.00±0.00 ^b
100% KM-11-10	10.33±0.88 ^a	0.00±0.00 ^b
25% PS-16+ 75% PUSA 0831	11.33±0.67 ^a	0.33±0.33 ^b
50% PS-16+ 50% PUSA 0831	11.00±0.58 ^a	1.00±0.58 ^b
75% PS-16+ 25% PUSA 0831	11.67±0.88 ^a	0.00±0.00 ^b
100% PS-16	10.67±0.88 ^a	0.00±0.00 ^b
100% PUSA 0831	12.33±0.88 ^a	7.00±0.58 ^a
LSD @ 5%	2.47	1.20
SE(d)	1.14	0.54
SE(m)	0.81	0.39

All values represent mean of three replications, Values represent Mean ± Standard Error.
Means in a column followed by different letter(s) are significantly different at *P* = 0.05

Table 4.28 Primer sequence, Temperature (⁰C), and Number of alleles of adzuki specific SSR markers transferable to other *Vigna* species.

Primer	Forward Primer	Reverse Primer	Motif	Temp. (⁰C)	Number of alleles	PIC value	Resolving power
CEDG021	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC	(AG)26	57	3	0.44	1.12
CEDG037	GAAGAAGAACCCTACCACAG	CACCAAAAACGTTCCCTCAG	(AG)16AC(AG)8	57	2	0.32	1.36
CEDG044	TCAGCAACCTTGCATTGCAG	TTTCCCGTCCACTTCTTCTAGG	(GT)10AT(AG)18	57	3	0.36	1.27
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	58	3	0.36	1.27
CEDG154	GTCCTTGTTTTCTCTCCATGG	CATCAGCTGTTCAACACCCTGTG	(AG)14	58	2	0.43	1.14
CEDG178	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	(AG)10G(AG)5	58	3	0.45	1.10
CEDG214	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC	(AG)4AA(AG)31	58	2	0.49	1.01
CEDG305	GCAGCTTCACATGCATAGTAC	GAACCTAACTTGGGTTGTCTGC	(AG)22	58	2	0.49	1.02

Catagorization of seed hardness in different green gram accessions



Catagorization of seed coat hardness in different green gram accessions

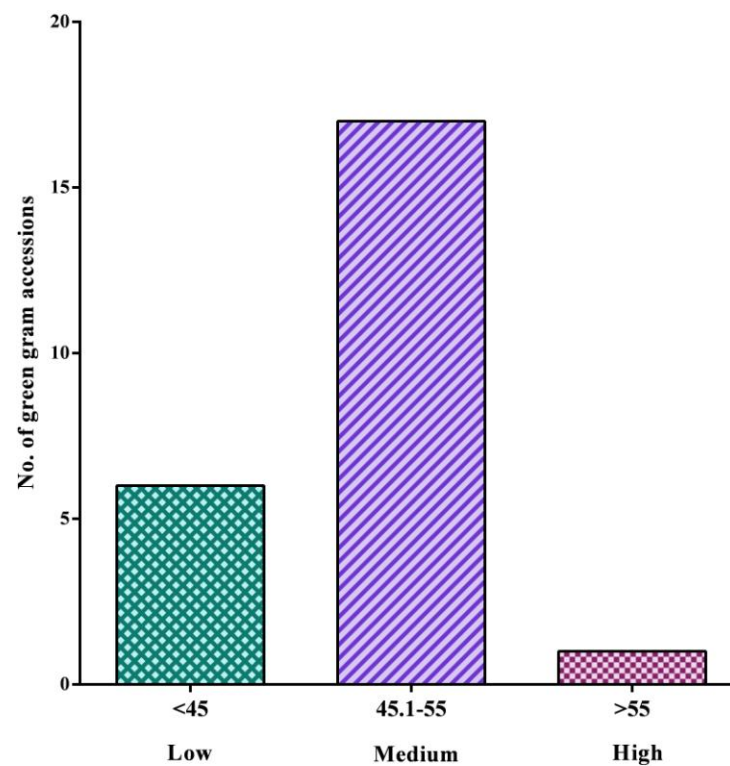
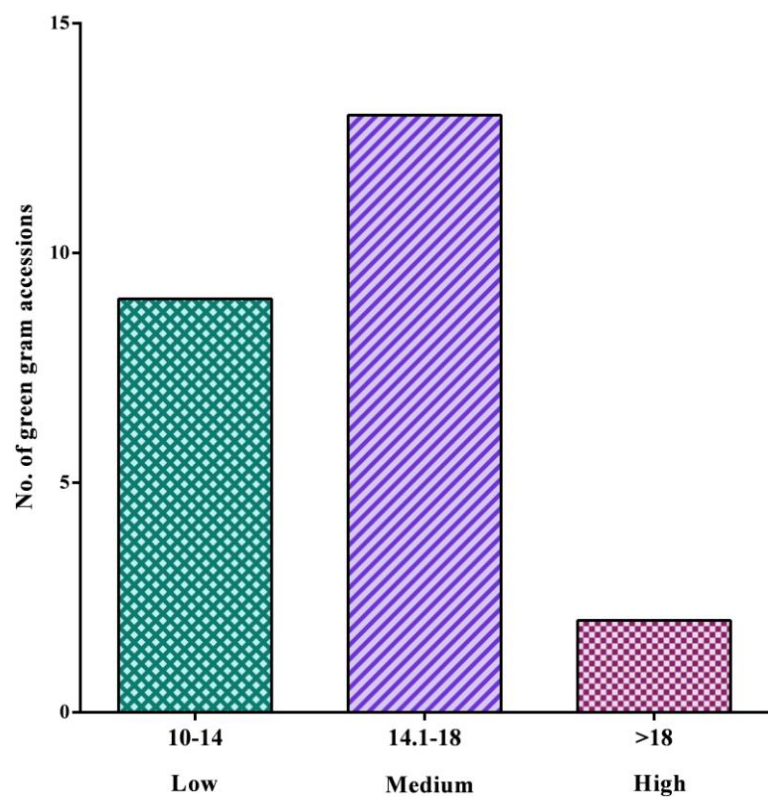


Fig 4.11 Frequency distribution of seed hardness (a) and seed coat hardness (b)

Catagorization of seed coat percentage in different green gram accessions



Catagorization of 100 seed weight in different green gram accessions

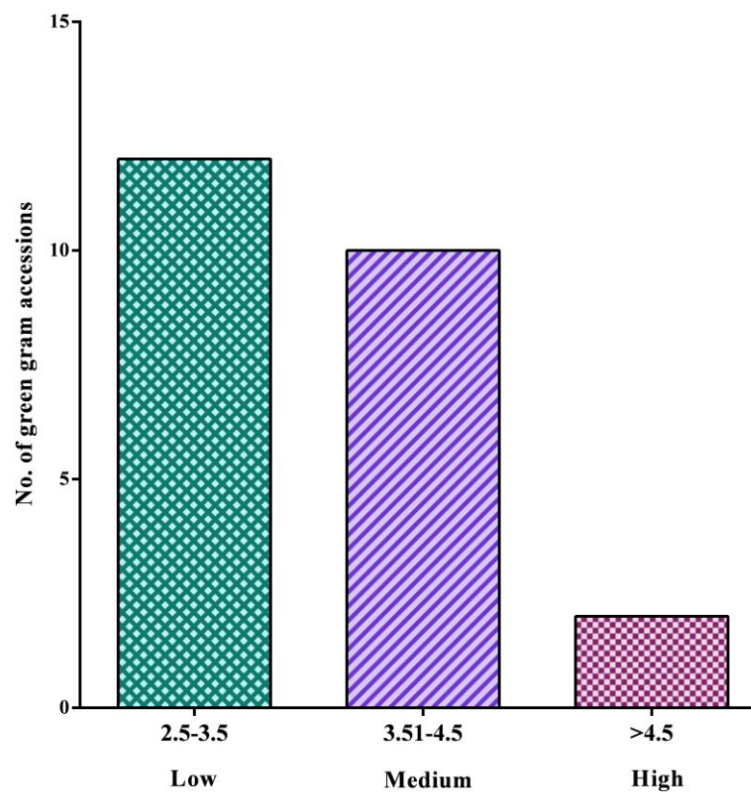
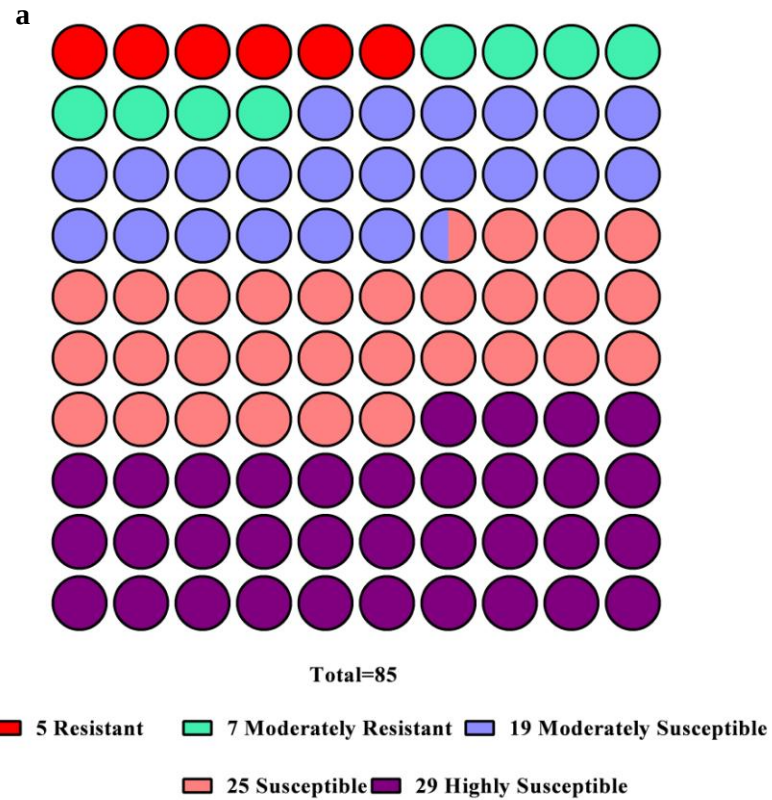


Fig 4.12 Frequency distribution of seed coat percentage (a) and 100-seed weight (b)

Reaction of green gram accessions to *C. maculatus* under free choice test



Reaction of different green gram accessions against *C. maculatus*

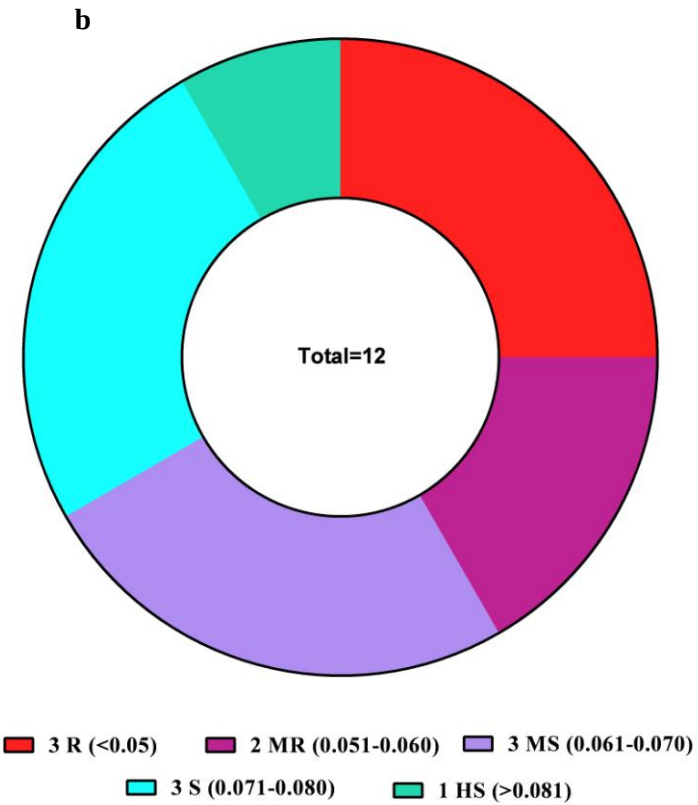
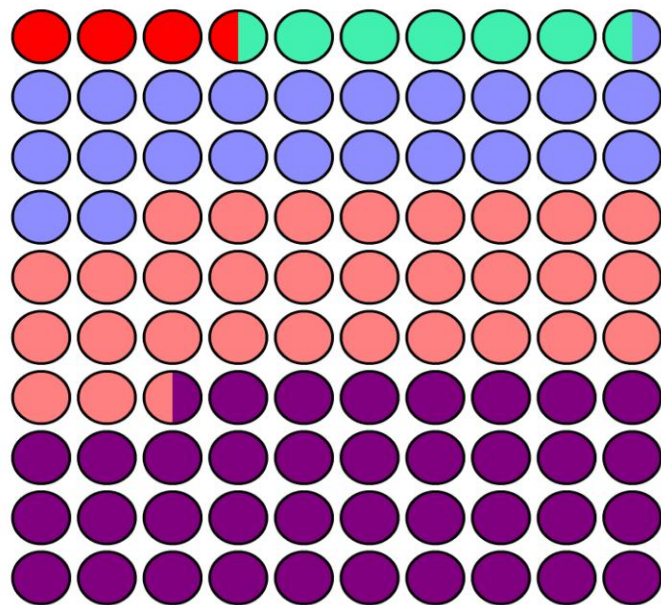


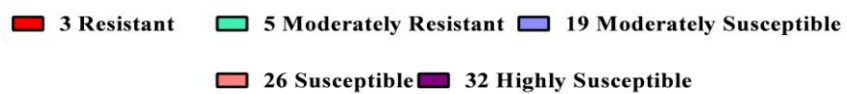
Fig: 4.1 Categorization of differential reaction of *C. maculatus* under free choice test (a) and force choice test (b)

Reaction of green gram accessions to *C. analis* under free choice test

a



Total=85



Reaction of different green gram accessions against *C. analis*

b

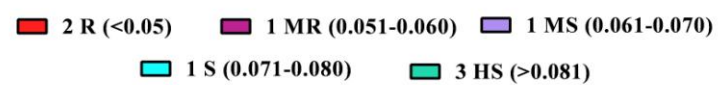
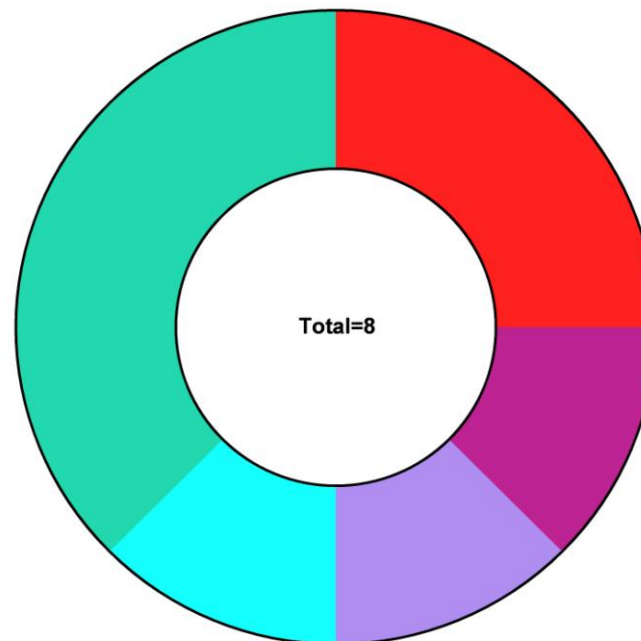
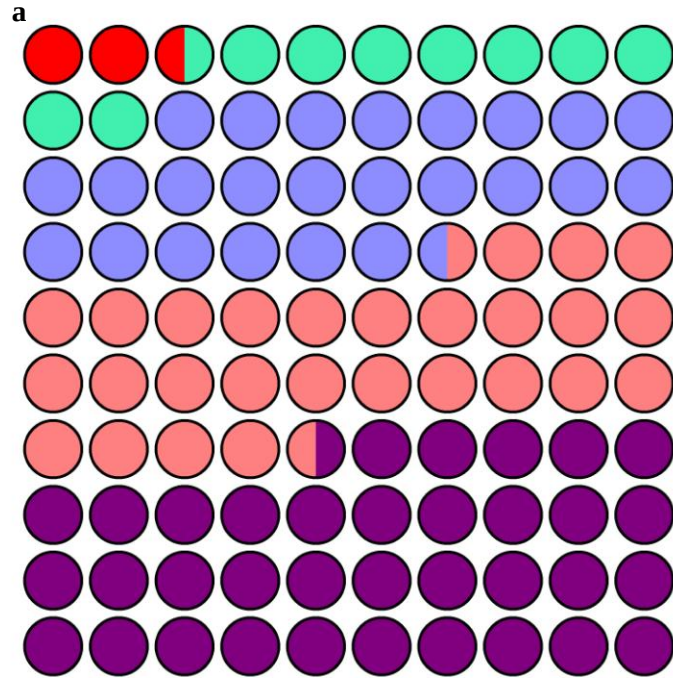


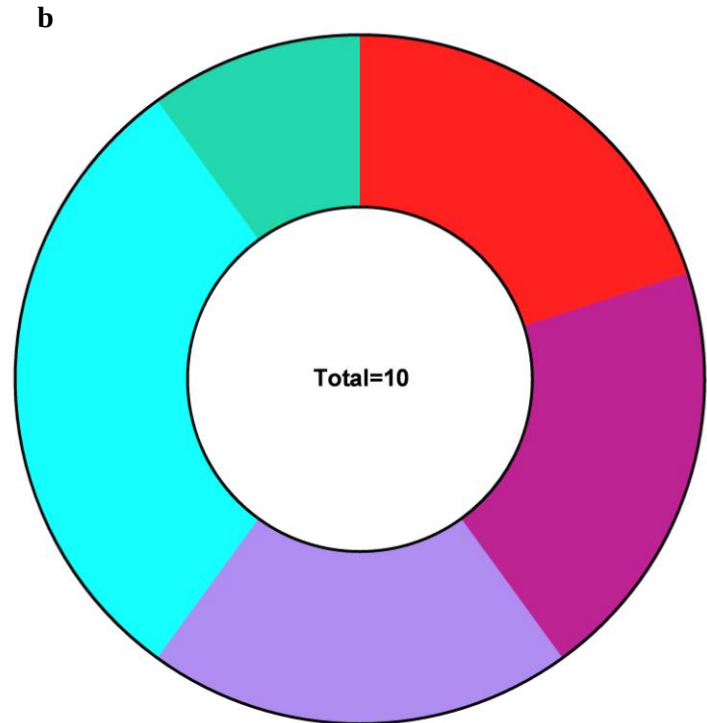
Fig: 4.2 Categorization of differential reaction of *C. analis* under free choice test (a) and force choice test (b)

Reaction of green gram accessions to *C. chinensis* under free choice test



■ 2 Resistant
 ■ 8 Moderately Resistant
 ■ 21 Moderately Susceptible
■ 24 Susceptible
 ■ 30 Highly Susceptible

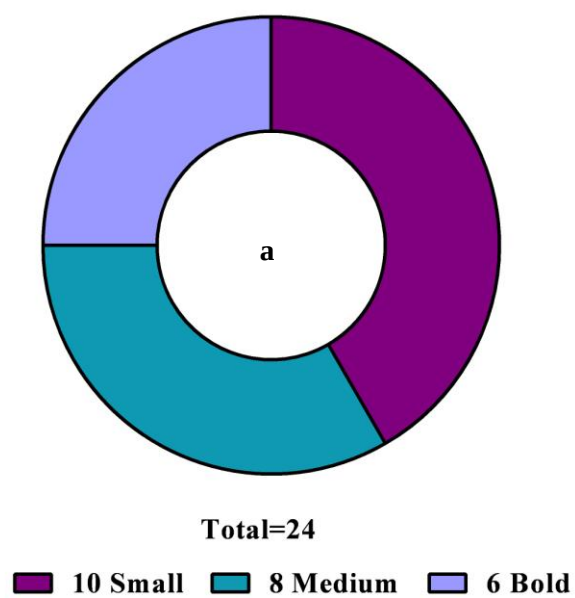
Reaction of different green gram accessions against *C. chinensis*



■ 2 R (<0.05)
 ■ 2 MR (0.051-0.060)
 ■ 2 MS (0.061-0.070)
■ 3 S (0.071-0.080)
■ 1 HS (>0.081)

Fig: 4.3 Categorization of differential reaction of *C. chinensis* under free choice test (a) and force choice test (b)

Catagorisation of seed stature in different green gram accessions



Catagorisation of seed lustre in green gram accessions

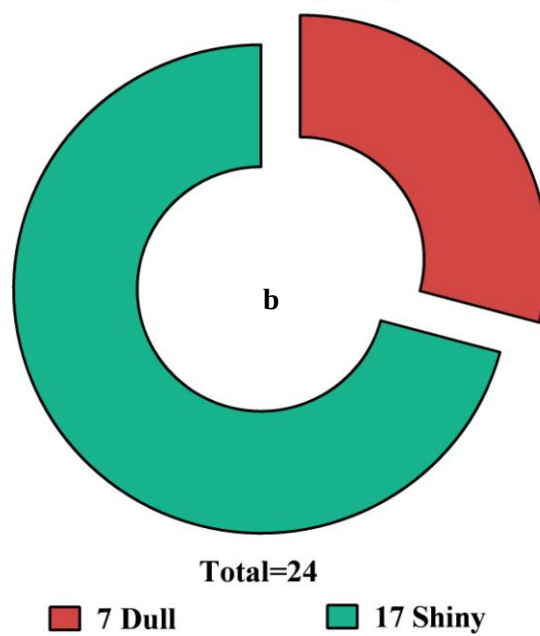


Fig 4.9 Frequency distribution of seed stature (a) and seed luster (b)

Catagorisation of seed colour in green gram accessions

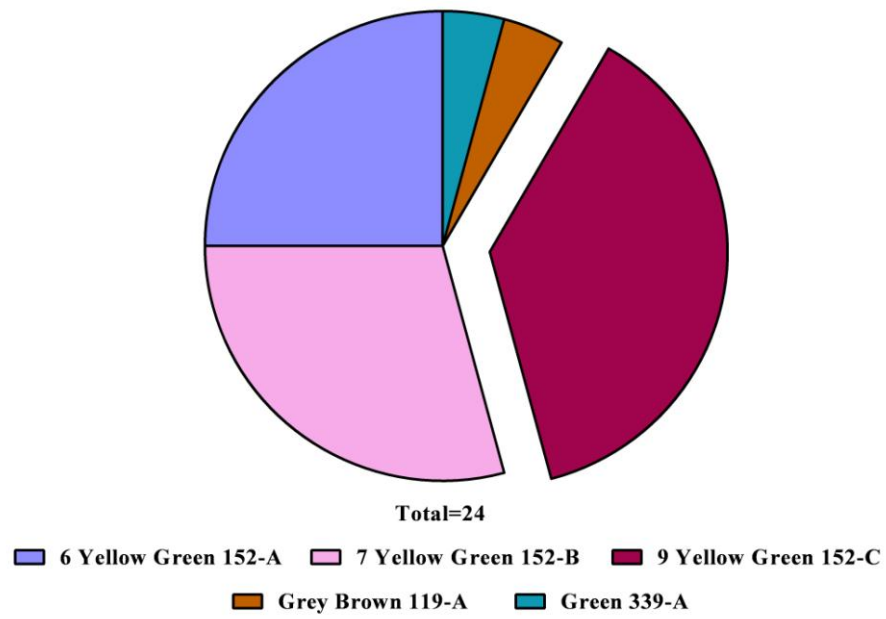


Fig 4.10 Frequency distribution of seed colour

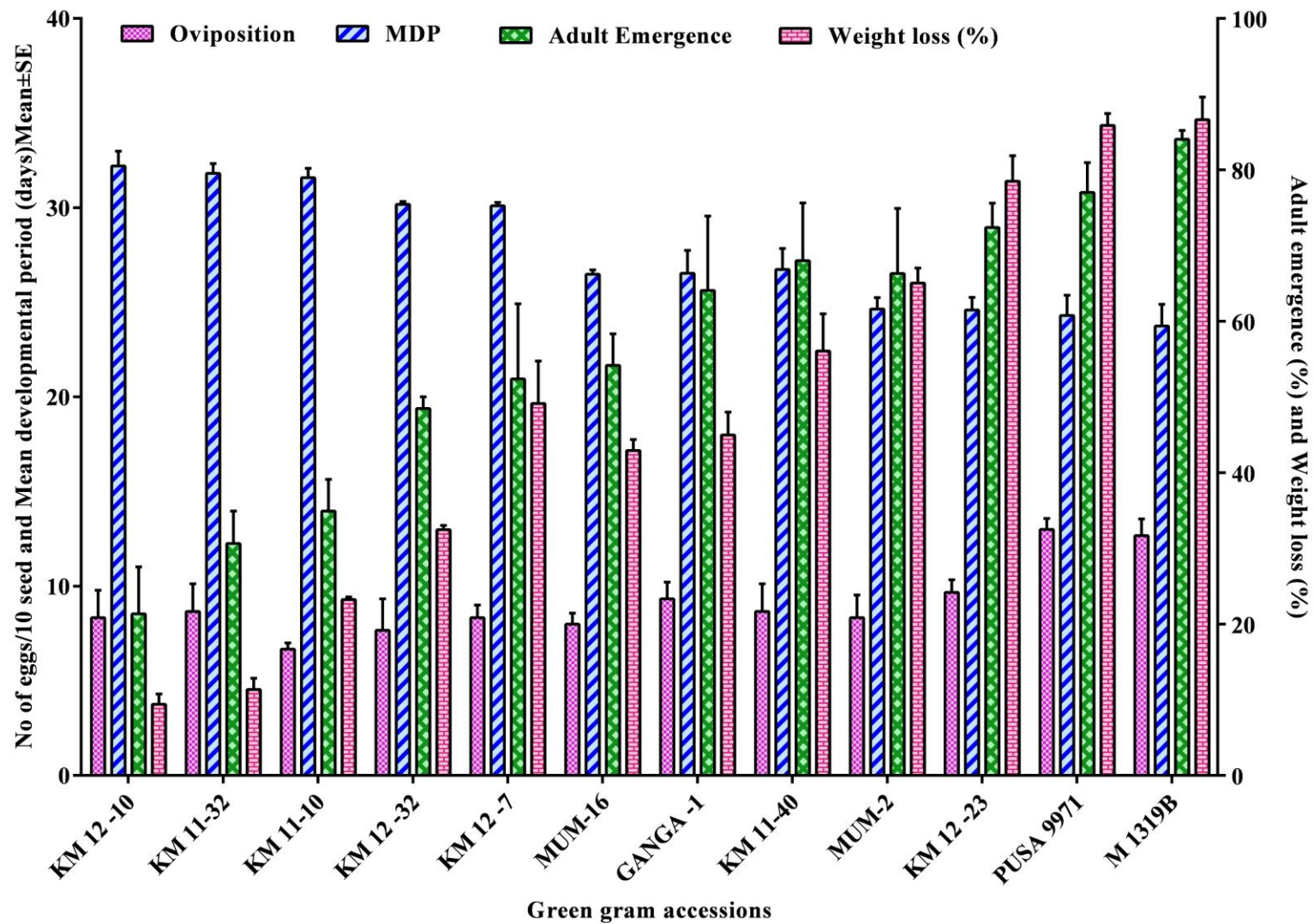


Fig 4.4 Reaction of green gram accessions to *C. maculatus* under 'force choice' test

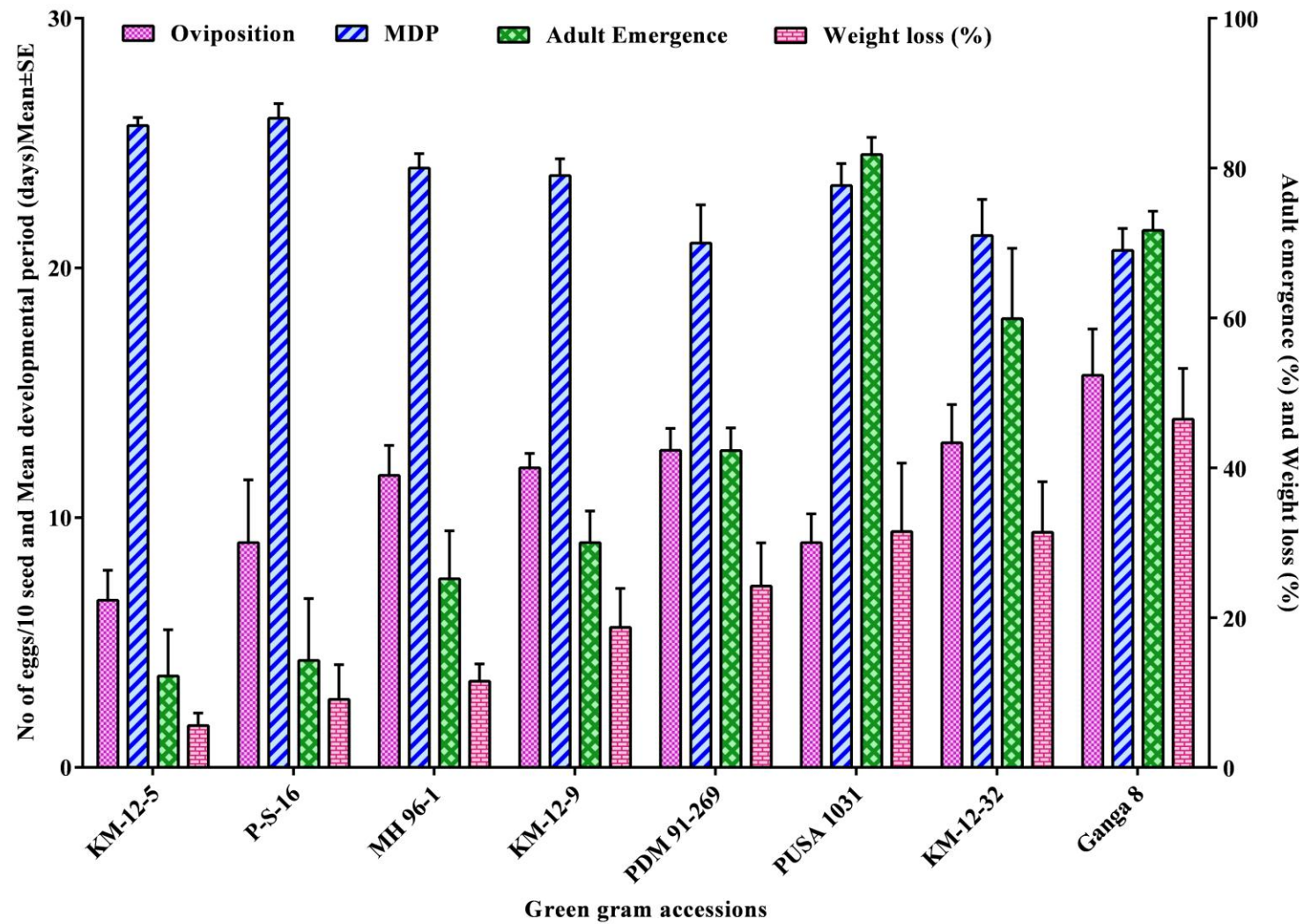


Fig 4.5 Reaction of green gram accessions to *C. analis* under 'force choice' test

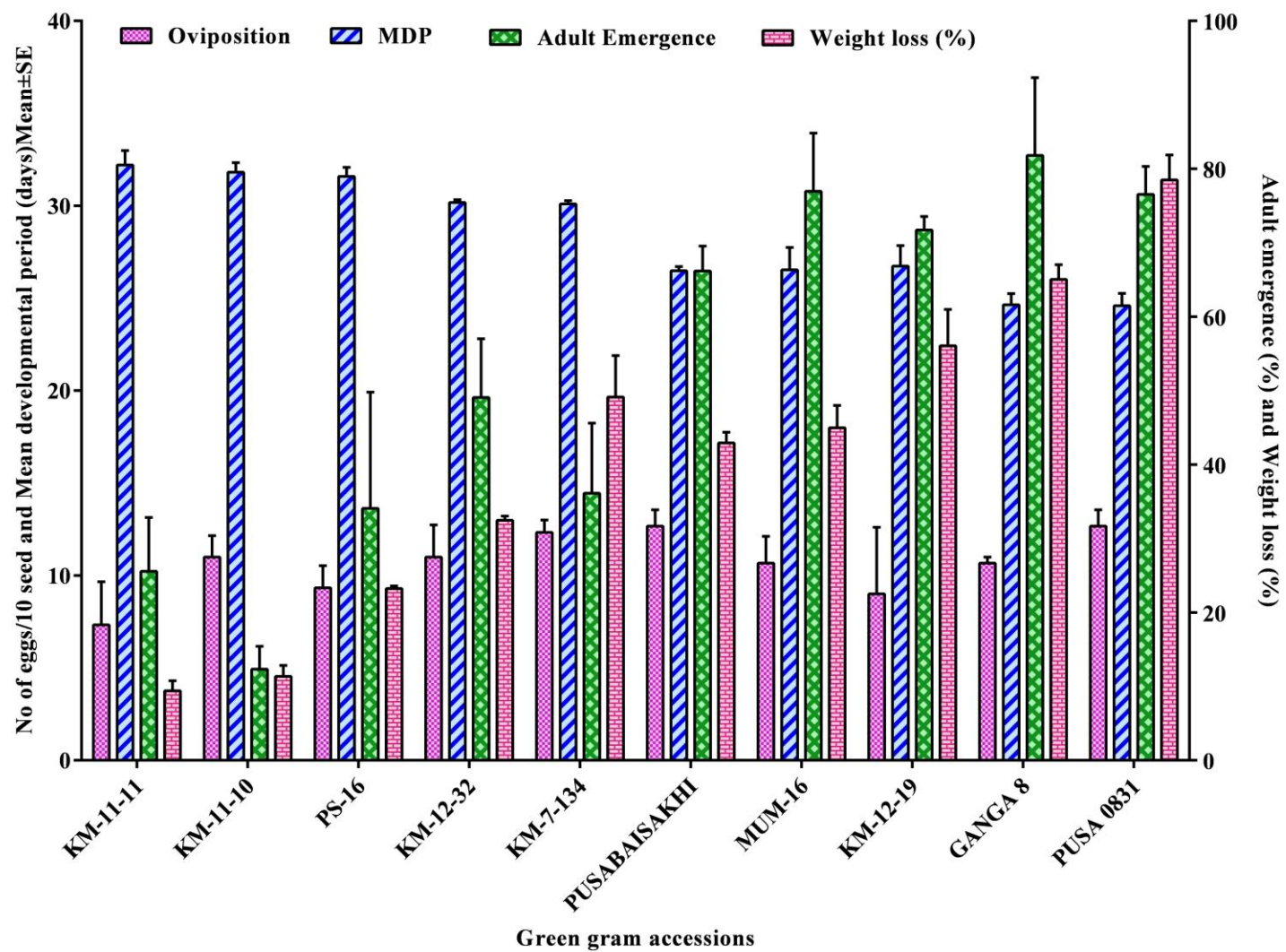


Fig 4.6 Reaction of green gram accessions to *C. chinensis* under 'force choice' test

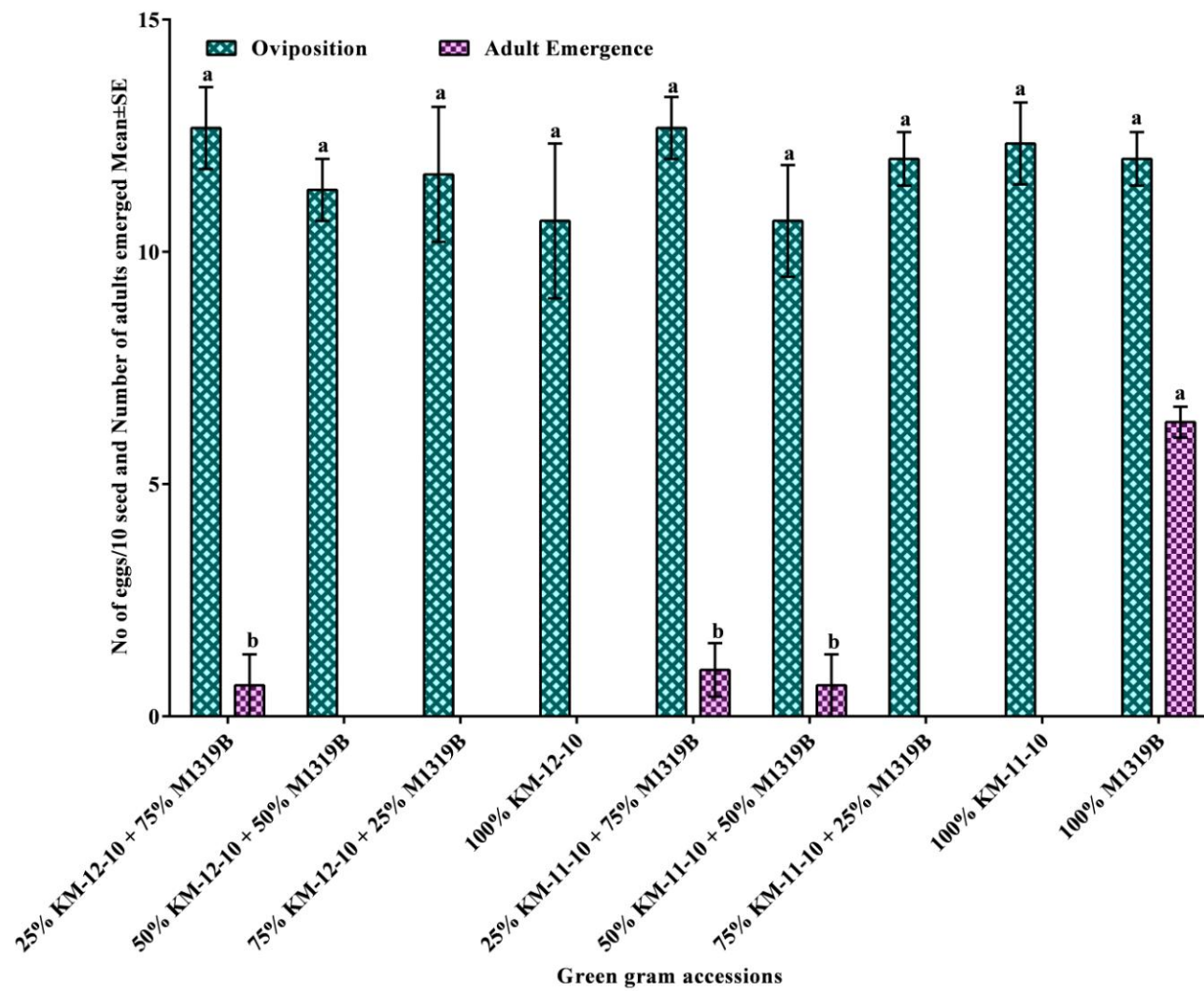


Fig 4.13 Artificial seed bioassay against *C. maculatus* under 'force choice' test

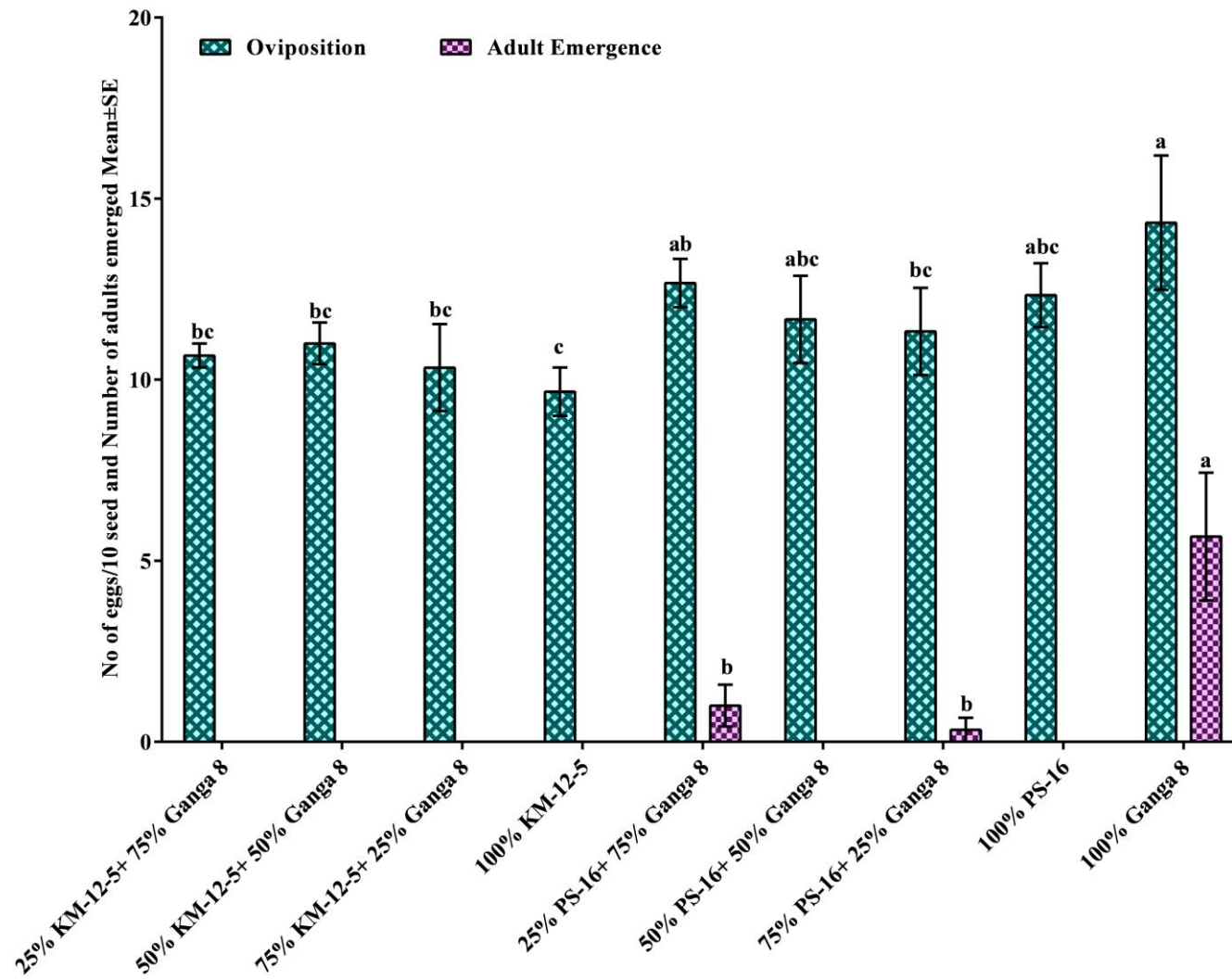


Fig 4.14 Artificial seed bioassay against *C. analis* under 'force choice' test

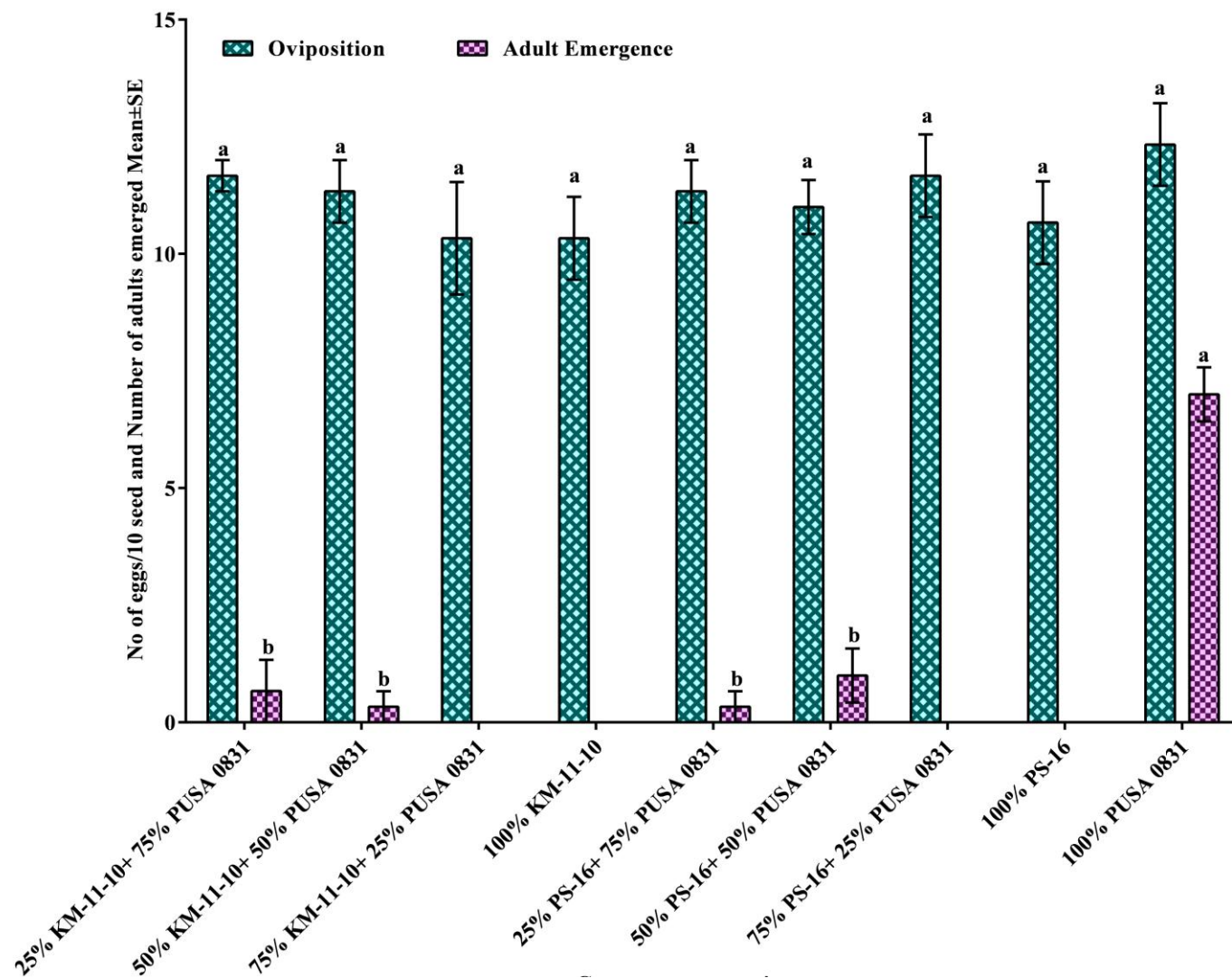


Fig 4.15 Artificial seed bioassay against *C. chinensis* under ‘force choice’ test

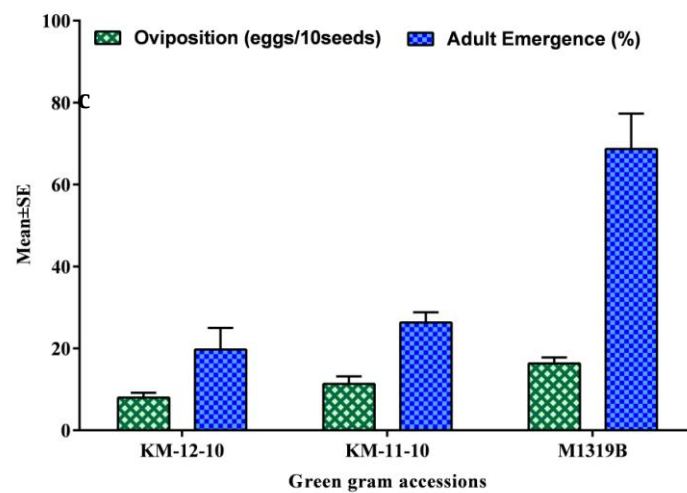
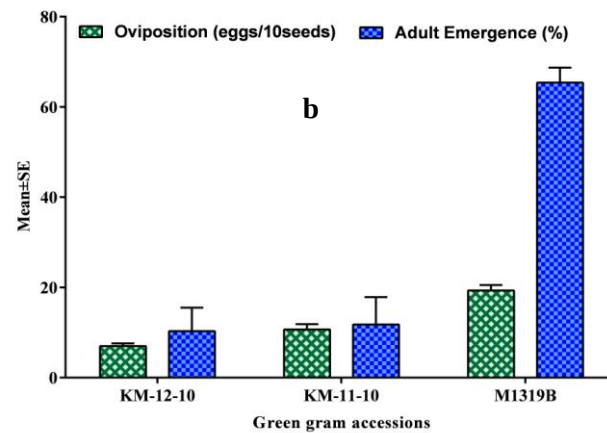
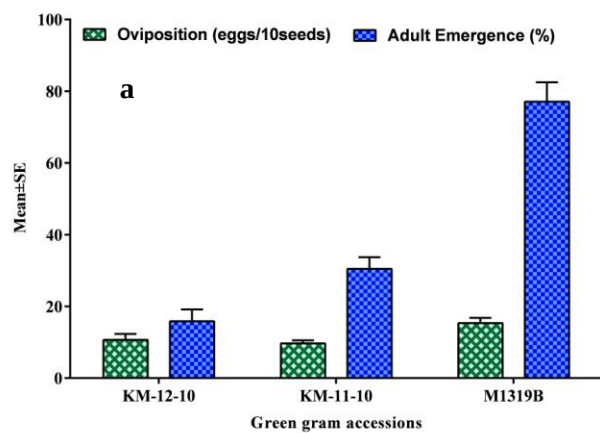


Fig 4.8 Force choice assay with decorticated seed against *C. maculatus* (a), *C. analis* (b) and *C. chinensis* (c)

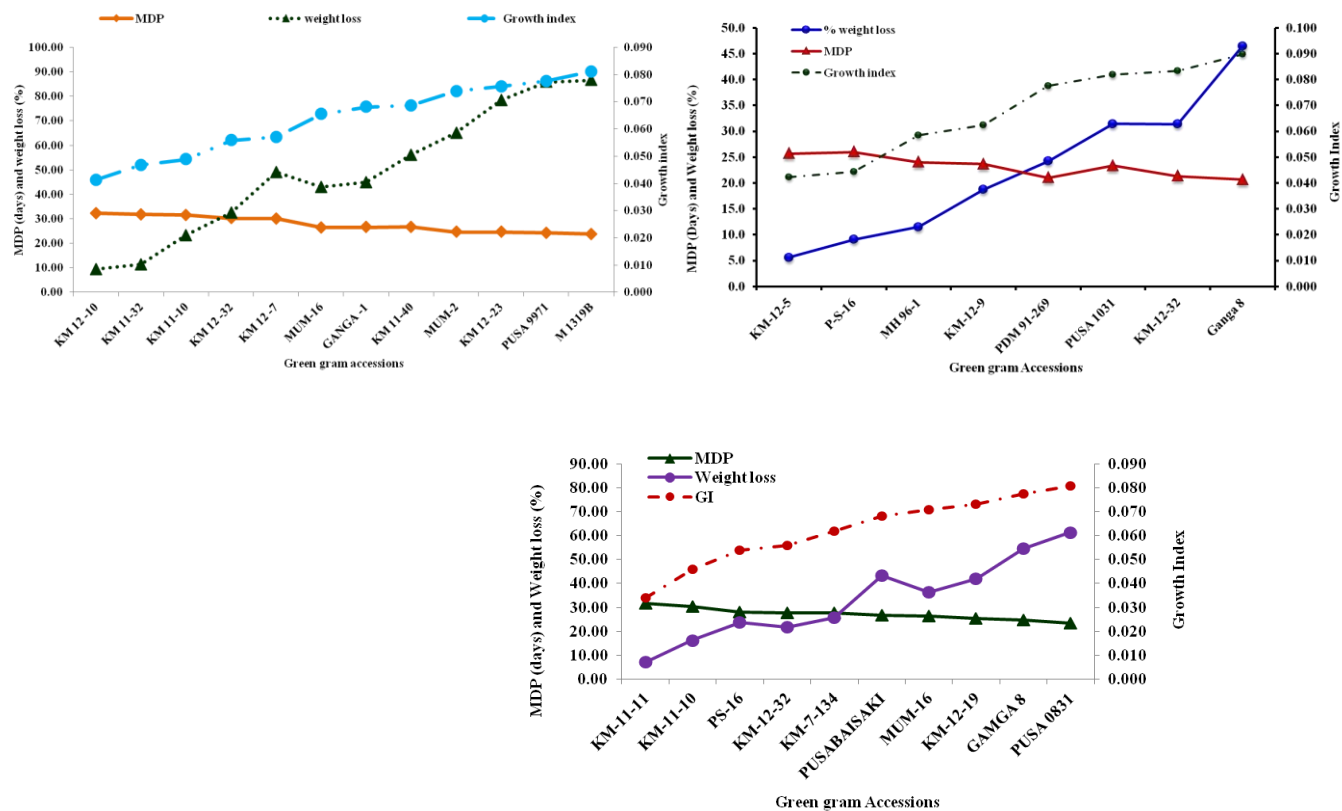


Fig 4.7 Correlation of GI of *C. maculatus* (a), *C. analis* (b) and *C. chinensis* (c) with MDP and Wight loss

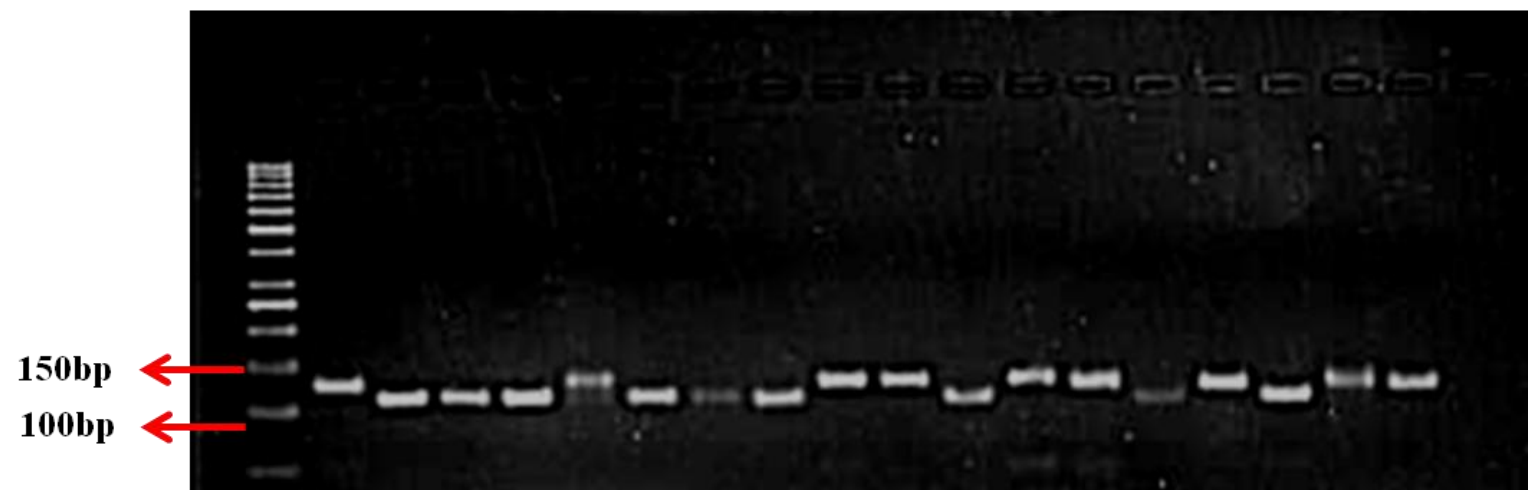


Plate 4.1 Detection of polymorphism using SSR markers in green gram accessions

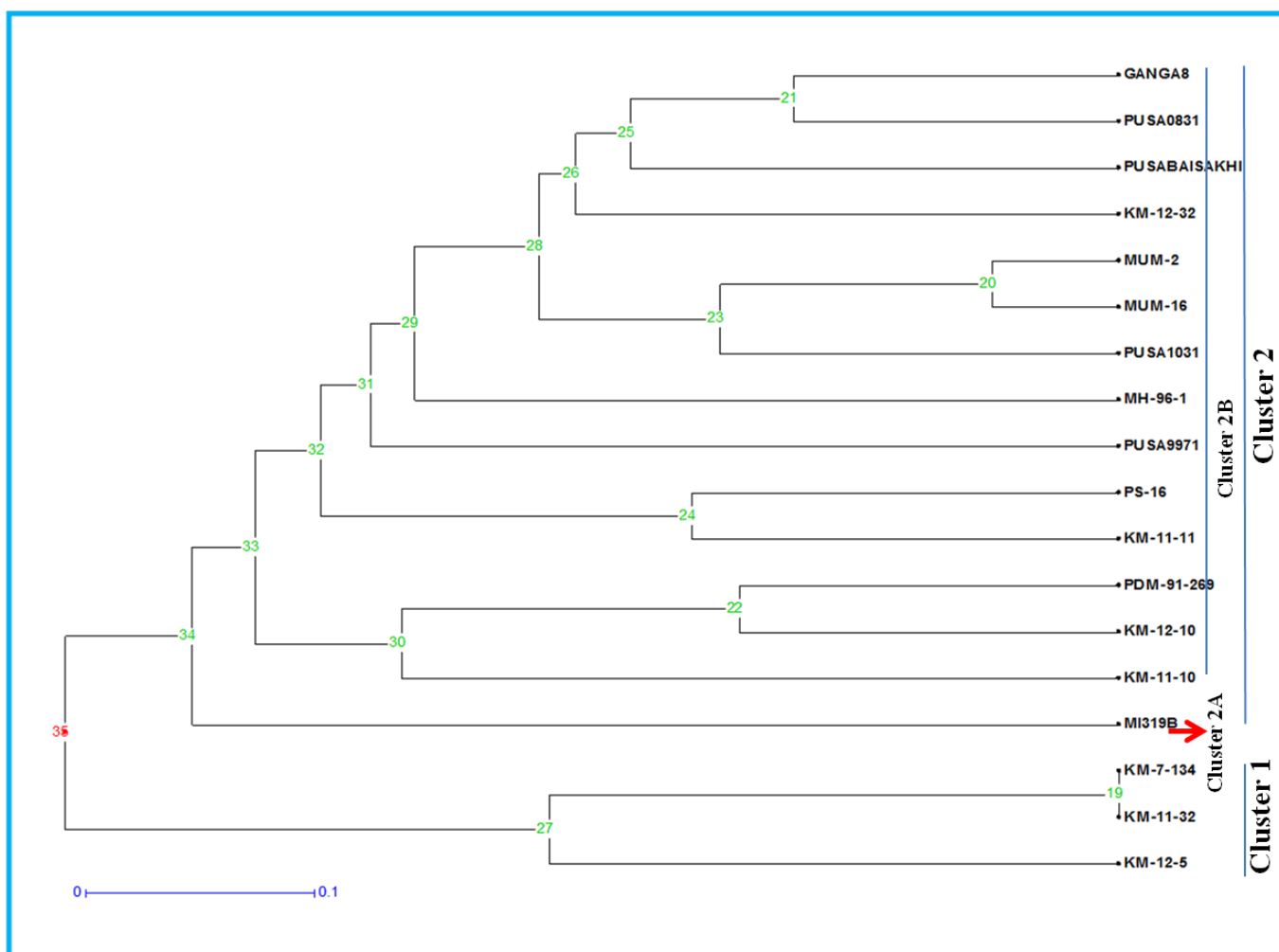


Plate 4.2 Clustering of mung bean derived from an unweighted pair group mean average (UPGMA) cluster analysis using Jaccard's similarity coefficient based on adzuki bean SSR primers.

Present investigation was carried out to study the susceptibility of different green gram accessions against three species of pulse beetle viz. *C. maculatus*, *C. analis* and *C. chinensis* laboratory screening was under taken with free and force test method for a range of cultivars to determine the susceptibility levels and to identify accessions that are having either some levels of resistance or lower susceptibility. Apart from these observations, studies were carried out to establish the role of physical and biochemical properties of these selected accessions in bruchid resistance. Further, the artificial bioassay was conducted to validate the screening results. Moreover, diversity analysis was performed to find out better accessions that could be utilised in further bruchids resistance breeding programs

The results obtained from the present investigations have been discussed in the light of pertinent literature under the following headings:

- 5.1. Evaluation of different green gram accessions against three species of pulse beetle through screening.
- 5.2. Comparative physical and biochemical parameters of the cultivars responsible for resistance.
- 5.3. Validation of screening results through artificial seeds.
- 5.4. DNA finger printing for diversity analysis of the green gram accession.

5.1 Evaluation of different green gram accessions against three species of pulse beetle.

In the free-choice test, the insects could infest the seed samples of their choice. The ability of a specific variety to repel insects (antixenosis) is usually measured with this method (Giga, 1995). Of the eighty five green gram accessions, only twelve, eight and ten accessions were found to be least susceptible against *C. maculatus*, *C. analis*

and *C. chinensis* respectively with low GI (<0.06) (Table 5.1). In both free and force choice test, no green gram accession was found immune to bruchid infestation, yet showed significant difference among the accessions in terms of oviposition, developmental period, adult emergence and seed weight loss (Table 5.3, 5.4, 5.5). The genotypes which were found to be least preferred under free choice test have shown differential reaction in their susceptibility when tested under force choice. Perhaps, changes in the susceptibility might be due to restricting the pest to their choice of the germplasm thereby forcing them to survive on the given genotypes. However certain accessions still maintained their susceptibility level despite the strained insect attack. Differential reaction with respect to oviposition, mean developmental period, weight loss and susceptibility index has been reported earlier in mung bean against *C. chinensis* (Hussian *et al.*, 1997) and *C. maculatus* (Lambrides and Imrie, 2000) and in dry beans against *Zebrotres subfasciatus* (Kananji, 2007).

Free choice test provided only a preliminary evaluation data of each accession with respect to bruchid biological parameters. Bruchid oviposits on the variety that attracts more in comparison to others, similarly development period and weight loss vary depends on the preference. Earlier, Duraimurugan *et al.* (2014) evaluated a total of 335 green gram and 140 black gram accessions under free choice test against *C. chinensis* and found that only eight green gram and nine black gram accessions exhibited GI less than 0.06. Likewise, Panzarino *et al.* (2011) studied the susceptibility of six chickpea genotypes against *C. maculatus*, of which a local variety with dark seed coat, Grumo Appula Black shown lower susceptibility. Similar kind of oviposition and growth pattern were observed in *Vigna* spp. and related pulses against bruchids by various workers (Srinivasan and Durairaj, 2000; Jha *et al.*, 2002; Kellouche *et al.*, 2004; Aslam *et al.*, 2006; Shaheen *et al.*, 2006; Asante and Mensah, 2007; Badoor *et al.*, 2009; Jha *et al.* 2011).

In force-choice test, the insects are restricted in their choice of seed sample. This method is widely used in laboratory screening of genotypes for resistance against storage pests (Giga, 1995). Based on the growth index, accessions were categorized as

resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible (Table 5.2). Three accessions viz., KM-12-10, KM-11-32 and KM-11-10 revealed lesser index of susceptibility (< 0.05) compared to M1319B with higher GI (> 0.081) against *C. maculatus*. Likewise, two accessions viz., (KM-12-5 and P-S-16) and (KM-11-10 and KM-11-11) revealed lesser susceptibility index (< 0.05) against *C. analis* and *C. chinensis* respectively. Those accessions which were found to be least preferred under free choice test had shifted to susceptible and highly susceptible category due to rigorous force choice test.

Similar categorisation of growth index was accomplished by Duraimurugan *et al.* (2014) who reported that eight out of 335 green gram accessions proved to be least preferred in the preliminary screening (free choice test) turned out to be moderately resistant, moderately susceptible and susceptible against *C. chinensis*. In contrast, Panzarino *et al.* (2011) opined that the biology of the cowpea weevil, *C. maculatus* was largely unaffected by the type of bioassay applied.

As GI is being calculated based on the bruchid biological parameters any change in the pattern of oviposition, MDP, adult emergence would adversely affect the susceptibility of the bruchid. In the present study, oviposition rate (no. of eggs) was found to be slightly higher under force choice test when compared to free choice test by all three species of bruchid under test, though least being deposited on the resistant cultivars (Table 5.3, 5.4, 5.6). The variation in egg laying might be attributed to some physical (seed size, seed coat texture) and biochemical parameters (Kashiwaba *et al.*, 2003; Somta *et al.*, 2006).

In general, higher oviposition rate was observed on the susceptible than resistant cultivars. The results of the present study are concomitant with some previous works, where bruchid laid maximum number of eggs on susceptible genotypes than the resistant one (Shafique and Ahmad, 2002; Shivanna *et al.*, 2011; Sarwar, 2012 and Badii *et al.*, 2013). However, the moderately resistant genotype such as MH-96-1 recorded more oviposition compare to most susceptible genotype PUSA 1031 though adult emergence, seed weight loss and growth index were found to be lower on this

accession than that of PUSA 1031 against *C. analis*. Likewise, Sarwar (2012) reported that resistant lentil harboured significantly lower number of eggs, inhibited adult progeny development thereby decreased the grain weight loss, indicating definite resistance against *C. analis*. This could be due to certain biochemical compounds present in the seeds which might have inhibited the growth and eventually death of the grub within the seeds (Southgate, 1979). Nevertheless, earlier studies also indicated that ovipositional preference is influenced by host availability to a greater extent and cannot be used to predict resistance as that of other variable such as adult emergence, MDP, percent weight loss and growth index (Redden and McGuire, 1983; Jackai and Asante, 2003).

Present study revealed that MDP (days) differed significantly between the selected green gram accessions against *C. maculatus*, *C. analis* and *C. Chinensis* (Table 5.3, 5.4, 5.6). The resistant lines were characterized by delayed, staggered and slow adult emergence while adult emergence in susceptible lines was early and extremely rapid. Singh *et al.* (1985) documented delayed development of *C. maculatus* in cowpea, where the per cent adult emergence was slower in resistant lines. In the susceptible varieties adults started emerging at 27 days after infestation (DAI) and about 86% emerged within three days, in contrast, the adult emergence in resistant lines started from 30 to 33 DAI and cumulative emergence up to 50 DAI ranged from 22.1 to 32.8%. Similarly, Shivanna *et al.*, (2011) also observed cowpea accession CP-17 was comparatively a least preferred host due to prolonged developmental period (34.67 days) and least adult survival (84.67 %) to *C. maculatus*. Similar trend of adult emergence in resistant genotypes of chickpea against *C. analis* was also reported (Shafique and Ahmad, 2005). Reduction in per cent adult emergence is an indication of the presence of antinutritional chemicals / antibiosis factors in seeds which may result in the prolongation of developmental period (Dongre *et al.*, 1996 and Somta *et al.*, 2006).

Present study exhibited higher percent seed weight loss in those varieties that had more GI than its counterpart. It has been documented earlier that tolerant varieties

experienced least damage and hence lesser weight loss against bruchid. Shivanna *et al.* (2011) found that CP-17 accession of cowpea was comparatively a least preferred host with maximum weight loss (0.96%) against *C. maculatus*. Similar kind of weight loss was exhibited in chickpea genotype ICC 4969 against *C. maculatus* by Erler *et al.* (2009). Also the number of adults released will determine seed weight loss. Delay in developmental period and prolonged adult emergence may lead to a considerable damage to seed and total weight loss during storage (Sulehrie *et al.*, 2003 and Chakraborty *et al.*, 2004). Lambrides and Imrie, (2000) reported that the tolerant varieties showed the least weight loss against bruchids.

Suitability of the food material/ genotype is determined on the basis of growth index (GI), an important parameter of insect growth and development. Genotypes with low GI were considered as resistant and those with high GI as susceptible. This is based on the assumption that few insect progenies would emerge out and also the progeny development would take a longer time in a resistant than in a susceptible genotype. Jha *et al.* (2011) also evaluated eight greengram accessions against three species of pulse beetles and observed that, PUSA Baisakhi, PS-10 and PS-16 was found to be resistant based on the growth index. Similar kind of results obtained in *C. chinensis* where four accessions were found to be moderately resistant with minimum growth index ranged 0.051 to 0.055 out of 335 greengram accessions screened (Duraimurugan *et al.*, 2014). Srinivasan and Durairaj (2007) also reported the lesser per cent survival (26.6 %), prolonged developmental period (40.7 and 36.6 days) and lesser index of suitability (0.0351 and 0.0389) of *C. maculatus* in green gram accessions PLS 262 and PLM 89 respectively. Similarly, Singh and Sharma (2001) studied thirteen varieties of chickpea against *C. chinensis* and found PG-5 was the most resistant variety with minimum GI (1.358) while GNG-663 was most susceptible with GI of 2.211. Earlier, Tripathi *et al.* (2012) examined reactions of 52 cowpea accessions to *C. chinensis* and *C. maculatus* reported three accessions were found to be resistant to both *C. maculatus* and *C. chinensis*. It has been reported that variables such as adult emergence, developmental period, seed weight loss and growth index are

the most reliable indicators for resistance of green gram to damage by bruchids (Redden and McGuire, 1983; Jackai and Asante, 2003).

Furthermore, correlation studies revealed a positive correlation between the oviposition and GI of all three species of bruchid tested (Table 5.6). Similarly percent adult emerged and weight loss was found have positive correlation with GI while, MDP was negatively correlated with the susceptibility index. However, no correlation exists between the preferential oviposition for different accessions and the subsequent larval development. Thus more the number of seeds, higher will be the oviposition (Dick and Credland, 1984). Moreover, bruchids have the unusual habit of laying eggs on unsuitable surfaces under conditions of host deprivation as observed by Wang and Horng, (2004). Tripathi *et al.* (2012) also evaluated 52 genotypes of cowpea for resistance to *C. chinensis* and reported that GI had significant negative relationship with mean development period and significant positive relationship with adult emergence and adult emergence had a positive relationship with weight loss. Kananji (2007) evaluated 42 Malawian dry bean genotypes for resistance to *Z. subfasciatus* and reported that the grain weight loss had highly significant and positively correlation with adult bruchid emergence as well as Dobie's susceptibility index. This could be attributed to the small size, seed coat thickness, seed coat width and presence of well-formed texture layer on the seed (Lambrides and Imrie, 2000).

Furthermore these tested genotypes when were ranked in order of their relative susceptibilities using parameters such as oviposition, adult emergence, seed weight loss and growth index. Genotypes like KM-12-10, KM-11-10 and KM-11-32 for *C. maculatus* (Table 5.7), KM-12-5 and P-S-16 for *C. analis* (Table 5.8), while KM-11-11 and KM-11-10 for *C. chinensis* (Table 5.9) were found to be least preferred. In support of the present findings, similar trend of results were obtained by Badii *et al.* (2013). In the insect-crop interactions presence of any of these factors *viz.*, no oviposition; disrupted embryo development; larvae failing to penetrate the testa; larvae dying within the cotyledon; failure of pupation or adult emergence and the reduced

fitness of adult insects triggered by the host plant would result in resistance (Simmonds *et al.*, 1989).

In the present study numbers of eggs were not reduced significantly on decorticated seeds as compared to eggs laid on whole seed, meanwhile larval growth and development was retarded and the number of adults that subsequently emerged from decorticated seeds was reduced for all the three *spp.* of pulse beetle.

Our results are in accordance with Singh *et al.* (1980) who explored the resistance to pulse beetle against different pulses. They also observed similar number of eggs laid and reduced adult emergence of *C. chinensis* with decorticated seeds of different pulses compared to that of whole seeds. They explained that it may be due to loss of some nutritive value of cotyledons during soaking as well as drying. Similarly Ali and Smith (2000) who assessed the effect of seed coat on the susceptibility of faba beans to *C. chinensis* and reported that percent adult emergence and susceptibility index were more on the decorticated seeds.

5.2. Comparative physical and biochemical parameters of the cultivars responsible for resistance.

Present study indicated that accessions colour, stature and lustre had no influence on the resistance or susceptibility of green gram accessions to beetles. Earlier, Kapila and Panji (1989) screened seeds of 17 cultivars of *Phaseolus vulgaris* for resistance to *Zabrotes subfaciatus* and concluded that neither size nor colour of the seed were important with regarding susceptibility. Similarly, Hussain *et al.* (1997) also observed that size, colour and protein content of the seed have no influence on the susceptibility of mung bean seeds to *C. chinensis*.

Correlation coefficient was worked out between GI of pulse beetle with physical and biochemical parameters which indicated that GI of all the three bruchids had positive relation with 100 seed weight and negative relation with seed coat hardness, whereas seed hardness had negative relationship with GI of *C. maculatus* and *C. chinensis*. Similarly the L: B ratio of the seed had positive relation with GI of

C. maculatus and *C. chinensis*, while it had negative relation against GI of *C. analis*. Protein and starch content showed positive while tannin had negative relation with GI of all three bruchids. GI of *C. maculatus* and *C. analis* were found to have negative relationship with α amylase inhibitor, whereas phenol had negative correlation with *C. chinensis* GI (Table 5.13, 5.14).

Larger grains provide more food and space for insect growth than that of smaller grains. In the present observation, smaller seeds offer more resistance to pests attack than the larger grains which was supported by the similar findings of Singh *et al.* (1974). Talekar and Lin (1992) investigated characteristics of resistance to *C. chinensis* in two mung bean and one black gram accessions and concluded that smaller seed size of the accessions was not responsible for the resistance. Pankaj *et al.* (2011) observed that the seed characters such as 100 seed weight, seed coat thickness, colour and texture of seed coat were not related with the ovipositional preference and host suitability of the pest to different pulse seeds.

Several and not completely understood factors affects ovipositional preference of bruchids (Southgate *et al.*, 1979; Sales *et al.*, 2005; Erler *et al.*, 2009, Swella and Mushobozy, 2009). Physical factors such as seed size, seed colour, seed hardness and type of testa in cowpea proved to be ineffective for oviposition by *C. maculatus* (Iloba, 1985; Edde and Amatobi, 2000 and 2003). In general, smooth, shiny and medium to bold seeds are preferred for oviposition by the bruchid than rough, hard and wrinkled seed coat (Ahmed *et al.*, 2003). Even though the texture of seed coat is an important factor in eliciting ovipositional responses but subsequent growth of the larvae appears to be regulated by apparent chemical constituents of the seed variety.

The presence of anti-nutritional factors in the cotyledons of resistant accessions of *V. umbellata* confirmed that the bruchid larvae were able to penetrate through the seed coat into cotyledons but failed to support a normal adult emergence. This was due to the mortality of first and second instars larva inside cotyledons (Kashiwaba *et al.*, 2003). In general, phenolics play a major role in protecting plants from insect herbivory (Corcuera, 1993; Simmonds, 2003). Resistant varieties had

higher α -amylase inhibitor activities than susceptible (Erlinda *et al.*, 1993). According to Lattanzio *et al.* (2005), during bruchid infestation in stored cowpea seeds, seed coat tannins are effectively involved in the biochemical defence mechanisms, which can deter, poison or starve bruchid larvae that feed on cowpea seeds. On the contrary, Desroches *et al.* (1994) observed that the presence of tannin did not influence the penetration rates in *C. chinensis*. The rate of larval penetration was through seed coats not significantly different with or without tannin. Even if tannins are generally considered to be deleterious to herbivores, insects can overcome this problem by modification of gut structure or the midgut environment (Harborne, 1999).

Southgate (1979) stated that resistance against bruchids might be a result of following one or more causes: seeds are too hard for the neonate larvae to penetrate. Seeds are either too small for the larva to reach its full size or has insufficient food. Sometimes seeds may contain toxic compounds either in their cotyledons or its seed coat. Similarly, Imrie *et al.* (1991) found that many small seeded mung bean genotypes had a high percentage of hard seed while large-seeded varieties did not show persistent hardseedness. Consequently, large seed cultivars might possess some physical properties related to seed coat that helped easy imbibition of water into the seeds (Afzal *et al.* 2003).

High phenotypic (PCV) and genotypic (GCV) coefficients were observed for several characters, with the maximum for seed hardness, hundred seed weight, seed volume and seed density (> 90%). Similarly Manju and Sreelathakumary (2002) and Cherian (2000) also observed high heritability of the fruit yield related traits in chilly and *Capsicum chinense* respectively. High heritability indicates the presence of huge number of fixable additive factors; hence selection of these traits may improve yield. Moreover high variability in turn offers good scope for selection. High heritability coupled with high genetic advance study revealed better for selection.

Likewise, Makeen *et al.*, (2007) found that analysis of variance revealed significant difference for all 14 characters among 56 accessions of mung bean. PCV and GCV were the highest in seeds per plant followed by pods per plant and Leaf area

index. Broad sense heritability estimates were higher than 50% in 100 seed weight (79.17%) and other yield related traits, suggesting that these characters were least influenced by the environmental factors and could be improved upon selection. Humphry *et al.* (2005) found a negative correlation between seed hardness and seed weight in mung bean.

5.3. Validation of screening results through artificial seeds

Artificial seed bioassay revealed that increasing percentage of resistant seed powder adversely affected the bruchid growth and development. The number of egg deposition and adult emergence declined as the proportion of resistant powder increased. Earlier, Somta *et al.*, (2008) also observed that artificial seeds comprising of resistant seed powder showed inhibited egg deposition and growth of pulse beetles. They suggested that biochemical(s) constituents present in the cotyledon might be responsible for the resistance (Shade *et al.*, 1986; Kashiwaba *et al.*, 2003). Use of artificial seeds with similar size and shape enable as to exclude seed physical difference (seed coat, size and hardness) as factors contributing to the resistance and to determine the potency of the resistance factors in the cotyledons.

5.4. Diversity analysis of green gram

From the present study it was evident that out of the eight SSR markers amplified, CEDG305 and CEDG214 revealed high PIC value of 0.49 apiece, indicating their complete transferability. Our results are in conformity with Dikshit *et al.*, (2012) were out of 78 mapped SSR markers, 41 amplified characteristic bands in at least one *Vigna* species with transferability percentage of 87.8 % in green gram. High interspecific transferability was found across the legume genera (Choudhary *et al.*, 2008). Likewise, Wang *et al.* (2009) reported the transferability of adzuki bean primers in mung bean and observed that 70% of the primer used generated clear and repeatable bands in mung bean.

The preceding discussion revealed that storage pests are the real challenge to agricultural researchers in general and more precisely to the entomologists and plant

breeders. Breeding elite genotypes with better genetic resistance to stored grain pest could be one of the viable alternatives to address storage insect pests in food legumes. Resistance to storage pests is often a rare phenomenon to occur in the in the cultivated species for direct exploitation, but may sometimes be revealed through screening of very large numbers of landraces. For example, Tvu 2027 cowpea was found resistant when screened among thousands of accessions (Singh and Singh, 1992). Therefore, it is appropriate to screen a wide array of wild species for resistance genes. Boosting yield potential with genetic manipulations is essential but, simultaneously, it is also essential to safeguard the produce from storage pests with the same genetic tool. It is not just a matter of choice but inevitably needs equal priority. It is a challenge to the and yet it is no simple challenge for respective researchers.

Table 5.1 Reaction of green gram accessions to all three species of bruchids on the basis of ‘free choice’ test

Category	Growth index	No. of greengram accessions		
		<i>C. maculatus</i>	<i>C. analis</i>	<i>C. chinensis</i>
Resistant	< 0.05	5	3	2
Moderately resistant	0.051-0.060	7	5	8
Moderately susceptible	0.061-0.070	19	19	21
Susceptible	0.071-0.080	25	26	24
Highly susceptible	> 0.081	29	32	30
Total	--	85		

Table 5.2 Comparison between reaction of green gram accessions against *C. maculatus* under free choice and force choice test.

All values represent mean of three replications, Values represent Mean $\pm$ Standard Error.

Accessions	FREE CHOICE TEST				FORCE CHOICE TEST			
	No. of eggs/10seed	Adult Emergence (%)	MDP (days)	Growth Index	No. of eggs/10seed	Adult Emergence (%)	MDP (days)	Growth Index
KM 12 -10	7.33 $\pm$ 0.33	26.79 $\pm$ 6.76	33.00 $\pm$ 0.58	0.043	8.33 $\pm$ 1.45	21.34 $\pm$ 6.22	32.21 $\pm$ 0.78	0.041
KM 11-32	6.00 $\pm$ 1.00	21.67 $\pm$ 11.67	31.33 $\pm$ 0.88	0.043	8.67 $\pm$ 1.45	30.64 $\pm$ 4.30	31.82 $\pm$ 0.51	0.047
KM 11-10	4.33 $\pm$ 0.67	31.11 $\pm$ 5.88	31.00 $\pm$ 0.58	0.048	6.67 $\pm$ 0.33	34.92 $\pm$ 4.20	31.59 $\pm$ 0.49	0.049
KM 12 -32	4.67 $\pm$ 1.20	35.32 $\pm$ 15.98	26.67 $\pm$ 1.120	0.058	7.67 $\pm$ 1.67	48.48 $\pm$ 1.52	30.18 $\pm$ 0.15	0.056
KM 12 -7	8.67 $\pm$ 0.88	32.75 $\pm$ 13.36	29.33 $\pm$ 0.67	0.052	8.33 $\pm$ 0.67	52.38 $\pm$ 9.91	30.10 $\pm$ 0.18	0.057
MUM-16	14.33 $\pm$ 0.88	62.96 $\pm$ 3.50	21.33 $\pm$ 0.67	0.058	8.00 $\pm$ 0.58	54.17 $\pm$ 4.17	26.49 $\pm$ 0.22	0.065
GANGA -1	8.33 $\pm$ 0.67	40.21 $\pm$ 9.72	28.67 $\pm$ 0.88	0.056	9.33 $\pm$ 0.88	64.06 $\pm$ 9.83	26.53 $\pm$ 1.22	0.068
KM-11-40	14.00 $\pm$ 1.16	69.64 $\pm$ 3.72	26.33 $\pm$ 0.67	0.060	8.67 $\pm$ 1.45	68.02 $\pm$ 7.61	26.73 $\pm$ 1.11	0.069
MUM-2	5.67 $\pm$ 1.20	35.00 $\pm$ 23.63	25.67 $\pm$ 0.88	0.060	8.33 $\pm$ 1.20	66.30 $\pm$ 8.61	24.64 $\pm$ 0.61	0.074
KM-12-23	8.00 $\pm$ 0.58	21.56 $\pm$ 5.33	29.33 $\pm$ 1.20	0.045	9.67 $\pm$ 0.67	72.39 $\pm$ 3.21	24.59 $\pm$ 0.67	0.076
PUSA 9971	7.67 $\pm$ 1.20	19.21 $\pm$ 7.17	24.00 $\pm$ 0.58	0.053	13.00 $\pm$ 0.58	77.02 $\pm$ 3.94	24.31 $\pm$ 1.07	0.078
M 1319B	10.00 $\pm$ 1.73	19.89 $\pm$ 5.07	23.67 $\pm$ 0.33	0.055	12.67 $\pm$ 0.88	84.05 $\pm$ 1.16	23.75 $\pm$ 1.15	0.081

Table 5.3 Comparison between reaction of green gram accessions against *C. analis* under free choice and force choice test.

All values represent mean of three replications, Values represent Mean $\pm$ Standard Error.

Accessions	FREE CHOICE TEST				FORCE CHOICE TEST			
	No. of eggs/10seed	Adult Emergence (%)	MDP (days)	Growth Index	No. of eggs/10seed	Adult Emergence (%)	MDP (days)	Growth Index
KM-12-5	4.00 $\pm$ 1.53	0.67 $\pm$ 0.33	26.67 $\pm$ 0.88	0.041	6.67 $\pm$ 1.20	12.22 $\pm$ 6.19	25.67 $\pm$ 0.33	0.042
P-S-16	4.67 $\pm$ 4.67	0.67 $\pm$ 0.67	27.33 $\pm$ 0.67	0.041	9.00 $\pm$ 2.52	14.29 $\pm$ 8.25	26.00 $\pm$ 0.58	0.044
MH 96-1	12.00 $\pm$ 1.16	2.00 $\pm$ 0.58	26.33 $\pm$ 0.33	0.059	11.67 $\pm$ 1.20	25.22 $\pm$ 6.37	24.00 $\pm$ 0.58	0.058
KM-12-9	6.67 $\pm$ 2.03	1.00 $\pm$ 0.58	24.00 $\pm$ 0.58	0.060	12.00 $\pm$ 0.58	30.04 $\pm$ 4.24	23.67 $\pm$ 0.67	0.062
PDM 91-269	7.33 $\pm$ 0.88	2.33 $\pm$ 0.88	25.67 $\pm$ 0.67	0.060	12.67 $\pm$ 0.88	42.32 $\pm$ 3.02	21.00 $\pm$ 1.53	0.077
PUSA 1031	9.00 $\pm$ 1.53	2.67 $\pm$ 0.88	26.67 $\pm$ 0.88	0.056	9.00 $\pm$ 1.16	81.77 $\pm$ 2.29	23.33 $\pm$ 0.88	0.082
KM 12 -32	7.00 $\pm$ 1.73	1.33 $\pm$ 0.33	22.67 $\pm$ 1.20	0.057	13.00 $\pm$ 1.53	59.90 $\pm$ 9.41	21.33 $\pm$ 1.45	0.083
GANGA -8	8.33 $\pm$ 2.96	2.00 $\pm$ 0.58	24.00 $\pm$ 0.58	0.058	15.67 $\pm$ 1.86	71.67 $\pm$ 2.54	20.67 $\pm$ 0.88	0.09
KM-12-5	4.00 $\pm$ 1.53	0.67 $\pm$ 0.33	26.67 $\pm$ 0.88	0.041	6.67 $\pm$ 1.20	12.22 $\pm$ 6.19	25.67 $\pm$ 0.33	0.042

Table 5.4 Comparison between reaction of green gram accessions against *C. chinensis* under free choice and force choice test.

All values represent mean of three replications, Values represent Mean $\pm$ Standard Error.

Accessions	FREE CHOICE TEST				FORCE CHOICE TEST			
	No. of eggs/10seed	Adult Emergence (%)	MDP (days)	Growth Index	No. of eggs/10seed	Adult Emergence (%)	MDP (days)	Growth Index
KM-11-11	8.00 $\pm$ 0.58	20.04 $\pm$ 6.67	29.33 $\pm$ 0.88	0.044	11.00 $\pm$ 1.16	12.33 $\pm$ 3.09	31.77 $\pm$ 2.00	0.034
KM 11-10	10.00 $\pm$ 0.58	10.07 $\pm$ 0.58	31.00 $\pm$ 0.58	0.032	7.33 $\pm$ 2.33	25.56 $\pm$ 7.29	30.30 $\pm$ 1.51	0.046
P-S-16	6.67 $\pm$ 0.88	20.36 $\pm$ 4.64	27.33 $\pm$ 0.67	0.048	9.33 $\pm$ 1.20	34.07 $\pm$ 15.73	28.20 $\pm$ 0.95	0.054
KM 12 -32	9.33 $\pm$ 0.33	28.52 $\pm$ 3.29	26.33 $\pm$ 0.67	0.055	12.33 $\pm$ 0.67	36.13 $\pm$ 9.47	27.70 $\pm$ 0.63	0.056
KM-7-134	9.67 $\pm$ 0.33	17.78 $\pm$ 7.78	24.00 $\pm$ 0.00	0.052	11.00 $\pm$ 1.73	49.08 $\pm$ 7.94	27.63 $\pm$ 1.61	0.061
PUSA Baisakhi	9.33 $\pm$ 0.33	14.44 $\pm$ 3.90	25.00 $\pm$ 0.58	0.046	12.67 $\pm$ 0.88	66.19 $\pm$ 3.37	26.77 $\pm$ 1.54	0.068
MUM-16	11.67 $\pm$ 1.20	28.92 $\pm$ 6.30	25.67 $\pm$ 0.33	0.057	10.67 $\pm$ 1.45	76.95 $\pm$ 7.88	26.40 $\pm$ 1.55	0.071
KM-12-19	9.33 $\pm$ 1.76	28.89 $\pm$ 10.60	25.33 $\pm$ 0.33	0.058	9.00 $\pm$ 3.61	71.73 $\pm$ 1.81	25.33 $\pm$ 1.82	0.073
GANGA -8	6.67 $\pm$ 2.19	24.70 $\pm$ 8.92	24.00 $\pm$ 0.58	0.058	10.67 $\pm$ 0.33	81.82 $\pm$ 10.50	24.63 $\pm$ 0.78	0.078
PUSA 0831	7.00 $\pm$ 1.73	23.10 $\pm$ 4.63	26.00 $\pm$ 0.00	0.052	12.67 $\pm$ 0.88	76.54 $\pm$ 3.77	23.33 $\pm$ 0.75	0.081

Table 5.5 Reaction of green gram accessions to all three species of bruchids on the basis of ‘force choice’ test

Category	Growth index	Green gram accessions		
		<i>C. maculatus</i>	<i>C. analis</i>	<i>C. chinensis</i>
Resistant	< 0.05	KM 12 -10, KM 11-32, KM 11-10	KM-12-5, P-S-16	KM-11-11, KM-11-10
Moderately resistant	0.051-0.060	KM 12 -32, KM 12 -7	MH 96-1	PS-16, KM-12-32
Moderately susceptible	0.061-0.070	MUM-16, GANGA -1, KM 11-40	KM-12-9	KM-7-134, PUSA Baisakhi
Susceptible	0.071-0.080	MUM-2, KM 12 -23, PUSA 9971	PDM 91-269	MUM-16
Highly susceptible	> 0.081	M 1319B	PUSA 1031, KM-12-32, Ganga 8	KM-12-19,GANGA 8, PUSA 0831
Total	--		85	

Table 5.6 Combined correlation matrix of green gram accessions against all three species of bruchids under force choice test.

Parameters	Growth index		
	<i>C. maculatus</i>	<i>C. analis</i>	<i>C. chinensis</i>
No. of eggs/10seed	0.688*	0.734*	0.332
% Adult Emergence	0.985**	0.952**	0.974**
Developmental Period (days)	-0.987**	-0.924**	-0.982**
% Weight loss	0.959**	0.931**	0.954**

Table 5.7 Ranking of different green gram accessions in order of relative susceptibility to *C. maculatus*

Accession	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Damage	Growth index	Total rank	Mean Rank
KM 12 -10	6	1	1	1	1	10	2
KM 11-32	8	2	2	2	2	16	3.2
KM 11-10	1	3	3	3	3	13	2.6
KM 12 -32	2	4	4	4	4	18	3.6
KM 12 -7	5	5	5	7	5	27	5.4
MUM-16	3	6	8	5	6	28	5.6
GANGA -1	9	7	7	6	7	36	7.2
KM 11-40	7	9	6	8	8	38	7.6
MUM-2	4	8	9	9	9	39	7.8
KM 12 -23	10	10	10	10	10	50	10
PUSA 9971	12	11	11	11	11	56	11.2
M 1319B	11	12	12	12	12	59	11.8

Infestation and damage; 1= least susceptible/infested, 8= most susceptible/infested

Table 5.8 Ranking of different green gram accessions in order of relative susceptibility to *C. analis*

Accession	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Damage	Growth index	Total rank	Mean Rank
KM-12-5	1	1	2	1	1	6	1.2
P-S-16	2	2	1	2	2	9	1.8
MH 96-1	4	3	3	3	3	16	3.2
KM-12-9	5	4	4	4	4	21	4.2
PDM 91-269	6	5	7	5	5	28	5.6
PUSA 1031	2	8	5	7	6	28	5.6
KM-12-32	7	6	6	6	7	32	6.4
Ganga 8	8	7	8	8	8	39	7.8

Infestation and damage; 1= least susceptible/infested, 8= most susceptible/infested

Table 5.9 Ranking of different green gram accessions in order of relative susceptibility to *C. chinensis*

Accession	No. of eggs/10seed	% Adult Emergence	Developmental Period (days)	% Damage	Growth index	Total rank	Mean Rank
KM-11-11	6	1	2	1	1	11	2.2
KM-11-10	1	2	1	2	2	8	1.6
PS-16	3	3	3	4	3	16	3.2
KM-12-32	8	4	5	3	4	24	4.8
KM-7-134	7	5	4	5	5	26	5.2
PUSA Baisakhi	9	6	6	8	6	35	7
MUM-16	5	9	7	8	7	36	7.2
KM-12-19	2	7	8	7	8	32	6.4
GAMGA 8	4	10	9	9	9	41	8.2
PUSA 0831	10	8	10	10	10	48	9.6

Infestation and damage; 1= least susceptible/infested, 10= most susceptible/infested

Table 5.10 Comparison between reactions of whole seed Vs decorticated seeds of selected green gram against accessions against *C. maculatus* under force choice test.

Parameters	<i>C. maculatus</i>			
	Whole seeds		Decorticated seeds	
	No. of eggs/10seed	% Adult Emergence	No. of eggs/10seed	% Adult Emergence
KM-12-10	8.33±1.45	21.34±6.22	10.67±1.67	15.87±3.30
KM-11-10	6.67±0.33	34.92±4.20	9.67±0.88	30.45±3.29
M1319B	12.67±0.88	84.05±1.16	15.33±1.45	77.09±5.38

Table 5.11 Comparison between reactions of whole seed Vs decorticated seeds of selected green gram against accessions against *C. analis* under force choice test.

Parameters	<i>C. analis</i>			
	Whole seeds		Decorticated seeds	
	No. of eggs/10seed	% Adult Emergence	No. of eggs/10seed	% Adult Emergence
KM-12-5	6.67±1.20	12.22±6.19	7.00±0.58	10.32±5.20
PS-16	9.00±2.52	14.29±8.25	10.67±1.20	11.79±6.05
Ganga 8	15.67±1.86	71.67±2.54	19.33±1.20	65.38±3.32

Table 5.12 Comparison between reactions of whole seed Vs decorticated seeds of selected green gram against accessions against *C. chinensis* under force choice test.

Parameters	<i>C. chinensis</i>			
	Whole seeds		Decorticated seeds	
	No. of eggs/10seed	% Adult Emergence	No. of eggs/10seed	% Adult Emergence
KM-11-10	7.33±2.33	25.56±7.29	8.00±1.15	19.72±5.28
PS-16	9.33±1.20	34.07±15.73	11.33±1.86	26.30±2.25
PUSA 0831	12.67±0.88	76.54±3.77	16.33±1.45	68.70±8.61

All values represent mean of three replications, Values represent Mean ± Standard Error.

Table 5.13 Comparison between different physical parameters against GI of all three species of bruchids.

Physical Parameters	Growth index		
	<i>C. maculatus</i>	<i>C. analis</i>	<i>C. chinensis</i>
L:B Ratio	0.525	-0.032	0.501
Hundred Seed Weight	0.507	0.518	0.284
Seed hardness	-0.225	0.015	-0.438
Seed coat hardness	-0.784**	-0.765*	-0.555
Seed coat percentage	0.486	-0.011	0.134

Table 5.14 Comparison between different biochemical parameters against GI of all three species of bruchids.

Biochemical Parameters	Growth index		
	<i>C. maculatus</i>	<i>C. analis</i>	<i>C. chinensis</i>
Protein	0.964**	0.624	0.823**
Crude protein	-0.344	0.565	0.545
Starch	0.580	0.362	0.095
TSS	0.206	-0.493	0.210
phenol	0.242	0.072	-0.598
Tannin	-0.933**	-0.801*	-0.742*
α amylase inhibitor	-0.701*	-0.291	0.277

he present study entitled “Factors for resistance in mung bean, *Vigna radiata* against *Callosobruchus* spp.” was carried out at Division of Entomology Indian Agricultural Research Institute, New Delhi. The salient finding of the present investigation have been summarized under the following heads:

1. Evaluation of bruchid resistance under ‘free choice’ test

- ❖ The susceptibility of eighty five green gram accessions were evaluated against three species of pulse beetle, viz., *Callosobruchus maculatus*, *C. analis* and *C. chinensis* under laboratory conditions.
- ❖ Accessions which exhibited lesser susceptibility index during the preliminary free choice test were further subjected to ‘force choice’ test for confirmation of bruchid resistance.
- ❖ No green gram accession was found immune to bruchid infestation but showed significant difference in terms of oviposition, developmental period, adult emergence and seed weight loss.
- ❖ Three accessions viz, KM-12-10, KM-11-32 and KM-11-10 revealed lesser per-cent adult emergence (21.3, 30.6 and 34.9% respectively), prolonged developmental period (32.2, 31.8 and 31.6 days respectively) and lesser index of susceptibility (0.041, 0.047 and 0.049 respectively) as compared to highly susceptible M1319B, which exhibited emergence of 84.1 %, developmental period of 23.8 days and index of suitability of 0.081 against *C. maculatus*.
- ❖ Whereas two accessions, KM-12-5 and P-S-16 revealed lesser per cent adult emergence of 12.2 and 14.3, respectively, prolonged developmental period (25.7 and 26.0 days, respectively) and lesser susceptibility index (0.042 and 0.044 respectively) in comparison to highly susceptible Ganga 8, which exhibited a per-cent survival of 71.7 per cent, developmental period of 20.7 days and susceptibility index of 0.09.
- ❖ Likewise for *C. chinensis*, KM-11-11 and KM-11-10 exhibited least adult emergence, higher MDP and lower GI as compared to Pusa 0831. Correlation between GI and other growth parameters of the three pulse beetle on different accessions indicated

that GI had negative relationship with mean developmental period and significant positive relationship with adult emergence and seed weight loss. Moreover, removal of the testa increased the oviposition rate on the decorticated seeds indicated seed coat properties hindered the egg deposition of pulse beetles.

2. Physical and biochemical factors responsible for bruchid resistance

- ❖ Selected green gram accessions were evaluated for their physical parameters like seed colour, luster, stature, length, breadth, seed volume, seed hardness, seed coat hardness, seed coat percentage and 100 seed weight and biochemical parameters namely soluble protein, crude proteins, starch, total soluble sugars (TSS), phenol, tannin and α amylase inhibitor (AI) to understand the physical and biochemical basis of resistance.
- ❖ Significant difference among the green gram accessions were observed for all physical and biochemical parameters. Based on the GI, accession KM-11-10 was found to be resistant to *C. maculatus* and *C. chinensis*, while P-S-16 was showed resistantance against *C. analis* and *C. chinensis*.
- ❖ Correlation coefficient was worked out between GI of pulse beetle with physical and biochemical parameters which indicated that GI of all the three bruchids had positive relation with 100 seed weight and negative relation with seed coat hardness, whereas seed hardness has negative relationship with GI of *C. maculatus* and *C. chinensis*.
- ❖ Protein and starch content showed positive while tannin had negative relation with GI of all three bruchids. GI of *C. maculatus* and *C. analis* were found to have negative relationship with α amylase inhibitor, whereas phenol had negative correlation with *C. chinensis* GI.

3. Evaluation of bruchid resistance using artificial seeds under ‘force choice’ test

- ❖ Artificial seed bioassay revealed that increasing percentage of resistant seed powder adversely affected the bruchid growth and development. The number of egg deposition and adult emergence declined as the proportion of resistant powder increased.

4. DNA finger printing of the selected greengram accessions

- ❖ The maximum number of SSR bands was amplified by the primer CEDG-305. In addition other primers CEDG037, CEDG044, CEDG127, CEDG154, CEDG178 and CEDG214 also were found to be very useful for diversity assessment of the green gram. UPGMA cluster analysis revealed clear separation of all the species with two main clusters.

Conclusion

On the basis of above findings, green gram accessions KM-11-10 and P-S-16 deserve special consideration for reducing the storage loss, as these were found relatively resistant against pulse beetle. Hence, these accessions can be effectively used as promising donors for developing bruchid resistant varieties which would ensure food security by reducing postharvest losses under storage conditions.

ABSTRACT

“Factors for resistance in mung bean, *Vigna radiata* against *Callosobruchus* spp.”

The susceptibility of eighty five green gram accessions were evaluated against three species of pulse beetle, viz., *Callosobruchus maculatus*, *C. analis* and *C. chinensis* under laboratory conditions. Accessions which exhibited lesser susceptibility index during the preliminary free choice test were further subjected to ‘force choice’ test for confirmation of bruchid resistance. No green gram accession was found immune to bruchid infestation but showed significant difference in terms of oviposition, developmental period, adult emergence and seed weight loss. Three accessions viz, KM-12-10, KM-11-32 and KM-11-10 revealed lesser per-cent adult emergence (21.3, 30.6 and 34.9% respectively), prolonged developmental period (32.2, 31.8 and 31.6 days respectively) and lesser index of susceptibility (0.041, 0.047 and 0.049 respectively) as compared to highly susceptible M1319B, which exhibited emergence of 84.1 %, developmental period of 23.8 days and index of suitability of 0.081 against *C. maculatus*. Whereas two accessions, KM-12-5 and P-S-16 revealed lesser per cent adult emergence of 12.2 and 14.3, respectively, prolonged developmental period (25.7 and 26.0 days, respectively) and lesser susceptibility index (0.042 and 0.044 respectively) in comparison to highly susceptible Ganga 8, which exhibited a per-cent survival of 71.7 per cent, developmental period of 20.7 days and susceptibility index of 0.09. Likewise for *C. chinensis*, KM-11-11 and KM-11-10 exhibited least adult emergence, higher MDP and lower GI as compared to Pusa 0831. Correlation between GI and other growth parameters of the three pulse beetle on different accessions indicated that GI had negative relationship with mean developmental period and significant positive relationship with adult emergence and seed weight loss. Moreover, removal of the testa increased the oviposition rate on the decorticated seeds indicated seed coat properties hindered the egg deposition of pulse beetles.

Selected green gram accessions were evaluated for their physical parameters like seed colour, luster, stature, length, breadth, seed volume, seed hardness, seed coat hardness, seed coat percentage and 100 seed weight and biochemical parameters namely soluble protein, crude proteins, starch, total soluble sugars (TSS), phenol, tannin and α amylase inhibitor (AI) to understand the physical and biochemical basis of resistance. Significant difference among the green gram accessions were observed for all physical and biochemical parameters. Based on the GI, accession KM-11-10 was found to be resistant to *C. maculatus* and *C. chinensis*, while P-S-16 was showed resistance against *C. analis* and *C. chinensis*. Correlation coefficient was worked out between GI of pulse beetle with

physical and biochemical parameters which indicated that GI of all the three bruchids had positive relation with 100 seed weight and negative relation with seed coat hardness, whereas seed hardness has negative relationship with GI of *C. maculatus* and *C. chinensis*. Protein and starch content showed positive while tannin had negative relation with GI of all three bruchids. GI of *C. maculatus* and *C. analis* were found to have negative relationship with α amylase inhibitor, whereas phenol had negative correlation with *C. chinensis* GI.

Artificial seed bioassay revealed that increasing percentage of resistant seed powder adversely affected the bruchid growth and development. The number of egg deposition and adult emergence declined as the proportion of resistant powder increased. The maximum number of SSR bands was amplified by the primer CEDG-305. In addition other primers CEDG037, CEDG044, CEDG127, CEDG154, CEDG178 and CEDG214 also were found to be very useful for diversity assessment of the green gram. UPGMA cluster analysis revealed clear separation of all the species with two main clusters.

On the basis of above findings, green gram accessions KM-11-10 and P-S-16 deserve special consideration for reducing the storage loss, as these were found relatively resistant against pulse beetle. Hence, these accessions can be effectively used as promising donors for developing bruchid resistant varieties which would ensure food security by reducing postharvest losses under storage conditions.

कैलोसोब्रुकस प्रजातियों के विरुद्ध मूँगबीन, वीग्ना रेडिएटा में प्रतिरोधिता – कारक

सार

प्रयोगशाला परिस्थितियों के अन्तर्गत दाल बीटल की तीन प्रजातियों यथा, *कैलोसोब्रुकस मैक्यूलेटस*, *कै. एनेलिस* एवं *कै. चाइनेनसिस*, के विरुद्ध मूँग के पचासी क्रों की सुग्राहिता का मूल्यांकन किया गया। प्राथमिक 'फ्री च्वाइस' परीक्षण के दौरान जिन क्रमों ने कम सुग्राहिता सूचकांक दर्शाया, उनका ब्रूकिड प्रतिरोधिता की पुष्टि हेतु आगे 'फोर्स च्वाइस' परीक्षण किया गया। ब्रूकिड प्रकोप हेतु मूँग का कोई भी क्रम प्रतिरक्षित (इम्यून) नहीं पाया गया किन्तु उन्होंने अंडरोपण, विकास-अवधि, वयस्क के निर्गमन एवं बीज भार में कमी के संदर्भ में महत्वपूर्ण अन्तर दर्शाए। *कै. मैक्यूलेटस* के विरुद्ध तीन क्रमों यथा, के एम-12-10, के एम -11-32 एवं के एम -11-10 ने कम प्रतिशत वयस्क निर्गमन (क्रमशः 21.3, 30.6 एवं 34.9%), अधिक लम्बी विकास-अवधि (क्रमशः 32.2, 31.8 एवं 31.6 दिन) तथा कम सुग्राहिता सूचकांक (क्रमशः 0.041, 0.047 एवं 0.049) दर्शाए जबकि इनकी तुलना में अत्यधिक सुग्राही एम 131 बी ने 84.1% निर्गमन, 23.8 दिन की विकास-अवधि एवं 0.081 का उपयुक्त सूचकांक दर्शाया। अत्यधिक सुग्राही गंगा 8 जिसने 71.7% की प्रतिशत उत्तरजीविता, 20.7 दिन की विकास अवधि एवं 0.09 का सुग्राहिता सूचकांक दर्शाया, की तुलना में दो क्रमों, के एम -12-5 एवं पी-एस-16 ने 12.2 एवं 14.3 का कम प्रतिशत वयस्क-निर्गमन, लम्बी विकास-अवधि (क्रमशः 25.7 एवं 26.0 दिन) तथा कम सुग्राहिता सूचकांक (क्रमशः 0.042 एवं 0.044) दर्शाए। इसी प्रकार से *कै. चाइनेनसिस* हेतु, पूसा 0831 की तुलना में, के एम - 11-11 एवं के एम-11-10 ने न्यूनतम वयस्क-निर्गमन, उच्चतर एम डी पी एवं कम जी आई दर्शाए। विभिन्न क्रमों पर तीन दाल बीटल के जी आई एवं अन्य वृद्धि प्राचलों के बीच सहसंबंध ने दर्शाया कि जी आई का, औसत विकास-अवधि के साथ ऋणात्मक सहसंबंध था तथा वयस्क-निर्गमन एवं बीज भार कमी के साथ महत्वपूर्ण धनात्मक संबंध था। इसके अतिरिक्त बीजावरण (टेस्टा) हटाने से कॉर्टेक्सरहित बीजों पर अण्डरोपण दर में बढ़ोतरी हुई जो दर्शाती है कि बीज खोल में निहित गुण, दाल की बीटल्स के अंडनिक्षेपण में बाधा पहुँचाते हैं।

प्रतिरोधिता के भौतिक एवं जैवरासायनिक आधार को समझने हेतु वरण किए गए मूँग क्रमों का उनके भौतिक प्राचलों यथा, बीज का रंग, चमक, कद, लम्बाई, मोटाई, बीज-आयतन, बीज की कठोरता, बीज खोल की कठोरता, बीज खोल-प्रतिशत एवं 100 दाना भार तथा जैवरासायनिक प्राचलों नामतः

घुलनशील प्रोटीन, अपरिष्कृत प्रोटीन्स, स्टार्च, कुल घुलनशील शर्कराएं (टी एस एस), फीनोल, टेनिन एवं एमायलेज संदमक (ए आई), का मूल्यांकन किया गया। सभी भौतिक एवं जैवरासायनिक प्राचलों हेतु मूँग के क्रमों के मध्य महत्वपूर्ण अन्तर देखे गए। जी आई के आधार पर, के एम -11-10 क्रम कै. मेक्यूलेटस एवं कै. चाइनेनसिस के विरुद्ध प्रतिरोधी पाया गया जबकि पी-एस-16 ने कै. एनालिस एवं कै. चाइनेनसिस के विरुद्ध प्रतिरोधिता दर्शायी। भौतिक एवं जैवरासायनिक प्राचलों के साथ दाल के बीटल के जी आई के बीच सहसंबंध गुणांक निकाला गया जिसने दर्शाया कि सभी तीनों ब्रूकिड्स के जी आई का 100 दाना भार के साथ धनात्मक सहसंबंध था जबकि बीज खोल-कठोरता के साथ ऋणात्मक संबंध था जबकि बीज-कठोरता का कै. मेक्यूलेटस एवं कै. चाइनेनसिस के जी आई के साथ ऋणात्मक संबंध था। सभी तीनों ब्रूकिड्स के जी आई के साथ प्रोटीन एवं स्टार्च अंश के धनात्मक जबकि टेनिन ने ऋणात्मक संबंध दर्शाया। कै. मेक्यूलेटस एवं कै. एनालिस के जी आई का एल्फा एमायलेज संदमक के साथ ऋणात्मक पाया गया जबकि फीनोल का कै. चाइनेनसिस के जी आई के साथ ऋणात्मक सहसंबंध था।

कृत्रिम बीज आमपन ने दर्शाया कि प्रतिरोधी बीज-पाउडर की बढ़ती प्रतिशतता, ब्रूकिड वृद्धि एवं विकास को दुष्प्रभावित करती है। प्रतिरोधी पाउडर का अनुपात बढ़ने के साथ-साथ अंड संग्रह की संख्या एवं वयस्क-निर्गमन में कमी हुई। प्राइमर सी ई डी जी-305 द्वारा एस एस आर बैंड्स की अधिकतम संख्या का प्रवर्धन हुआ। इसके अतिरिक्त, अन्य प्राइमर्स यथा सी ई डी जी 037, सी ई डी जी 044, सी ई डी जी 127, सी ई डी जी 154, सी ई डी जी 178 एवं सी ई डी जी 214 भी मूँग के विविधता -मूल्यांकन हेतु बहुत उपयोगी पाए गए। यू पी जी एमए समुच्चय विश्लेषण ने दो मुख्य समुच्चयों सहित सभी प्रजातियों का स्पष्ट विलगन दर्शाया।

उपर्युक्त परिणामों के आधार पर, भण्डारण-क्षति को कम करने के लिए मूँग के क्रमों, के एम -11-10 एवं पी-एस-16 की ओर विशेष रूप से ध्यान दिया जाना चाहिए क्योंकि ये दाल के बीटल के विरुद्ध अपेक्षाकृत प्रतिरोधी पाए गए हैं। इसलिए ब्रूकिड प्रतिरोधी किस्में विकसित करने के लिए श्रेष्ठ दाताओं (डोनर्स) के रूप में इन क्रमों का प्रभावी उपयोग किया जा सकता है ताकि भण्डारण परिस्थितियों के अन्तर्गत कटाई-उपरांत क्षति को कम कर खाद्य सुरक्षा को सुनिश्चित किया जा सके।

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