

**GENETIC ENHANCEMENT OF HOST PLANT
RESISTANCE TO *Spodoptera litura* (Fab.)
IN GROUNDNUT**

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AUGUST, 2003

**GENETIC ENHANCEMENT OF HOST PLANT
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IN GROUNDNUT**

*Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfilment of the requirements for the
degree of*

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in

GENETICS AND PLANT BREEDING

By

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C E R T I F I C A T E

This is to certify that the thesis entitled "**GENETIC ENHANCEMENT OF HOST PLANT RESISTANCE TO *Spodoptera litura* (Fab.) IN GROUNDNUT**" submitted by **Mr. RAJENDRA PRASAD M. N.** for the degree of **DOCTOR OF PHILOSOPHY** in **GENETICS AND PLANT BREEDING** to the University of Agricultural Sciences, Dharwad is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or other similar titles.

DHARWAD
August 2003


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CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I.	INTRODUCTION	01-04
II.	REVIEW OF LITERATURE	05-22
III.	MATERIAL AND METHODS	23-42
IV.	EXPERIMENTAL RESULTS	43-94
V.	DISCUSSION	95-114
VI.	SUMMARY	115-121
VII.	REFERENCES	122-137
	APPENDICES	138-155

LIST OF TABLES

Table No.	Title	Page No.
1.	Germplasm registered as <i>Spodoptera litura</i> resistant.	11
2.	<i>Arachis</i> spp. tested (Laboratory assay) at ICRISAT center for resistance to <i>Spodoptera litura</i> with an indication of the level of resistance	13
3.	Pedigree of the selected groundnut cultivars for evaluation.	24
4.	Salient features of parents used in hybridization programme	26
5.	List of primers used for RAPD analysis	40
6a.	Analysis of variance for <i>Spodoptera</i> damage in groundnut germplasm	44
6b.	Components of genetic variation for <i>Spodoptera</i> damage in groundnut germplasm	44
7.	Mean performance of groundnut germplasm for <i>Spodoptera</i> damage	46
8a.	Analysis of variance for leaf area damage (cm ²) and gain in weight (%) by <i>Spodoptera</i> larvae in laboratory	47
8b.	Mean performance of the selected groundnut genotypes for leaf area damage (cm ²) and gain in weight (%) of the <i>Spodoptera</i> larvae in laboratory rearing	47
9.	Variance and components of variability for <i>Spodoptera</i> resistance in selected groundnut genotypes	49
10.	Mean performance of the selected groundnut genotypes for <i>Spodoptera</i> resistance parameters and days to maturity	51
11a.	Analysis of variance for life history parameters of <i>Spodoptera litura</i> (F.)	53
11b.	Genetic components of variation for life history parameters of <i>Spodoptera litura</i> (F.)	53
12.	Life history parameters of <i>Spodoptera litura</i> on selected groundnut genotypes	54
13.	Components of variance for damage due to <i>Spodoptera</i> (%) in various crosses	57
14.	Components of variance for gain in weight (%) by larvae of <i>Spodoptera</i> in various crosses	59
15.	Components of variance for damage due to thrips in different crosses	61
16.	Components of variance for damage due to late leaf spot in various crosses	62
17.	Components of variance for pod yield in various crosses	64
18.	Components of variance for shelling out turn in various crosses	65

Contd...

Table No.	Title	Page No.
19.	Components of variance for hundred seed mass in various crosses	67
20.	Correlation coefficients for pest and disease resistance parameters and plant yield in different crosses	68
21.	Frequency of superior segregants for multiple traits in various crosses	71
22.	Performance of <i>Spodoptera</i> resistant lines for thrips, late leaf spot and productivity parameters	72
23.	Genetic components of variation for insect resistance, disease resistance and productivity parameters in selected progenies of different crosses	75
24.	Analysis of variance for stability of productivity parameters in <i>Spodoptera</i> resistant groundnut genotypes	77
25.	Performance of <i>Spodoptera</i> resistant progenies for various biotic stresses	78
26.	Stability parameters for various productivity traits in <i>Spodoptera</i> resistant lines	81
27.	Correlation coefficients for insect resistance, disease resistance and productivity parameters in selected progenies of different crosses	83
28.	Analysis of variance for stability of productivity parameters in single plant selections in groundnut	85
29.	Performance of single plant selections for pest, disease and productivity parameters	86
30.	Stability parameters for various productivity traits in single plant selections	87
31.	Biochemical parameters in selected groundnut genotypes	90
32.	Lectin specific activity in the progenies of the cross TAG 24 X R 9227 vis-a-vis <i>Spodoptera</i> resistance	91
33.	Analysis of RAPD patterns in selected groundnut genotypes	93
34.	Genetic similarity as DICE co efficient based on pooled data of RAPD profiles of selected groundnut genotypes	94
35.	Performance of progenies of different crosses for various biotic stresses and productivity parameters	108

LIST OF FIGURES

Figure No.	Title	Between pages
1.	Distribution of progenies in different crosses for gain in weight by <i>Spodoptera</i> larvae	69-70
2.	Distribution of progenies in different crosses for damage due to <i>Spodoptera</i> larvae	69-70
3.	Dendrogram obtained from pooled data of RAPD profiles of selected groundnut genotypes	93-94
4.	Genetic components of variance for damage due to <i>Spodoptera</i> in groundnut germplasm	96-97
5.	<i>Spodoptera</i> damage in groundnut germplasm	96-97
6.	Performance of groundnut genotypes for gain in weight by III instar larvae of <i>Spodoptera litura</i>	98-99
7.	Effect of groundnut genotypes on mortality of <i>Spodoptera litura</i> at different stages	101-102
8.	Effect of groundnut genotypes on gain in weight of <i>Spodoptera</i> larvae at different stages	101-102
9.	Effect of groundnut genotypes on pupation and fecundity of <i>Spodoptera litura</i>	101-102
10.	Reaction of parents for insects and disease	104-105
11.	Distribution of progenies for <i>Spodoptera</i> resistance parameters in different crosses	105-106
12.	Enhancement of resistance to <i>Spodoptera</i> in superior segregates	108-109

LIST OF PLATES

Plate No.	Title	Between pages
1.	Artificial infestation of <i>Spodoptera litura</i> in field	28-29
2a.	<i>Spodoptera litura</i> damage : Closeup view	28-29
2b.	<i>Spodoptera litura</i> damage : Field view	28-29
3a.	Egg masses of <i>Spodoptera litura</i>	29-30
3b.	Rearing <i>Spodoptera litura</i> in laboratory	29-30
3c.	A setup for egg laying by moths in laboratory	30-31
3d.	Pupal deformities	30-31
4.	Genotypic effect on larval development at 13 days after hatching	101-102
5.	Genotypic differences for the occurrence of malformed pupae	101-102
6.	Variation in parents of feeding by starved larvae of <i>Spodoptera litura</i>	103-104
7.	Variation in for <i>S. litura</i> damage in progenies	104-105
8.	Differential response of progenies to thrips	106-107
9.	Variation for late leaf spot resistance in segregating progenies	106-107
10.	Variation for rust resistance in segregating progenies	106-107
11a.	RAPD pattern in selected groundnut genotypes	112-113
11b.	RAPD pattern in selected groundnut genotypes	112-113

APPENDICES

Appendix No.	Title	Page No.
1.	Mean monthly data at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad	138
2.	Mean performance of the progenies of different crosses for pest resistance, disease resistance and productivity parameters in different crosses	139
3.	Performance of <i>Spodoptera</i> resistant segregants for productivity over environments	152
4.	Performance of single plant selections for productivity across seasons	154

LIST OF ABBREVIATIONS USED

AFLP	:	Amplified fragment length polymorphism
CTAB	:	N-Cetyl N, N, N-trimethyl ammonium bromide
DAH	:	Days after hatching
DS	:	Damage due to <i>Spodoptera litura</i>
EDTA	:	Ethylene diamine tetra acetic acid
ETL	:	Economic thresh hold level
GIW	:	Gain in weight by larvae
GMO	:	Genetically modified organisms
GN	:	GPBD 4 X NC Ac 343
HSM	:	Hundred seed mass
IPM	:	Integrated pest management
LAD	:	Leaf area damaged
LLS	:	Late leaf spot
MG	:	28-2 X GPBD 4
MLP	:	Mean larval period
MN	:	28-2 X NC Ac 343
MR	:	28-2 X R 9227
PCR	:	Polymerase chain reaction
PI	:	Protease inhibitor
PVP	:	Poly vinyl Pyrrolidone
PY	:	Pod yield
RAPD	:	Random amplified polymorphic DNA
RG	:	R 9227 X GPBD 4
RN	:	R 9227 X NC Ac 343
SP	:	Shelling out turn
SSR	:	Simple sequence repeats
TCA	:	Tri chloroacetic acid
TN	:	TAG 24 X NC Ac 343
TR	:	TAG 24 X R 9227

Introduction

I. INTRODUCTION

The cultivated groundnut (*Arachis hypogaea* L.) is an important oilseed crop of the world covering an area of 25.5 m ha with a production of 35.1 m t (Damodaram and Hegde, 2002). Groundnut is rich in oil and proteins and crushed for cooking oil. It is also used in the form of edible products. Protein rich meal is used for livestock and haulms are important source of fodder, especially in developing countries.

Among groundnut producing nations, India sows the highest area (8.0 m ha; 33%) and is the second largest producer (7.3m t; 28%) next to China (34%). Groundnut is the major contributor (33 %) of total oil seed production in India. Of late, the production has declined due to stiff competition from cheaper oils. This has been aggravated by today's liberalization process under the WTO. However, one of the greatest strengths of groundnut is that it is an important food legume having good demand as snack food in domestic as well as export market. India has good opportunity to enhance its export of hand picked selection (HPS) grade of groundnut to international market. Hence, more emphasis to improve and exploit groundnut as a food crop is given to make groundnut farming more competitive and remunerative.

Though, India is a leading producer of groundnut, the productivity is very low (756 Kg ha⁻¹) as compared to USA (3393 Kg ha⁻¹) and China (3134 Kg ha⁻¹) [Damodaram and Hegde, 2002]. Low productivity is mainly attributed to abiotic and biotic stresses affecting the crop. Among the biotic stresses defoliating insect, *Spodoptera litura* (F.) has become a major pest, because of which the yield losses are reported up to 71 per cent in the states of Karnataka and Andhra Pradesh (Amin, 1983). In Karnataka,

transitional tract, Dharwad has been identified as hot spot for *S.litura* during rainy season, where yield loss in groundnut to the tune of 66.6 per cent has been reported (Kulkarni, 1989).

At present, the pest is mainly controlled by insecticide application. Apart from increasing the cost of cultivation, the continuous use of pesticides has resulted in serious impact on biological ecosystem including resurgence of pesticide resistant insect population and biomagnification of pesticide residues in food and feed. The problem of development of resistance to pesticides is more acute in *S. litura* because of its polyphagous nature and rapid multiplication (Ramakrishna *et al.*, 1984 and Armes *et al.*, 1997). The integrated management of the pest with biological agents and resistant varieties appears to be the best strategy. With more importance given to groundnut as a food and export-earning crop, the problem of pesticide residue is of great concern. Host plant resistance occupies a prime position as it avoids pesticide usage in pest management. The varieties with pest resistance will have a major role in increasing the productivity of the crop and to make it more competitive for organic food markets of the world.

In recent years, the genetically modified crops (GMOs) with insecticidal genes have been developed in many crops including groundnut to manage insect pests. However, this has been met with wide spread resistance to accept them among the consumers. Uncertainty of the performance of these crops also exists. Possibility of extensive planting of the transgenic crops affecting non target animals such as predators and parasitoids persists. Additional problem is the development of resistance by the insects to toxins. Reports indicate that long term exposure to Bt transgenic crops cause allergic reactions in field workers. Hence, in the

world of environmental consciousness more emphasis should be given for exploitation of the inherent resistance present in the crop species through breeding.

Insect resistant cultivars can go to the farmers as a package that can combine an effective management strategy with good yield and desirable agronomic qualities, which are essentially cost free to the farmers. Thus, resistant cultivars facilitate an economic and environmentally sound management of pests and promote sustainable agriculture.

Other major biotic stresses affecting the crop are thrips and foliar diseases. Thrips attain the pest status in groundnut as sap feeders or vectors of viral diseases. Foliar diseases, late leaf spot along with rust can cause yield loss up to 70 per cent. Therefore, resistance to *S.litura* should not be considered in isolation and breeding efforts should aim at combining multiple pest and disease resistance with productivity.

In groundnut, erect bunch and early maturing Spanish types are most popular and widely cultivated because of ease in cultivation and harvesting. But, they are highly susceptible to pests and suffer heavily. Research efforts have been successful in identifying germplasm resistant to *S.litura* but most of them suffer from undesirable pod and kernel attributes, late maturity and susceptibility to other pest and diseases. Hence, there is a strong need to develop and identify new germplasm combining high level of resistance to *S.litura* including resistance to other pests and diseases with desirable agronomic attributes besides productivity in groundnut.

The migratory nature of the insect, non-uniform infestation in the field, complex nature of resistance and its association with poor agronomic features have made resistance breeding slow and difficult. Understanding

the mechanism of resistance will greatly enhance the efficiency of breeding. Identification of biochemical and molecular markers associated with resistance would help in increasing the precision and efficiency of resistance breeding. Considering the above information and analysis the present investigation envisaged.

1. Screening groundnut germplasm for resistance to *Spodoptera litura* (F.)
2. Standardization of a new criterion for large scale screening in the laboratory
3. Detailed evaluation of resistance in selected groundnut genotypes
4. Understanding the mechanism of resistance by assessing growth and development of the insect on host genotypes
5. Enhancing the resistance and combining it with other desirable agronomic features through hybridization
6. Assessing biochemical basis of resistance and
7. Assessing the molecular diversity of groundnut genotypes with varying levels of resistance

Review of Literature

II. REVIEW OF LITERATURE

Groundnut is an important oil seed crop of tropical and sub-tropical regions of the world. The environment in which groundnut is cultivated is an assortment of several biotic and abiotic components. One of the major reasons for the low productivity in groundnut in developing countries is the occurrence of biotic stresses. The major biotic stresses that groundnut crop generally encounters are disease and insect pests. Groundnut is attacked by soil insects, insects that live on the leaves and flowers and post harvest insect pests, among these insects that live on the leaves and flowers are important, as they cause maximum yield loss when compared to others.

In India, more than 90 species of insects attack groundnut. However, only a few are economically important over large areas. The leaf miner (*Aproaerema modicella* (Deventer)), tobacco caterpillar (*Spodoptera litura* (F.)), hairy caterpillars (*Amsacta* spp.), aphids (*Aphis craccivora* (Koch.)), Jassids (*Empoasca* Spp.), thrips(*Scirtothrips dorsalis*(Hood.), *Franklimella schultzei* (Trybom.), *Thrips palmi* (Kary) and termites (*Odontotermes* spp.) are recognized as important destructive pests on groundnut (Amin and Mohammad, 1980).

The literature pertaining to

1. Importance of *S. litura* in groundnut
2. Role of integrated pest management
3. Host plant resistance to *S. litura* and thrips
4. Biology and development of *S. litura* on different groundnut genotypes
5. Mechanisms of resistance to *S. litura* and
6. Molecular diversity in groundnut are presented in this chapter.

2.1 IMPORTANCE OF *S. litura* IN GROUNDNUT

Spodoptera litura is a polyphagous foliage-feeding insect found throughout the tropical and subtropical parts of the world (Feakin, 1973). In India, it was first reported in 1980 as an increasingly important pest of groundnut (Amin and Mohammad, 1980). The larvae feed gregariously on leaves and fresh growth causing extensive damage.

All India Co-ordinated Research Project (AICRP) on groundnut has identified five centers Viz., Dharwad, Jalgaon, Jagital, Raichur and Vriddhachalam for screening the genotypes against pests. Out of these, Dharwad and Vriddhachalam have recorded on an average 50 per cent defoliation due to *S. litura* since last five years (AICRP Annual progress reports 1998-2002). At Dharwad, the incidence of defoliation was noticed during vegetative stage and continued till harvesting. The maximum damage was seen at flowering and pod formation stage indicating the prevalence of pest infestation throughout the entire crop period. Hence, Dharwad has been identified as hot spot for *S. litura* and provides excellent conditions for screening of genotypes against this pest.

In groundnut, even a slight damage to tips by *S. litura* reduces the yield by 4-10 per cent and medium or heavy defoliation of leaves reduces the yield by 13-17 per cent (Teich, 1969). The yield loss varied from 13-71 per cent in the states of Karnataka and Andhra Pradesh (Amin, 1983). In Karnataka, Dharwad has been identified as a hotspot for *S. litura*, where in, the yield losses ranged from 22 per cent (Patil *et al.*, 1994) to 66.60 per cent (Kulkarni, 1989). Depending on the infestation, the yield losses from 19.6 to 27.14 per cent have been reported. (Panchabhavi and Nethradhani Raj, 1987 and Kennedy *et al.*, 1992).

The density of the insect and stage of the crop influences the extent of yield loss (Dhir *et al.*, 1992). One larva per plant at the seedling and flowering stage would cause significant yield loss. At seedling stage, one

larva per plant consumed about 54.7 per cent leaf area and reduced pod yield by 25.8 per cent. At flowering stage, one larva per plant consumed about 38.8 per cent leaf area and resulted in yield loss of 5.7 per cent. The presence of 3 egg masses of an average size (each containing approximately 250 eggs) in 15 meter source area may cause significant loss of pod and haulm of groundnut (Panchabhavi and Nethradhani Raj, 1987).

2.2 ROLE OF INTEGRATED PEST MANAGEMENT

World wide insect damage to food crops costs farmers and consumers millions of dollars each year. Many insect control strategies have been employed. It is increasingly being realized that reliance upon a single control strategy is not advisable. Heavy reliance upon insecticides has created a host of problems such as resurgence of pest populations, emergence of secondary pest outbreaks as a consequence of elimination of beneficial insect species, increased cost of food production and serious environmental pollution. Because most insect pests have high reproductive rates, they can eventually adapt to natural and synthetic pesticides, making complete eradication highly unlikely. Control measures therefore must aim at containment of insect populations rather than their eradication. This thinking led to the development of integrated pest management (IPM). IPM aims at keeping pest population below economic threshold level (ETL) through an ecologically sound, economically practicable, socially acceptable and environmentally sustainable technology. Host plant resistance along with natural biological and cultural control measures is the basic component of an IPM system.

2.2.1 Insecticide resistance in *Spodoptera* spp.

Extensive research on development of insecticidal resistance by *Spodoptera* spp. has been done in Cotton. In Egypt, *S. littoralis* has developed resistance to more than 20 (Methyl parathion, DDT, Monocrotophos, Phosalone, Chlorpyrifos, Pyrethroids, Carbamates etc.,) commonly used insecticides (El-Dahan *et al.*, 1985, El-Guindy *et al.*, 1986a

and b, El-Sayeed and Abdullah, 1988, El-Sayeed *et al.*, 1991 and Issa *et al.*, 1986) while, at Florida *S. frugiperda* attacking maize has developed resistance to more than 15 insecticides (Yu, 1992). Development of insecticide resistance by *S. exigua* and *S. littoralis* has also been reported in tomato and potato at California and Cyprus respectively (Brewers and Trumble 1989 and Charalambous and Iordanou 1997). The development of insecticide resistance in *Spodoptera spp.* is due to reduced penetration, action of detoxifying enzymes, target site insensitivity, enhanced metabolism and also to some extent to behavioral resistance.

2.2.1.1 Status in India

S. litura was one of the first pest of agricultural importance in India to develop resistance to insecticides. By 1965 resistance to benzene hexachloride (BHC) was reported in field populations from Rajasthan (Srivastava and Joshi, 1965) and by the early 1970s to endosulfan and carbaryl in Haryana (Verma *et al.*, 1971). In the early 1980's populations in the south Indian state of Andhra Pradesh were shown to be highly resistant to lindane, endosulfan, carbaryl and malathion (Ramakrishna *et al.*, 1984). Populations from Bapatla (Andhra Pradesh) also showed resistance to chlorinated hydrocarbons, organophosphates and carbamates indicating the insecticidal resistance (Mayuravalli *et al.*, 1987). It was concluded from the study in 1996 at ICRISAT that *S. litura* in Andhra Pradesh has developed significant levels of resistance to cypermethrin, fenvalerate and monocrotophos, but resistance to endosulfan, quinalphos and methomyl was moderate to low. It appears however, that resistance may be increasing with time as indicated by the generally high resistance levels to all chemical class reported in strains tested in 1996 (Armes *et al.*, 1997). Of late populations in Karnataka were shown to have insensitivity to cypermethrin, quinolphos, endosulfon, monocrotophos and carbaryl (Ramegowda and Basavana Goud, 2001). Under these circumstances, the development of resistant/tolerant variety assumes greater significance in the management of *S. litura*.

2.3 HOST PLANT RESISTANCE

Host plant resistance is the relative amount of qualities inherited by the plant, which influences the ultimate degree of damage done by the insect in the field (Painter, 1951).

The genus *Arachis* displays several of these resistance factors. Therefore, host plant resistance represents the key for major advances in insect pest management

2.3.1 Host plant resistance to *Spodoptera litura*

Host plant resistance can be exploited as an effective and environment friendly component of pest management. To detect and exploit it has been a high priority for groundnut breeder.

During *Kharif* 1995-97 at Jalgaon, Maharashtra, India, *S. litura* larval damage to foliage was recorded in 32 genotypes. The lowest leaf damage (5%) was recorded in ICGV 86156, 86400, 86528, 87128, 87141, 87290, 87411 and 91214 (Dharne and Patel, 2000). Fifty groundnut genotypes were screened for resistance to *Spodoptera litura* in All India Co-ordinated Research Project on Oil Seeds at Dharwad (Anon., 1985). Due to low level of infestation during *kharif* 1985, majority of entries (40) fell under injury rating of 3. However, 7 entries were under injury rating of 2 and three entries sustained higher damage with a rating of 4. At Hissar, GC-79 and GC-333 were observed to be free from *S. litura* damage (Anon., 1985). Of the 50 cultivated groundnut genotypes screened for resistance to defoliator, 22 entries fell below injury rating scale of 2, thirteen below rating 3, three below rating 4, six below rating 5 and one below rating 6 (Anon, 1986).

Fifteen *Arachis hypogaea* genotypes were screened in laboratory using choice test for resistance to *H. armigera* (3rd instar) and *S. litura* (1st, 3rd and 5th instar). Of them, only BG 2 a Virginia bunch variety was resistant to both the pests (Singh *et al.*, 1993). Based on oviposition and

feeding performance test conducted with the seven cultivars, NC Ac 2243 was least preferred, while the UPL Pn 2 was the most preferred and EG Pn13 occupied an intermediate position (Xie Jia Li, 1987). The cultivar C-501 was most resistant and M-145 was the most susceptible among the nine groundnut varieties tested in laboratory at Pantnagar on the basis of growth and development of *S. litura* (Tiwari *et al.*, 1989).

Preliminary screening to assess the resistance to *S. litura* was carried out in the field on six Virginia bunch and 18 Virginia runner accessions and the highest resistance was recorded by Virginia runner varieties NC Ac 17840, NFG 79 and EC 21989 (Rajgopal *et al.*, 1988). In a screening study by Patil *et al.* (1991), entries ICGVs 87264 and 86598 have recorded the least damage (<17.5%) and entries ICGVs 86598 and 86125 have also recorded less damage (<27.5%). Other entries, ICGVs 86350, 86276 and 87287 showed promise for resistance with least damage (17.5%) at two stages of screening (75 and 90 DAS). RG 97 was another promising line resistant to *S. litura*.

Groundnut genotype DER (Dharwad Early Runner) treated with ethyl methane sulfonate, yielded many Valencia mutants with multiple resistance to foliar diseases. These mutants were systematically screened for *S. litura* in 1996. Based on leaf area damage, three mutants (28-2, 45 and 110) were identified as resistant to *S. litura* (Prasad, *et al.*, 1998).

Screening of groundnut germplasm and advanced breeding lines in the All India Co-ordinated Project has resulted in new sources of resistance to *S. litura* with better agronomic background – ICGVs 86364, 91172, 86400, 86402, 91112, M 335 (Anon., 1998), ICGVs 91167, 86364, 86590, ICGS 44 and ICG 2271 (Anon., 1999). Several years of screening germplasm collections at National Research Center for Groundnut, Junagadh resulted in genotypes *viz.*, NRCG 5724, 2615, 9773, 8313 and 8673 possessing resistance/tolerance to *S. litura* (Basu, 2003). Some of the elite groundnut genotypes resistant to *S. litura* have been registered as improved germplasm (Table 1).

Table 1: Germplasm registered as *Spodoptera litura* resistant.

	Genotypes	Pedigree	References
1.	ICGV 86031	F334A-B14 X NCAC2214	Dwivedi <i>et al.</i> (1993)
2.	ICGV 86590	X-14-4-13-19-B X PI 259747	Anon. (1992)
3.	ICGV 87165	PI 261942 X PI 594923	Moss <i>et al.</i> (1997)
4.	ICGV 86699	Single plant selection from CS29	Reddy <i>et al.</i> (1996)
5.	PI 619171	PI 261942 X GKP 10017	Stalker and Lynch (2002)
6.	PI 619172	PI 261942 X PI 262141	Stalker and Lynch (2002)
7.	PI 619173	PI 261942 X PI 262141	Stalker and Lynch (2002)
8.	PI 619174	PI 261942 X PI 262141	Stalker and Lynch (2002)
9.	Mutant 28-2	EMS mutant from VL 1	Gowda <i>et al.</i> 2002

2.3.2 Host plant resistance in *Arachis* spp. for *Spodoptera litura*

Many wild species of *Arachis* have varied levels of insect resistance. The extent of resistance in wild species is presented in Table 2. This represents a formidable amount of potentially exploitable materials, especially when it is realised that the levels of resistance far exceed that detected in *A. hypogaea*.

Most wild species have not been exploited in resistance breeding due to the ploidy incompatibility in turn crossing barrier. Though some species belonging to section *Arachis* have been utilized in the improvement of *A. hypogaea* for resistance to other pests and diseases, no systematic efforts have been made to utilize the resistance to *Spodoptera*.

2.4 BIOLOGY AND DEVELOPMENT OF *SPODOPTERA LITURA* (F.) ON DIFFERENT GROUNDNUT GENOTYPES

Spodoptera litura is a polyphagous pest attacking 112 species of plants belonging to 44 families (Moussa *et al.*, 1960). Therefore, vast numbers of reports are available on the biology of this insect on different host plants. However, literature pertaining to biology and development of *S. litura* on groundnut is presented below.

The biology of *S. litura* was studied on different groundnut genotypes along with a wild tetraploid *A. monticola* (Patil *et al.*, 1995). The survival was least on the wild species whereas it was maximum on Dh-3-30. The survivability on ICGVs 86031 and 87264 was comparatively less followed by ICGVs 87165, 86350, and 86699. The larval duration was less on susceptible genotypes indicating higher rate of multiplication while, it was more in resistant genotypes. The genotype ICGV 86031 showed similar response in another laboratory study by Sreenivasa *et al.* (1997). Larval mortality was highest on ICGV 86031 (82.8%) followed by ICGV 86350 (53.32%) and Dh-3-30 (43.02%). Development was shortest on Dh-3-30 (32.25 days), and longest on ICGV 86031 (37.5 days) with adults surviving a maximum of 9.5 days. Of the nine groundnut varieties tested in the

Table 2: *Arachis* spp. tested (Laboratory assay) at ICRISAT center for resistance to *Spodoptera litura* with an indication of the level of resistance (Wightman and Ranga Rao, 1994)

<i>Arachis</i> species	Collection Numbers	Reaction to armyworm (<i>S. litura</i>)
<i>A. apressipila</i>	10002	HR
<i>A. batizogaea</i>	GKP 9667 (316)	HR
<i>A. cardensaii</i>	36034 YF	S
<i>A. chacoensis</i>	10602	R
<i>A. chacoensis</i>	10602(5)	HR
<i>A. paraguariensis</i>	30134	HR
<i>A. paraguariensis</i>	30109	HR
<i>A. paraguariensis</i>	GKPSC 30124	HR
<i>A. pseudovillosa</i>	9634	R
<i>Arachis</i> sp.	30007	R
<i>Arachis</i> sp.	A77/133	HR
<i>Arachis</i> sp.	GKP 9578 (312)	S
<i>Arachis</i> sp.	GKP 9797	HR
<i>Arachis</i> sp.	GKPSCS 30135 (21)	HR
<i>Arachis</i> sp.	GKP 9893 (321)	HR
<i>Arachis</i> sp.	GKP 9629 (315)	HR
<i>Arachis</i> sp.	GKP 9572 (311)	HR
<i>Arachis</i> sp.	9921 (100)	HR
<i>Arachis</i> sp.	KG 30012 (339)	HR
<i>Arachis</i> sp.	GKPSC 30116/19	HR
<i>Arachis</i> sp.	GKPSC 30114	HR
<i>Arachis</i> sp.	GKPSCS 30114 (18)	HR
<i>Arachis</i> sp.	GKP 9553 (90)	HR
<i>Arachis</i> sp.	GKPSCS 30135	HR
<i>Arachis</i> sp.	GKPSCS 30138 (3)	HR
<i>A. chacoense</i>	GKP 10602	HR
<i>A. glabrata</i>	HL 189	HR
<i>A. glabrata</i>	HLK He 571 (91B)	HR
<i>A. hagenbeckii</i>	A 45/114	HR
<i>A. hagenbeckii</i>	A 27/117	HR
<i>A. hagenbeckii</i>	2A 5	HR
<i>A. kemf-mercadoi</i>	30085	HR
<i>A. monticola</i>	7264	R
<i>Arachis</i> sp.	GKPSCS 30132 (1)	HR
<i>Arachis</i> sp.	GKPSC 30122	HR
<i>Arachis</i> sp.	GKPSC (30120 (13)	HR
<i>Arachis</i> sp.	Manfredi 5	HR
<i>A. stenophylla</i>	30126	HR
<i>A. sternospema</i>	HLK408	S
<i>A. villosa</i>	----	HR
<i>A. hypogaea</i>	TMV 2	S
<i>A. hypogaea</i>	EC 36892	S

HR : Highly resistant, R – Resistant,

S – Susceptible.

laboratory for resistance to *S. litura*, C-501 was most resistant and M-145 was the most susceptible (Tiwari *et al.*, 1989). On the basis of growth and development parameters, the varieties could be grouped in to six groups.

In a laboratory feeding bioassay, larval weight of fall armyworm at 10 days of development ranged from 75.7 mg (Southern runner) to 26.5 mg (Florunner) indicating wide diversity of growth response on various groundnut genotypes (Todd *et al.*, 1991). But survival was very high on all the genotypes tested. Larvae fed with Florunner (resistant) required an average 3.7 more days to develop compared with larvae fed with curly leaf.

Spodoptera litura reared on EMS derived Valencia mutants at Dharwad, Karnataka, showed that mutant 28-2 and 45 consistently showed less leaf damage, high mortality, low weight and low gain in weight of larvae compared to susceptible check (JL 24) and parents (DER and VL 1) at all the stages. The mortality and gain in weight was very much pronounced on neonate larvae. The resistance effect of these mutants also extended the larval period by three days and had pronounced effect on the fecundity of moths indicating resistance in the mutants (Prasad *et al.*, 2000). Leuk and Skinner (1971) reported that the mean length of the life cycle of *S. frugiperda* was shorter (29 days) for the susceptible Starr than South eastern runner (33.3 days). The mean percentage of the moth emergence was significantly less for larvae fed on foliage of the resistant cultivar, south eastern runner and the mortality of the total insects fed with the foliages was higher at all the stages of larval development and pupation on south eastern runner than Starr.

Longer larval duration, lower fecundity and egg hatchability were observed on NC Ac 2243 (Xie Jia Li, 1987). The duration of the generation was short and the intrinsic rate of increase was higher on the varieties of dwarf mutants and Pol 2 than on any other cultivar (Tiwari *et al.*, 1981). But the pupal weights of male *S. frugiperda* tend to be greater than female on Florunner groundnut (Garner and Lynch, 1981). On the same cultivar, leaf consumption was significantly greater on 40 days old plants than on

60 days old plants. Consumption declined significantly as the age of the leaves increased and larval mortality rates were higher on older leaves. Larvae preferred to feed on younger leaves.

Several wild species of *Arachis* disrupted larval development and increased mortality of *S. frugiperda* (Lynch *et al.*, 1981). Whilst the majority of *A. hypogaea* cultivars were susceptible to *S. litura*, efforts to identify resistance to this pest in cultivated groundnuts have shown that, some cultivated genotypes of *A. hypogaea* possess low levels of resistance (Wightman *et al.*, 1990) and Stevenson *et al.* (1993a) and Lynch *et al.* (1981) have indicated that many wild species of *Arachis* were highly resistant to attack by larvae of *S. litura*.

The mortality of neonate larvae on TMV 2 was significantly lower than that on any other species of *Arachis*. Overall, the mortality and development of larvae recorded in the field were similar to those recorded for larvae on excised leaves of the same species in laboratory. When neonate larvae were exposed to excised leaves of *A. batizogaea*, *A. kemphmercadoui*, *A. appresila*, *A. stenophylla*, and *A. villosa*, mortality was greater than 94 per cent compared to less than 20 per cent on TMV 2. Diets containing the chemical extracts of dried leaves of *A. kemphmercadoui*, *A. paraguariensis*, *A. appresipila*, *A. chacoensis*, *A. glabrata* and *A. pseudovillosa* resulted in low larval weight gain (Stevenson *et al.*, 1993b). Differences in the rate of development of larvae on *Arachis* species were readily apparent. At 5 days, larvae on all species had entered 2nd instar with the exception of larvae on *A. burkartii*, which was still in the 1st instar. Larval growth was significantly faster on *A. monticola* than on *A. cordensaii*, *A. chacoense*, *A. correntina*, *A. lignosa*, *A. villosulicarpa* and *A. villosa*. Ten days onwards differences in larval growth rates were even more pronounced. Larval survival to pupation also tends to be related to the rate of larval development. Survival was greatest (90%) on *A. monticola*, but only few (15-20%) larvae were able to complete larval development on *A. villosulicarpa*, *A. cardensii*, *A. lignosa* and *A. correntina*. None of the larvae completed their development on *A. burkartii*, *A. villosa* and *A. repens*. They

consumed the greatest amount of leaf tissue and required longest development period. The pupae weighed significantly lower than that of all the other wild species (Lynch *et al.*, 1981).

2.5 MECHANISM OF RESISTANCE

Resistance may vary between two extreme immunity and high susceptibility. In addition to the level, mechanism of resistance is also important for its complete understanding. Factors that determine the resistance of host plants to insect establishment include presence of structural barriers, chemicals and nutritional imbalances.

2.5.1 Morphological and anatomical feature as mechanism of resistance

Structural and morphological factors can serve as defence mechanisms for plants when herbivores come in contact with them. The most common factors that impart resistance are leaf toughness, cell wall composition, proliferation of wounded tissue, pubescence, etc.

Bioassay carried out with larvae to detect the mechanism of resistance (Wightman and Ranga Rao, 1994) revealed no antibiosis effect on II-VI instar larvae when fed with matured leaves of ICGV 86031. Padgham (cf. Wightman and Ranga Rao) also found that newly hatched larvae had a marked propensity to vacate the leaves of this variety in the first two hours of free life. This suggests the resistance factors that influence the neonates is associated with the leaf surface because the feeding activity is restricted to scraping of the leaf surface.

Although, the groundnut variety C-501 fulfilled the nutritional requirements of *Spodoptera litura*, it was less suitable as a food plant, possibly on account of the texture of its leaves and/or the pubescence (Tiwari *et al.*, 1991). The same factor also influenced the life cycle of *S. litura* on genotype NC Ac 2243 (Xie Jia Li, 1987). Information on leaf morphology of some 21 *Arachis hypogaea* lines along with check JL 24

suggested that genotypes with long, narrow leaves and profuse hairiness of stems and leaves was less preferred by *S. litura* larvae than other morphological types (Vasanthi and Padmavathamma, 2000).

2.5.2 Biochemical basis of resistance

It is known that biochemicals play an important role in the defence against insect herbivores as they act as insect repellent, feeding inhibitors and/or toxins, thus protecting plants at different phases of growth. But, adequate information on behavioral and physiological responses of insects to plant chemicals is needed. The chemicals responsible for insect resistance in crop plants have been identified only in a few instances and currently efforts are being made to investigate these factors.

2.5.2.1 Phenols

Phenols include a large group of organic aromatic compounds and are known to provide resistance to the plants against insects. Four genotypes resistant to *S. litura* and the susceptible control TMV 2 were analysed by HPLC for phenolic acids. Of the 8 phenolic acids detected, 7 were present in all the genotypes. The highest phenol content was found in the susceptible genotypes TMV 2 and M13 (Singh and Sachan, 1992). Biochemical analysis of samples from accessions differing in resistance revealed that, there was no relationship between nitrogen, phenolics, potash and sodium contents and leaf damage (Rajgopal *et al.*, 1988). The resistant groundnut mutants (28-2, 45 and 110) differed significantly for phenols and orthodihydroxy phenols, but were not associated with any of the insect resistance parameters (Prasad, 1997).

2.5.2.2 Polar phenolic compounds

The potential role of quercetin-3-diglycosides and coffeoylquinic acid in the resistance of crop plants to the attack of lepidopteran insects is well established (Elliger *et al.*, 1981, Isman and Duffey, 1982; Duffey and Felton, 1991). Both rutin and chlorogenic acid severely inhibited larval

development when presented to larvae at the same concentration as the equivalent compounds found in the foliage of *A. chacoensis*, *A. paraguariensis*, and hybrid (*A. chacoensis* x *A. hypogaea*) which suggested that, these compounds are at least responsible for the resistance to groundnuts to the larvae of *S. litura* (Stevenson, 1993). Further, analysis of the coffeoylquinic acids in *A. paraguariensis* revealed the presence of 1-coffeoyl-4-deoxyquinic acid (1-cdQA), 3-coffeoylquinic acid (3-CQA) and 5-coffeoylquinic acid (5-CQA Chlorogenic acid) (Stevenson *et al.*, 1993a). Development of *S. litura* larvae fed with diets treated with 1-cd QA, 3-CQA and 5-CQA was severely inhibited compared to the larvae fed on untreated diet.

2.5.2.3 Cuticular lipids

Many herbivore insects seem to select their host plant on the basis of the chemical and ultra structural characteristics of the plant surface. Certain plant cuticular lipids can contribute to insect resistance by affecting insect feeding behaviour.

Fall army worm that were fed with a diet containing foliage of *A. ipaensis* from which the cuticular lipids had been extracted had increased larval weights and earlier pupation and adult emergence compared to the individuals reared on diet with unextracted foliage. The developmental parameters of fall armyworm that were fed on diet containing the lipids that had been extracted from the foliage of *A. ipaensis* did not differ from those of insects fed on control diet (Yang *et al.*, 1995).

2.5.2.4 Plant protease inhibitor

Protease inhibitors have been found in several crop species of plants. These proteins may normally be present in plant tissues (Boulter *et al.*, 1990; Gatehouse *et al.*, 1991), or they may be induced as a result of injury (Broadway *et al.*, 1986). When ingested by insects, protease inhibitors inhibit the activity of digestive enzymes, such as trypsin and chymotrypsin

resulting in improper digestion of proteins, starvation and death. The inhibition of digestive enzymes not only has a direct effect on insect's nutritional status, but is also thought to lead to secondary effects. Wherever synthesis of digestive enzymes occur as a feedback mechanism in an attempt to utilize ingested food leading to further short comings in recycling of amino acids used for gut protein synthesis.

The most promising protease inhibitor is the cowpea trypsin inhibitor (CPTI) from *Vigna unguiculata* L. (Boulter *et al.*, 1990; Hoffmann *et al.*, 1992). The gene for CPTI has been transferred to tobacco and has provided resistance to wide range of insects among the lepidoptera, coleoptera and orthoptera. Protease inhibitors from tomato (Tomato inhibitor II) and potato (PI-I and PI-II) have been transferred to tobacco and enhanced resistance to *Spodoptera exigua* was observed (Jongsma *et al.*, 1995).

The leaves of groundnut genotypes, GG 2 and PBS 105 contained 7260 and 5923 units of trypsin inhibitor per gram of fresh weight, respectively (Nandagopal *et al.*, 1997). The specific inhibitor activities were 1952 and 1431 units mg⁻¹ protein, while the levels of endogenous proteinases were 600 and 1200 units per gram of fresh leaf weight in GG 2 and PBS 105, respectively. Thus it was shown that there exists a trypsin inhibitor in the leaves of groundnut but did not ascertain whether these inhibitors are effective against *S. litura*. The augmentation of genes coding trypsin inhibitors in plants for enhancing resistance can be achieved either by conventional breeding or recombinant DNA technology

2.5.2.5 Lectins

Lectins form a large and diverse group of proteins, which are found throughout the range of living organisms, but are identified by a common property of specific binding to carbohydrate residues, either as free sugars, or more commonly, as part of oligo-or polysaccharides. They are distinguished from enzymes by having no action on the carbohydrate other than binding to it. Most lectin molecules contain multiple binding sites and

thus can cross-link oligo-or polysaccharides. They are also called agglutinins for this reason, since the cross-linking of carbohydrate side chains on cell surface glycoproteins leads to the formation of aggregates of cells, and a visual agglutination of cells such as red blood cells.

Lectins when ingested along with food, binds to specific receptor glycoproteins (determined by the carbohydrate binding specificity of the lectin) on the surface of the epithelial cells lining the gut. This leads to potential disruption of the mid gut. Lectin also passes across these cells and appears in the haemolymph leading to the possibility of systemic effects. Lectin binding can also be observed in the Malpighian tubules and may occur to chemoreceptors in mouthparts leading to antifeedant effects (Gatehouse, *et al.*, 1996)

The insecticidal properties of plant lectins have been demonstrated in numerous studies where purified proteins were fed to insects in artificial diet bioassays. Seventeen commercially available plant lectins were screened for insecticidal activity against the storage pest *Callisobruchus maculatus* (Murdock *et al.*, 1990). Five lectins were found to cause a significant delay in larval development at dietary levels of 0.2 % and 1.0 %. Czalpa and Lang (1996) took a similar approach when they screened a range of lectins for activity against the Southern corn rootworm and European corn borer. Artificial diet bioassays have shown that, snowdrop lectin (GNA) significantly decreased growth and development of tomato moth larvae (*Lacanobia oleracea*) at 2 % of total protein, although little effect on survival was observed (Fitches *et al.*, 1997). Transgenic plants expressing pea lectin up to 1.0 % of total protein showed reduced larval biomass and leaf damage by *Heliothis virescens* as compared to control plant (Boulter *et al.*, 1990)

Extensive studies on distribution of lectins in jumbo Virginia and Spanish varieties of the peanut indicated the temporal distribution of lectins in roots, stems, hypocotyls and leaves in both the varieties of groundnut (Pueppke, 1981). The absolute amount of lectins (as $\bar{\text{I}}$ g/plant in

a given tissue) was usually greater in the Spanish (601 g /plant) than in jumbo Virginia plants (421 g /plant). He screened 4652 genotypes of genus *Arachis*. Peanut lectin was present in each of the 4556 lines of cultivated peanuts. Although seeds of most of the wild *Arachis* species also contained peanut lectin, extracts from seeds of *Arachis* spp. 33828, 338292 and 338294(from Brazil) and *Arachis villosa* 261871(from Argentina) reproducibly failed to form immuno precipitates. This suggests the natural variation present for lectins in peanuts. However, the insecticidal properties of lectins have to be tested against a target pest in order to explore them for genetic improvement of groundnut with respect to resistance breeding.

2.6 MOLECULAR DIVERSITY IN GROUNDNUT USING RAPD ASSAY

Molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programmes. However, their application in groundnut enhancement is lagging behind because of limited knowledge of the genome. Extensive variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al.*, 1992), but abundant DNA polymorphism has been observed only in wild diploid *Arachis* species (Kochert *et al.*, 1991; Halward *et al.*, 1993). It is surprising that, such a diverse variation for phenotypic characteristics in cultivated species did not show significant variability at the DNA level. However, Halward *et al.* (1992) observed very low levels of DNA polymorphism in cultivated groundnut germplasm, using different techniques such as restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) analysis.

No variation in banding pattern was observed among the cultivars and germplasm lines of *A. hypogaea* using RAPD technique (Halward *et al.*, 1992), whereas the wild *Arachis* species were uniquely identified with most primers tested. Bhagwat *et al.* (1997) studied the variation in RAPD profiles between groundnut cultivar Spanish Improved and its mutants developed through X-ray irradiation. Twelve RAPD primers produced 1182 fragments of which 65 fragments were polymorphic (5.5%) thus giving a ratio of 1.51

polymorphic bands per primer. The identification of 9 groundnut varieties by polymerase chain reaction, 223 RAPD molecular markers were detected; Of them, 183 markers were polymorphic and 83 markers were unique. Two primers B10 and B16 proved very powerful for detecting genetic variation among the 9 groundnut varieties (Huang and Tsai, 1997). Further, of the 48-oligonucleotide primers only 7 (14.6%) yielded polymorphic amplification products in a RAPD assay involving 70 selected groundnut genotypes (Subramanian *et al.*, 2000). Twenty-one random primers revealed 42.7 per cent polymorphism among 220 genetic loci amplified from 13 accessions (Raina *et al.*, 2001). Further study by Dwivedi *et al.* (2001) detected substantial differences in DNA profiles among 26 cultivated ground accessions. However, these differences could not be related to the known biological information about the accessions falling into different clusters. Of the 18 RAPD assays conducted to study genetic diversity among germplasm with varying levels of resistance to early leaf spot, the primers G 20, J 17, V 15, and V 16 produced greater polymorphic differences. The accessions were grouped into three distinct clusters and crossing of diverse genotypes was suggested for the development of recombinant inbred lines to identify DNA markers linked with early leaf spot resistance (Dwivedi and Gurtu, 2002)

Thus, detection of polymorphism in cultivated groundnut opens up the possibility of development of its molecular map by judicious selection of genotypes that show DNA polymorphism. This approach will be useful for developing marker assisted selection as a tool for genetic enhancement of groundnut for desirable traits like pest and disease resistance.

Material and Methods

III. MATERIAL AND METHODS

The information on the experimental material utilized and the methods adopted to fulfill the objectives of the current investigations are given in this chapter.

3.1 EXPERIMENTAL MATERIAL

Experiment 1: Screening germplasm for resistance to *S. litura*

Thirty nine groundnut germplasm comprising of interspecific derivatives (7), advanced breeding lines (19), mutants (4) and popular cultivars (9) were screened against *S. litura* under artificial infestation during 1998 rainy season. Each genotype was grown in three rows of 2.5 m length with a spacing of 30 cm between rows and 10 cm between plants. The treatments were replicated twice and leaf area damaged due to *S. litura* was visually scored.

Experiment 2: Standardization of a new criterion for large scale screening

Different leaves on the main stem (I, II and III leaves) from top in four genotypes comprising of two resistant (28-2 and NC Ac 343) and two susceptible (TAG 24 and JL 24) genotypes were used for feeding III, IV and V instar larvae of *S. litura* during 2000 rainy season. Leaf area damaged and gain in weight by larvae after feeding were recorded.

Experiment 3: Evaluation of selected groundnut genotypes against *S. litura*

Thirteen selected groundnut genotypes were evaluated against *S. litura* during 1999 rainy season. The identity and pedigree of these genotypes is presented in Table 3. Each of the selected genotypes was sown in five rows of 5 m length with a spacing of 30 cm X 10 cm. The experiment was replicated twice. In field, damage due to *S. litura* was visually assessed

Table 3: Pedigree of the selected groundnut cultivars for evaluation

Genotype	Botanical type	Pedigree
NC Ac 343	VB	NC Bunch / PI 121067
Mutant 28-2	SB	EMS Mutant of VL 1
R 9227	SB	(ICGS 7 X NC Ac 2214) X ICGV 86031
ICGV 86031	SB	F 334 A-B-14 X NC Ac 2214
ICGV 86699	VB	[(<i>A. batizocai</i> x <i>A. duranensis</i>) x <i>A. hypogaea</i> (Cv. NC 2)] CS 29
ICGV 87165	VB	[<i>A. hypogaea</i> var. <i>fastigiata</i> (PI 261942) x <i>A. cardensaii</i>] CS 9
ICGV 86590	VL	X 14-4-B-19-B X PI 259747
GPBD 4	SB	KRG 1 X ICGV 86855
JL 24	SB	Selection from EC 94943
TMV2	SB	Mass selection from 'Gudhiatham bunch'
TG 26	SB	BARCG 1 X TG 23
TAG 24	SB	TGS 2 X TGE 1
TG 49	SB	TG 28 A X TG 26

SB : Spanish bunch, VB – Virginia bunch, VL – Valencia.

under artificial infestation. In laboratory, gain in weight by larvae was recorded as in experiment 2.

Experiment 4: Life history parameters of *Spodoptera litura* (F.) on selected groundnut genotypes

The experimental material comprised of four resistant genotypes (28-2, NC Ac 343, ICGV 86031 and R 9227) and three susceptible genotypes (TAG 24, GPBD 4 and JL 24). The experiment was conducted during 1999 rainy season.

Experiment 5: Enhancement of resistance to *Spodoptera* through hybridization

Five parents involving three resistant (28-2, R 9227 and NC Ac 343) and two susceptible (TAG 24 and GPBD 4) with good agronomic features were hybridized randomly to produce eight crosses *viz.*, 28-2 X R 9227, 28-2 X NC Ac 343, 28-2 X GPBD 4, R 9227 X NC Ac 343, R 9227 X GPBD 4, R 9227 X TAG 24, TAG 24 X NC Ac 343 and GPBD 4 X NC Ac 343 during rainy season of 1999. F_{2:3} progenies were sown in 1 m rows and assessed for *S. litura* damage under artificial infestation in field and the gain in weight by larvae in laboratory during rainy season of 2001. The experiment was replicated twice. The pedigree and characters of parents used in hybridization programme are presented in Table 4.

Experiment 6: Biochemical basis of resistance to *S. litura*

Thirteen selected groundnut genotypes (Table 3) were analyzed for protease inhibitors and lectins during 2003 rainy season.

Experiment 7: Molecular diversity in selected groundnut genotypes

Thirteen popular groundnut cultivars (Table 3) were assessed for DNA polymorphism through RAPD assay.

3.2 EXPERIMENTAL SITE

The field experiments were conducted at the Botanical Garden of University of Agricultural Sciences, Dharwad, which is located in the

Table 4: Salient features of parents used in hybridization programme (Naidu, 2003).

Parents	LLS	Rust	<i>Sclerotium</i>	<i>S. litura</i>	Bud necrosis	Thrips	Iron chlorosis	Days to maturity
GPBD 4	+	+	-	-	-	-	-	105
Mutant 28-2	+	-	-	+	-	+	-	107
NC Ac 343	-	-	+	+	-	+	-	120
R 9227	-	+	+	+	+	-	+	105
TAG 24	-	-	-	-	-	-	+	100

+ Resistance

- Susceptible

transitional tract of Karnataka state at 15°13' N latitude, 75°07' E longitude and at an altitude of 678 m above mean sea level. The experimental material was raised in a randomized block design with two replications under unprotected conditions. The soil type was medium black with pH range of 7.0–7.5. Insect rearing and molecular work was carried out at Division of Genetics and Plant Breeding and Biotechnology, UAS, Dharwad, while biochemical analysis was carried out at Department of Biochemistry, Karnataka University, Dharwad.

3.3 CLIMATIC CONDITIONS

The monthly meteorological data obtained from meteorological observatory attached to the Agricultural College, Main Agricultural Research Station, UAS, Dharwad, with regard to temperature, relative humidity, and rainfall during the course of investigation is presented in appendix-I. The weather conditions of rainy season 1998 was characterized by average rainfall, less variation in temperature and high relative humidity which provided excellent conditions for the outbreak of *Spodoptera litura*. This helped in meaningful screening of the material.

3.4 CULTIVATION PRACTICES

The seed bed was prepared to fine tilth before taking up sowing. The recommended package of practice for groundnut cultivation was adopted.

3.5 METHODOLOGIES ADOPTED

Experiment 1 : Screening germplasm for resistance to *S. litura*

1. Artificial infestation of *S. litura*

Egg masses of *S. litura* were collected from the unsprayed neighboring fields. Uniform egg masses were selected and pinned on to abaxial surface of young leaves (egg mass facing the leaf surface) (Plate 1) in each genotype of 3 rows of 2.5 m length in, when the crop was 50-55

days old. The leaf area damaged by *S. litura* was visually scored when the crop was 70 days old.

2. Leaf area damaged due to *S. litura* (DS)

Five leaflets on the main stem from top to bottom were used for the estimation of leaf area damaged. Only top five leaflets were selected as the damage was confined to young leaflets showing differences among entries (Plate 2). The leaf area damaged (in per cent) was visually assessed. In each entry, five plants were separately estimated for leaf area damage and the average was computed.

Experiment 2: Standardization of a new criterion for large scale screening

The experimental material was raised in the field during 1999 rainy season. All the cultural practices as recommended were adopted, except spraying of insecticides. Care was also taken to protect the plants from drifting of insecticide sprays from neighboring fields. The I, II, and III leaves from the top of 40-45 days old plants were used for feeding of III, IV and V instar larvae. Thus, the experiment comprised of nine combinations of treatments *viz.*, I leaf with III instar, IV instar and V instar, II leaf with III instar, IV instar and V instar and III leaf with III instar IV instar and V instar. The treatments were replicated thrice.

Egg masses of *S. litura* were collected from unsprayed groundnut fields. Uniform egg masses were surface sterilized with 4 per cent formaldehyde, washed 3-4 times with distilled water and kept in sterilized petriplates (5 cm diameter) for hatching on a moist filter paper. Freshly hatched larvae were provided with fresh castor leaves and transferred to a disinfected plastic tray of diameter 12 inches and height 5 inches. The open top was covered by muslin cloth with rubber band. Fresh leaves were provided twice daily, once in the late morning and another at early evening after cleaning the tray.



a. An egg mass

b. Pinning of an egg mass



c. Pinned egg mass

d. Hatching of larvae

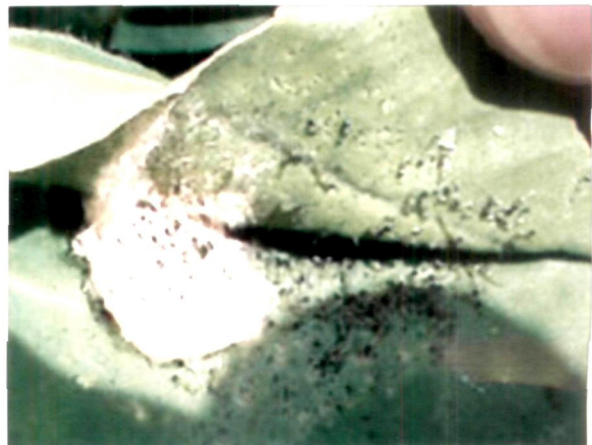




Plate 2a: *Spodoptera litura* damage : Closeup view



Plate 2b: *Spodoptera litura* damage : Field view

Five larvae (24 hours pre-starved) of respective instars were allowed to feed on respective leaves in a petriplate with moist filter paper for 24 hours. The initial weight of the larvae before feeding and after feeding was recorded. The difference in weight was expressed as percent gain in weight (GIW) over initial weight. Leaf area (cm²) before and after feeding for each treatment was recorded using leaf area meter (ELICOR -15). The difference in leaf area was expressed as leaf area consumed (cm²) by the larvae.

Experiment 3: Evaluation of selected groundnut genotypes against *S. litura*

Artificial infestation of *S. litura*

Three egg masses (collected as described in previous experiment) were pinned on to the leaves at every 5 rows of 5 m length plot at 50-55 days old crop. The following parameters were recorded.

i. Leaf area damaged due to *S. litura* (DS):

This parameter was scored when the crop was 70 days old and the damage was expressed in percentage as described in experiment 1.

ii. Gain in weight by larvae (GIW):

The gain in weight by larvae was recorded when the crop was 45-50 days old and was expressed in percentage as described in experiment 2.

Experiment 4: Life history parameters of *Spodoptera litura* (F.) on selected groundnut genotypes

The experimental material was raised in the field during 1999 rainy season as given in experiment 2.

Egg masses of *S. litura* were collected from unsprayed groundnut fields (Plate 3a). Uniform egg masses were surface sterilized with 4 per cent formaldehyde, washed 3-4 times with distilled water and kept in sterilized petriplates (5 cm diameter) for hatching on a moist filter paper. Third leaf



Plate 3a: Egg masses of *Spodoptera litura*



Plate 3b: Rearing *Spodoptera litura* in laboratory

from top was selected for rearing. Neonate larvae were provided with fresh leaves and transferred to a disinfected plastic tray of diameter 12 inches and height 5 inches. The open top was covered by muslin cloth with rubber band (Plate 3b). Fresh leaves were provided twice daily, once in the late morning and another at early evening after cleaning the tray. Before pupation, larvae were transferred to another plastic tray containing sterilized sawdust. Pupae were kept for moth emergence in cages (35 X 25 X 45 cm). Diluted honey (10%) was provided as adult food in small vials with cotton wad (Plate 3c). Groundnut plants placed in conical flask with water were kept inside the cage for egg laying. The following parameters were recorded.

1. Number of larvae

In each replication the larval number was counted and the average was computed. The same procedure was followed at 5, 10 and 15 days after hatching (DAH) of eggs.

2. Mortality of larvae (%)

This was computed by assuming 350 eggs/mass as initial value and expressed in percentage.

3. Weight of larvae (g)

Randomly ten larvae were taken in each replication. They were weighed and average was computed. The same was followed at 5, 10 and 15 days after hatching of eggs.

4. Gain in weight (%)

The weight difference between two successive intervals was divided by the initial weight and expressed in percentage.



Plate 3c: A setup for egg laying by moths in laboratory



Plate 3d: Pupal deformities

5. Mean larval period (days)

This was calculated by the formula

$$\text{Larval duration} = \frac{\sum \text{Individual pupa} \times \text{Number of days taken for pupation}}{\text{Total number of pupae}}$$

6. Number of pupae (%)

The number of pupae formed were counted in each replication and expressed as percentage of larval number at 15 DAH.

7. Pupal weight (g)

The weight of pupae was computed by dividing total weight of pupae by total number of pupae.

8. Malformed pupae (%)

The total number of malformed pupae characterized by larval head capsule retention and incomplete sclerotization (Plate 3d) were counted in each replication and expressed as percentage of total number of pupae.

9. Moth emergence (%)

The number of moths emerged were counted and expressed as percentage of total number of pupae.

10. Number of eggs per mass

Five uniform egg masses were selected. The eggs were counted using magnifying lens and average eggs per mass were computed.

Experiment 5: Enhancement of resistance to *Spodoptera* through hybridization

The crosses were effected and the hybrids were advanced to get F₁ (F₂ seeds) and F₂ generation. The F₂ generation was advanced to F₃ and further through plant to progeny. The work carried out is as under

Year	Season	Nature of work carried out
1999	Rainy season	Eight crosses were generated using five parents
	Post rainy season	F ₁ plants were raised
2000	Rainy season	F ₂ pants were raised
	Post rainy season	-----
2001	Rainy season	481 F ₃ progenies of all the crosses viz., (28-2 x R 9227 : 92 R 9227 x NC Ac 343 :136 28-2 x NC Ac 343 : 36 R 9227 x GPBD 4 : 55 28-2 x GPBD 4 : 32 TAG 24 x NC Ac 343 : 63 GPBD 4 x NCAc 343: 20 & TAG 24 x R 9227 : 47 were screened for damage due to <i>Spodoptera</i> in the field under artificial infestation and were also screened in laboratory for gain in weight by larvae. The progenies were also scored for damage due to thrips and late leaf spot in the field besides productivity
	Post rainy season	1. 39 progenies selected from F ₃ generation based on low DS and GIW were evaluated for thrips and rust resistance besides productivity 2. 25 single plant selections based on good agronomic attributes were evaluated for productivity.
2002	Rainy season	1. The 39 superior resistant progenies were again assessed for gain in weight of <i>Spodoptera</i> larvae in laboratory besides evaluating for productivity. 2. 25 single plant were evaluated for productivity
	Post rainy season	The 39 superior resistant progenies and 25 single plant selections were evaluated for productivity

The experiment was replicated twice.

1. Artificial infestation in field

The segregating material was raised in single rows of 1 m length and replicated twice in a randomized block design. Egg mass (as in Experiment 1) was pinned onto leaves at every three rows when the crop was 50-55 days old.

2. Screening in laboratory

A large number of egg masses of *S. litura* were collected from unsprayed fields. The egg masses were surface sterilized with 4 per cent formaldehyde and washed with sterile distilled water 3-4 times and kept for hatching on a moist filter paper in petriplate. Freshly hatched larvae were fed with castor leaves. Second and third instar larvae were reared in disinfected plastic trays of 12 inches diameter and height of 5 inches. Fresh castor leaves were provided twice a day at late morning and early evening. All the necessary care was taken to get healthy and disease free larvae.

The third instar larvae of one day old were selected and starved for 24 hrs. Five starved larvae were allowed to feed on the leaves of individual genotype in a petriplate of 10 inches diameter. Larvae were allowed to feed for 24 hours. The weight of the larvae before and after feeding was recorded. The observations recorded included

i. Leaf area damaged due to *S. litura* (%)

This parameter was recorded in the field as described in experiment 1.

ii. Gain in weight (%)

In laboratory, weight of larvae before and after feeding was noted and the difference was expressed in percentage of initial weight.

iii. Thrips damage

Thrips damage was scored using 1- 5 scale (Prasad *et al.*, 1998)

iv. Late leaf spot and rust

The disease damage was assessed using 1-9 scale (Subba Rao *et al.*, 1990)

v. Plant yield (PY)

The dry weight of pods of each line was weighed and average weight per plant was computed.

vi. Shelling out turn (SP)

One hundred gram of pods of a genotype were taken and shelling out turn was calculated by the following formula

$$SP = \frac{\text{Kernel weight (g)}}{\text{Pod weight (100 g)}} \times 100$$

vii. Hundred kernel weight (HKW)

One hundred matured seeds were weighed to get hundred kernel weight (g).

Experiment 6 : Biochemical basis of resistance to *S. litura*

1. Estimation of trypsin inhibitor activity

Reagents :

1. Phosphate buffer (0.1 M, pH 7.2)
2. Casein in phosphate buffer (1.0%)
3. Trichloro acetic acid (TCA) (5%)
4. Stock trypsin solution: 10 mg of Bovine trypsin (Sigma, type III, T8042) dissolved in 10 ml of 0.001 M HCl and stored in deep freezer.
5. Working trypsin standard was prepared by diluting one ml of stock to 10 ml with 0.001 M HCl.

Preparation of the sample

Five grams of seed was ground into fine powder and defatted by soxhlet apparatus. One gram of the defatted powdered sample was extracted with pet ether (40-60°C) dissolved in 20 ml of phosphate buffer pH 7.2 by keeping and kept for overnight at 4°C on an orbital shaker (at 200 rpm for extraction). The resulting extract was centrifuged at 5,000 rpm for 20 minutes and the protein content, Trypsin inhibitory activity and lectin activity was determined as described.

Trypsin inhibitor activity

Trypsin inhibitory activity in the seed extracts was determined essentially according to Kakade *et al.*, 1969. An aliquot of 100 µl sample extract was pipetted in a triplicate set of test tubes and the volume was made up to 100 µl with phosphate buffer.

To which Trypsin (100 µl) was added and incubated for 10 minutes at 37°C in circulating water bath. Reaction was started by adding casein (1000 µl) as substrate and the reaction was allowed for 20 minutes by adding 3.0 ml of 5% TCA and the precipitate formed after 30 minutes was centrifuged off at 5000rpm. Residual enzyme activity was estimated by reading at 280nm(double beam Spectrophotometer: Hitachi 150-200) using the inhibitor reference as blank, and compared with enzyme reference.

An aliquot of 1.0 ml of the seed extract was drawn into separate set of test tubes and analyzed for protein content using FCR employing the methods of Lowry *et al.* (1951).

Enzyme control : Enzyme control was maintained throughout by taking 0.9 ml buffer and 1.0 ml casein and 0.1 ml enzyme source. The proteolysis reaction was similarly carried out and terminated.

Enzyme blank : Inhibitor blank consisted of 100 µl enzyme, 900 µl, buffer and 1000 µl Casein. The reaction was carried out as described for inhibitor

assay. However, the seed extract (100 μ l) was added after the addition of 3.0 ml TCA. Absorbance of the supernatant was read at 280nm.

Expression of trypsin inhibitor activity: One trypsin inhibitor unit (TIU) was arbitrarily defined as decrease of 0.01 absorbance units at 280 nm with reference to the enzyme control.

2. Determination of lectin activity

Preparation of the sample

The samples were extracted as described in the estimation of trypsin inhibitor activity.

Preparation of erythrocytes

Lectin activity in the seed extracts was determined by haemagglutination assay using trypsinised and desialylated erythrocytes. Erythrocytes for the haemagglutination assay were prepared by the method described by Lis and Sharon (1976). O positive blood collected, from healthy humans, mixed in an equal volume of Alsever's solution (2.05 g glucose, 0.8 g sodium citrate and 0.42 g NaCl, dissolved in 100.0 ml of distilled water and pH was adjusted to 6.1 by the addition of citric acid) was centrifuged at 1000 rpm for 5 min at room temperature. The plasma was separated and the erythrocytes were repeatedly washed with saline by centrifugation. Washed erythrocytes were suspended in PBS, and the absorbance of the cell suspension was adjusted to 2.5 at 620 nm by diluting suitably with PBS. Resulting cell suspension was incubated with 0.025 per cent (w/v) trypsin at 37°C. After incubating for one hour, erythrocytes were repeatedly washed by centrifugation using saline to remove the traces of trypsin, trypsinized erythrocytes were finally suspended in PBS. Thus, prepared typified erythrocytes were desialylated using Sialidase / neuramidase (10 μ g/ml) suspended in saline and the absorbance was adjusted to 2.0 at 620nm.

Hemagglutination assay

Hemagglutination activity in the plant extract was assayed by the serial two fold dilution technique of Liener and Hill (1953) in microtitre assay plates having U-bottom, with some modifications. Saline (50 μ l) was added to all wells of a row and the lectin sample (50 μ l) was added to the first well of the assay plate containing 50 μ l of saline. The contents of the first well (100 μ l) were mixed well to give two fold dilutions in the second well. The process was repeated serially for the remaining wells. Thus, the lectin extract was serially two fold diluted to which 50 μ l of trypsinized erythrocyte suspension was added and gently mixed on a rotary shaker for five minutes. After incubation for one hour at 37°C, the plates were visually examined for hemagglutination. The highest dilution of the extract, causing visible hemagglutination was regarded as the titre.

The protein content in the highest dilution of the extract causing visible hemagglutination was regarded as haemagglutination(HAU) titre. The protein content in the highest dilution causing visible agglutination is referred to as 'one unit' of hemagglutinin activity. It is otherwise expressed as MCA (minimum concentration of protein required for agglutination). The specific hemagglutination activity is expressed as unit mg^{-1} protein.

Estimation of protein

Protein content in the seed extracts was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard.

Experiment 7: Molecular diversity in selected groundnut genotypes

1. DNA extraction

DNA was extracted based on a previously reported CTAB method (Sahai-Maroo *et al.*, 1984) with some modifications. Leaves were ground to a fine powder in the presence of liquid nitrogen and transferred to a sterile tube containing 9 ml of pre-heated (65°C) 2 X CTAB extraction buffer (100 mM Tris-HCl buffer pH 8, 700 mM NaCl, 20 mM EDTA pH 8, 2%

hexadecyltrimethyl-ammonium bromide, 1% β -mercaptoethanol and 1% sodium bisulphite). 200 mg polyvinylpyrrolidone (PVP) 10 per g of leaf tissue was added and mixed gently. The contents were incubated for 90 min at 65°C in a water bath with occasional shaking during incubation. The tubes were kept for 10 min to allow them to return to room temperature. An equal quantity (9 ml) of chloroform and amyl alcohol solution, prepared in a ratio of 24:1 was added to the tubes and they were rotated on a tube rotator for 10 min and centrifuged at 5000 rpm at 15°C for 20 min. The aqueous phase was transferred to a clean tube and the chloroform and amyl alcohol solution step was repeated. Nucleic acids were precipitated by adding 0.6 ml chilled isopropanol to the aqueous phase after incubating at -20°C for 20 min. The DNA was spooled using glass Pasteur pipettes and transferred to a new sterile tube containing 2 ml of T₁₀E₁ buffer (50 ml T₁₀E₁ + 1 ml RNase 10 mg / ml) and left overnight at room temperature. Contents were later on incubated at 37°C for 30 min. 150 μ l of 5 M NaCl was added to the tube and kept at 4°C. An equal volume (150 μ l) of solution of phenol, chloroform and amyl alcohol, prepared in a ratio of 25:24:1, was added to the tube and mixed gently and the tube was centrifuged at 5000 rpm at 2°C. The clear phase was once again cleaned by another phenol, chloroform, and isoamyl alcohol solution, washed and spun at 2°C. The aqueous phase was transferred to new tubes and DNA was precipitated using 2 to 4 ml of pure chilled ethanol. Tubes were kept at -20°C for 10 min. The DNA precipitate was removed and washed with 2 ml of 0.2 M sodium acetate in 76% alcohol for 20 min followed by 1 ml of 10 mM ammonium acetate in 76% alcohol for 1 min. The DNA pellet was further washed with 70% alcohol for 30 min and re-centrifuged. The tubes were allowed to drain and dried at room temperature for 2 to 3 hr and re suspended in 200 to 500 μ l of TE buffer. The quality and concentration of DNA was assessed by a spectrophotometer and also by gel electrophoresis using 0.8% agarose with known concentrations of uncut lambda DNA.

$$\text{DNA } (\mu\text{g/ml}) = \text{OD}_{260} \times \text{dilution factor} \times 50 \mu\text{g}$$

OD₂₆₀/ OD₂₈₀ ratio was used to assess the purity of DNA. A ratio of 1.6 or less indicates that, there may be proteins and/or other UV absorbers in the sample whereas ratio higher than 2.0 indicates sample may be contaminated with chloroform or phenol.

RAPD-PCR

Requirements

1. Template DNA

Purified genomic DNA extracts (20 ng) from the selected individual genotypes of groundnut were used as template DNA.

2. Random primers

Commercial kits of random decamer DNA primers were obtained from Operon Technologies Inc. Alamedas, USA. A total of 35 Random decamer DNA primers were used for RAPD-PCR (Table 5).

3. Taq DNA polymerase

Taq DNA polymerase and 10X Taq dilution buffer were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore.

4. dNTPs

Individual dNTPs such as dATP, dGTP, dCTP and dTTP were obtained from M/s. Bangalore Genei Pvt. Ltd., Bangalore.

5. Thermocycler

Eppendorf, Master cycler gradient supplied by Eppendorf, 2231, Hamburg Germany was used for cyclic amplification of DNA.

Table 5: List of primers used for RAPD analysis

Sl. No.	Primer Code	Sequence (5'—3')
01	OPF 01	ACGGATCCTG
02	OPF 02	GAGGATCCCT
03	OPF 03	CCTGATCACC
04	OPF 04	GGTGATCAGG
05	OPF 05	CCGAATTCCC
06	OPF 06	GGGAATTCGG
07	OPF 07	CCGATATCCC
08	OPF 08	GGGATATCGG
09	OPF 09	CCAAGCTTCC
10	OPF 10	GGAAGCTTGG
11	OPF 11	TTGGTACCCC
12	OPF 12	ACGGTACCAG
13	OPF 13	GGCTGCAGAA
14	OPF 14	TGCTGCAGGT
15	OPF 15	CCAGTACTCC
16	OPF 16	GGAGTACTGG
17	OPF 17	AACCCGGGAA
18	OPF 18	TTCCCGGGTT
19	OPF 19	CCTCTAGACC
20	OPF 20	GGTCTAGAGG
21	OPJ 05	CTCCATGGGG
22	OPJ 06	TCGTTCCGCA
23	OPJ 07	CCTCTCGACA
24	OPJ 08	CATACCGTGG
25	OPJ 09	TGAGCCTCAC
26	OPJ 10	AAGCCCGAGG
27	OPJ 11	ACTCCTGCGA
28	OPJ 12	GTCCCGTGGT
29	OPJ 13	CCACACTACC
30	OPJ 14	CACCCGGATG
31	OPJ 15	TGTAGCAGGG
32	OPJ 16	CTGCTTAGGG
33	OPJ 18	TGGTCGCAGA
34	OPJ 19	GGACACCACT
35	OPJ 20	AAGCGGCCTC

6. Reaction mix

One primer at a time was used to study the polymorphism between genotypes by RAPD-PCR assay with genomic DNA as template. Master mix required was prepared afresh together to avoid handling errors. The master mix was distributed to 13 tubes (24 μ l per tube) and 1 μ l of template DNA from the respective genotypes was added to make the total reaction volume to 25 μ l.

7. PCR-amplification

The PCR-amplification was done by following the method proposed by Bhagwat *et al.*, 1997 with certain modifications.

Stage	Step	Temperature (°C)	Duration (Min.)	Number of cycles
I	1. Denaturation	94	4	1
II	1. Denaturation	94	1	40
	2. Annealing	37	1	
	3. Extension	72	2	
III	1. Extension	72	10	1
	2. Dump	4	-	-

After the completion of required cycles of amplification the samples were stored at 4°C in a refrigerator and the contents were loaded on to agarose gels for electrophoresis.

Separation of amplified products by agarose gel electrophoresis

15 μ l of the amplified products from each tube along with 2 μ l of loading dye were separated on 1.2 per cent agarose gel using 1X TAE buffer of pH 8.0 and photographed using gel documentation system (Uvitec, Cambridge, England).

Analysis of RAPD profiles

The amplification profiles for all the primers were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0) generating the 0, 1 matrice.

The genetic similarity was estimated by computing DICE coefficient using NTSYS pc-2.0 software programme (Dice, 1945 and Nei and Li, 1979). The clustering was done and dendrograms were drawn through unweighted pair group arithmetic mean (UPGMA). The polymorphism was assessed using the following formula

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.6 STATISTICAL ANALYSIS

The field data recorded during rainy season of 1998, 1999, 2001 and 2002 and post rainy season of 2001 and 2002 regarding pest, diseases and yield parameters was subjected to statistical analysis ANOVA (Randomized complete block design (RCBD) as detailed by Panse and Sukathme (1967). Further, from this analysis, parameters of variation, *viz.*, phenotypic and genotypic coefficients of variation, heritability and genetic advance were derived using appropriate procedures. Correlation was worked out (Singh and Chaudhury, 1979) to determine the nature of association among characters.

G × E interactions of yield and yield attributing characters of *Spodoptera* resistance lines and single plant selections recorded during rainy and post rainy season of 2001 and 2002 were computed following the procedure of Eberhart and Russell (1966). If a genotype is said to be stable when it shows high mean, unit regression and zero mean square deviation.

Experimental Results

IV. EXPERIMENTAL RESULTS

The experiments conducted in the present study included the

- 4.1 Screening germplasm for resistance to *Spodoptera litura*
- 4.2 Standardization of a new criterion for large scale screening
- 4.3 Evaluation of selected groundnut genotypes against *S. litura*
- 4.4 Development and life history parameters of *S. litura* on selected groundnut genotypes
- 4.5 Enhancing the resistance through hybridization
- 4.6 Biochemical basis of resistance
- 4.7 Molecular diversity in selected groundnut genotypes

4.1 SCREENING GERMPLASM FOR RESISTANCE TO *S. litura*

Thirty-nine groundnut germplasm comprising interspecific derivatives (7), advanced breeding lines (19), mutants (4) and released cultivars (9) were screened for resistance to *S. litura* during 1998 rainy season, at Dharwad. The condition for screening was excellent as there was severe outbreak of *Spodoptera* during the season.

Analysis of Variance

Analysis of variance indicated significant variation among genotypes for damage due to *Spodoptera* (Table 6a). The extent of variability was moderate (25.7 and 22.4%) as revealed by PCV and GCV. Heritability was high (76.3%) indicating the heritable nature of the variation. Genetic advance was moderate (40.4%) revealing scope for selection for the *Spodoptera* resistance in the germplasm (Table 6b).

Table 6a: Analysis of variance for *Spodoptera* damage in groundnut germplasm

Source of variation	df	MSS
Replication	1	11.48
Genotypes	38	372.75**
Error	38	50.06

** - indicate significance at 1 per cent level of probability

Table 6b: Components of genetic variation for *Spodoptera* damage in groundnut germplasm

Components	Damage due to <i>Spodoptera</i>
Minimum	34.3
Maximum	80.7
Mean	56.7
PCV	25.7
GCV	22.4
H	76.3
GAM	40.4

Mean Performance

The mutants *viz.*, 28-2, 45 and 110 constituted good source of resistance to *Spodoptera* as they recorded lesser damage (36, 34 and 45 %) compared to all other groups of genotypes (Table 7). All the released cultivars except ICGV 86590(42%) and TAG 24(41%) suffered heavily (63 to 84%)revealing susceptibility to *Spodoptera* but matured early. Maximum defoliation was seen in GPBD 4 (81%), KRG1 (82%), Dh 40(84%) and JL 24(78%). All the inter-specific derivatives were susceptible to *Spodoptera* compared to mutants (50-81%) and matured late except GPBD 4. Among the advanced breeding lines, NC Ac 343 (36%), ICGV 91180 (34%), ICGV 86031(41%) and R 9241(41%) suffered less and were on par with mutants for resistance to *Spodoptera*. ICGV 86031 and R 9241 matured early whereas NC Ac 343 and ICGV 91180 matured late (121days).

4.2 STANDARDIZATION OF A NEW CRITERION FOR LARGE SCALE SCREENING

Leaves (I, II and III from top) from resistant (28-2 and NC Ac 343) and susceptible (JL 24 and TAG 24) genotypes were fed to III, IV and V instar larvae of *Spodoptera* during rainy season, 2000. The parameters leaf area damaged (cm²) and gain in weight (%) by larvae were recorded.

The resistant and susceptible genotypes did not differed significantly for the leaf area damaged and gain in weight by larvae at IV and V instars irrespective of leaves used for feeding. However, significant differences were observed among resistant and susceptible genotypes for both the parameters in III instar on different leaves used for feeding (Table 8a).

The susceptible and resistant genotypes did not differ significantly for leaf area damaged when III instar larvae were fed with the first leaf. While the genotypes differed significantly when II and III leaf was used for feeding, but the magnitude of difference between resistant and susceptible genotypes was less as compared to gain in weight. For gain in weight by

Table 7: Mean performance of groundnut germplasm for *Spodoptera* damage

Genotype	Damage due to <i>Spodoptera</i>	Days to maturity
Interspecific derivatives		
ICGV 86699	61.67 g-n	122
ICGV 87165	52.00 b-j	121
ICGV 88256	65.67 i-o	122
ICGV 93023	62.50 g-n	121
A 30 b	50.17 a-j	121
B 37c	72.00 i-p	115
GPBD 4	80.67 op	105
Advanced breeding lines		
ICGV 86031	41.00 a-d	101
ICGV 87264	50.00 a-j	105
ICGV 87807	49.33 a-i	119
ICGV 90266	60.44 g-m	122
ICGV 91173	55.50 d-i	121
ICGV 91177	58.33 e-m	122
ICGV 91180	34.33 a	121
ICGV 92188	54.33 d-k	121
ICGV 93008	53.33 c-j	121
ICGV 93020	55.33 d-l	121
ICGV 93021	46.17 a-h	121
NC Ac 343	35.67 a-b	120
NC Ac 17090	46.83 a-h	105
ICGV 96262	54.67 d-k	122
ICGV 96266	58.33 e-m	121
Dh 73	59.33 f-m	105
R 8972	74.33 m-p	101
R 9214	41.00 a-d	101
R 9227	60.00 g-m	105
Mutants		
VL 1	59.44 g-m	105
28-2	36.33 a-c	107
45	34.33 a	107
110	45.00 a-g	107
Released cultivars		
ICGV 86590	42.17 a-f	105
K 134	70.67 k-p	101
KRG 1	82.33 op	101
JL 24	77.83 n-p	101
TMV 2	73.33 m-p	101
Dh 8	63.00 h-n	101
Dh 40	83.67 p	101
R 8808	67.00 j-p	101
TAG 24	41.33 a-e	101
Mean	56.65	
CV (%)	12.49	

Figure(s) with same alphabet (s) do not differ significantly at 5% level of probability

Table 8a: Analysis of variance for leaf area damage (cm²) and gain in weight (%) by *Spodoptera* larvae in laboratory

Source of variation	d.f.	III Instar		IV Instar		V Instar	
		LAD (cm ²)	GIW (%)	LAD (cm ²)	GIW (%)	LAD (cm ²)	GIW (%)
Genotype	3	67.4	5421.6 **	2593	380.3	84.81	1431.0
Leaves	2	88.0	2860.4 **	375.2	326.8	170.4	61.7
Genotype x Leaves	6	103.7	181.5	312.7	496.4	85.9	310.7
Error	24	53.9	75.1	1358.4	386.8	620.4	2657.4
C.V. (%)		30.1	09.6	78.89	39.9	18.2	24.20

** Indicates significance at 1 % probability LAD: Leaf area damage GIW: Gain in weight by larvae

Table 8b: Mean performance of the selected groundnut genotypes for leaf area damage (cm²) and gain in weight (%) of the *Spodoptera* larvae in laboratory rearing

Instars	Genotypes	Leaf area damage (cm ²)			Gain in Weight (%)		
		I Leaf	II Leaf	III Leaf	I Leaf	II Leaf	III Leaf
III	28-2	23.0	23.6	16.6	61.6 ^a	65.6 ^{ab}	83.0 ^{bc}
	NC Ac 343	23.3	18.6	28.0	60.5 ^a	59.6 ^a	84.0 ^{bc}
	TAG 24	27.3	28.3	21.6	92.6 ^c	102.0 ^{cd}	134.6 ^e
	JL 24	24.0	37.6	20.3	92.6 ^c	120.0 ^{de}	127.0 ^e
IV	28-2	28.0	34.3	39.6	45.0	39.0	64.6
	NC Ac 343	43.3	34.6	32.3	41.0	50.6	31.3
	TAG 24	54.3	42.6	37.6	69.6	44.0	57.0
	JL 24	81.6	51.3	80.6	64.3	45.3	39.6
V	28-2	138.3	142.0	139.0	211.0	207.0	215.0
	NC Ac 343	136.0	130.0	135.0	97.0	208.0	183.0
	TAG 24	126.0	141.3	139.0	218.0	211.0	229.0
	JL 24	131.0	146.0	142.0	229.0	215.0	231.0

Figure(s) with same alphabet (s) do not differ significantly at 1% level of probability

larvae, significant differences were recorded among resistant and susceptible genotypes when III instar larvae were fed with different leaves.

Among the resistant genotypes, 28-2 (61.6%) was on par with NC Ac 343 (60.5%), while TAG 24 (92.6) and JL 24 (92.6%) showed similar gain in weight and did not differ between each other individually when I leaf was used for feeding III instar larvae (Table 8b). But when II leaf was fed to III instar larvae, NC Ac 343 (59.6%) differed significantly compared to 28-2 (65.6%). Both the susceptible genotypes JL 24 (120%) and TAG 24 (102%) differed significantly between each other. The same trend was observed when III leaf was used for larval feeding but the magnitude of difference among the genotypes was less compared to II leaf.

Therefore, gain in weight (III stadium larvae and II leaf) can be exploited in large scale screening of germplasm against *Spodoptera* as it indicated the gradation of resistance in four genotypes used. It also showed low coefficient of variation revealing less error and better reproducibility of results compared to leaf area damaged, which again indicated the superiority of GIW over leaf area damage.

4.3 EVALUATION OF SELECTED GROUNDNUT CULTIVARS AGAINST *S. litura*

Thirteen selected groundnut genotypes (from experiment 1) were evaluated for damage due to *Spodoptera* (DS) and gain in weight by *Spodoptera* larvae (GIW) during 1999 rainy season.

Analysis of Variance

Analysis of variance indicated highly significant genotypic variation for the characters evaluated (Table 9a). The extent of variability was moderate for both DS and GIW as indicated by phenotypic and genotypic coefficient of variation (22-35%). Genetic advance (21%) and heritability were moderate (46%) for DS. Genetic advance was high for GIW (68%) due

Table 9: Variance and components of variability for *Spodoptera* resistance in selected groundnut Genotypes

a: ANOVA

Source of Variance	df	Variables (MSS)	
		DS	GIW
Replication	1	9.37	101.56
Treatments	15	94.58*	4206.9**
Error	15	35.24	121.59

* & ** indicate the significance at 5% and 1% probability respectively.

b: Genetic components of variance

Components	Variables	
	DS(%)	GIW(%)
Minimum	18	58
Maximum	44	197
GM	36	133
PCV	22.34	34.87
GCV	15.11	33.88
H	46.0	94.0
GAM	21.03	68.00

DS: Damage due to *Spodoptera* GIW : Gain in weight by larvae

to high heritability (94%) and moderate level of variation revealing better scope for selection for *Spodoptera* resistance (Table 9b).

Mean performance

The mean performance of the entries is presented in Table 10. The mutant 28-2 (18%) and NC Ac 343 (25%) suffered least damage and were significantly superior to all the other entries. The entries R 9227, ICGV 86031, ICGV 86699 and ICGV 87165 showed moderate damage (33-35%). However, the popular cultivars except TAG 24 and ICGV 86590 showed high damage (41-44%). *Spodoptera* larvae reared on genotypes NC Ac 343 (59%), 28-2 (71%) and R 9227 (61%) recorded less gain in weight compared to other genotypes. The gain in weight was moderate in genotypes ICGV 87165, ICGV 86031, ICGV 86699, ICGV 86590 and GPBD 4 (88-158%) for GIW and was high in popular cultivars, viz., JL 24, TMV 2, TG 26 and TG 49 (174-198%).

The genotypes NC Ac 343 (120) and ICGV 87165 (121) matured late, while TG 26 (96) and TG 49 (96) matured early, whereas the remaining entries matured in 105 days. The correlation study revealed significant positive association between field damage and gain in weight (Table 10) hence, the criterion of gain in weight could be exploited in the development of *Spodoptera* resistant lines instead of solely depending on field damage.

4.4 DEVELOPMENT AND LIFE HISTORY PARAMETERS OF *S. litura* ON SELECTED GROUNDNUT GENOTYPES

Growth and development of an insect on different genotypes is an important criterion in investigating mechanism of resistance. Hence, *was* reared in laboratory on selected groundnut genotypes comprising resistant (NC Ac 343, mutant 28-2, R 9227 and ICGV 86031) and susceptible (TAG 24, GPBD 4 and JL 24) genotypes during 1999 rainy season. Based on growth and development of *S. litura* on different genotypes viz., larval number, larval weight, gain in weight by larvae at different intervals,

Table 10: Mean performance of the selected groundnut genotypes for *Spodoptera* resistance parameters and days to maturity

Genotypes	DS (%)	GIW (%)	Days to maturity
NC Ac 343	25 ab	59 a	120
MUTANT 28-2	18 a	71 a	107
R 9227	33 bd	61 a	105
ICGV 86031	36 bd	88 ab	101
ICGV 86590	36 bd	134 cd	105
ICGV 86699	39 cd	111 bc	122
ICGV 87165	35 bd	158 de	121
TAG 24	31 bc	174 cf	101
GPBD 4	42 cd	150 de	105
JL-24	44 cd	193 f	101
TMV 2	43 cd	194 f	101
TG 26	42 cd	153 de	96
TG 49	41 cd	198 f	96
GM	36	133	-
CV (%)	16.46	8.26	-
CD (5% and 1%)	12.62	32.47	

DS: Damage due to *Spodoptera* GIW : Gain in weight by larvae

Figure(s) with same alphabet (s) do not differ significantly at 1% and 5% level of probability

Note: Phenotypic correlation coefficient between DS and GIW : 0.58 *

percentage of pupae formed, percentage of malformed pupae, pupal weight, mean larval period and number of eggs laid was computed.

Analysis of variance indicated significant variation for all the parameters (Table 11a). This variation was of heritable nature as revealed by different components of variation *viz.*, PCV, GCV, H and GAM (Table 11b).

As revealed by PCV, variation was high for weight of larvae at all the stages, gain in weight by larvae at both the intervals and percentage of malformed pupae (37.7 to 112.4%). Variation was moderate for number of larvae at all the stages and number of eggs per mass (18.6 to 25.8%) and variation was low for larval mortality at all the stages, weight of pupae and mean larval period (2-12.5%). Heritability was very high (>72%) for all the growth and developmental parameters except mean larval period. The genetic advance for larval weight parameters, all the pupal characteristics and eggs per mass was due to high heritability and high magnitude of variation while, it was moderate to low in rest of the parameters due to low magnitude of variation.

Mean performance

Mean performance of the selected genotypes is presented in Table 12. The entry mutant 28-2 (106, 91, 80 and 70, 74, 77%), NC Ac 343 (107, 100, 61 and 69, 71, 83%) and R 9227 (110, 98, 84 and 68, 72, 76%) recorded significantly less number of larvae and high larval mortality at all the stages compared to susceptible genotypes (JL 24, GPBD 4 and TAG 24). Among resistant genotypes, mutant 28-2 showed significantly less larval number and high larval mortality at 5 DAH and 10 DAH while, NC Ac 343 recorded less larval number and high larval mortality at 15 DAH.

The genotype ICGV 86031 showed significantly less larval weight (0.02, 0.21 and 2.55 g) at all the three stages while, mutant 28-2 recorded less larval weight (0.02 and 2.55 g) at two stages *viz.*, 5 DAH and 15 DAH compared to the rest of the genotypes. The genotypes NC Ac 343 and

Table 11a: Analysis of variance for life history parameters of *Spodoptera litura* (F.)

Source of variation	Larval number			Larval mortality (%)			Larval weight (g)			Gain in weight (g)		Pupae (%)	Mal formed pupae (%)	Pupal weight (%)	MLP (Days)	No. of eggs/mass
	5 DAH	10 DAH	15 DAH	5 DAH	10 DAH	15 DAH	5 DAH	10 DAH	15 DAH	10 DAH	15 DAH					
Replication	21.00	3.18	4.4	2.3	0.19	0.18	0.00001	0.00023	0.032	0.000004	0.018	10.33	0.17	0.0002	0.33	358.2
Genotypes	217.05**	1377.7**	1050.8**	177.58**	112.8**	88.2**	0.005**	0.081**	8.1**	0.091**	6.26**	888.9**	845.5**	0.001**	1.73	3089.2**
Error	14.38	5.5	7.8	1.3	0.63	0.80	0.0802	0.00018	0.032	0.0001	0.007	10.72	0.311	0.0001	1.72	37.06

**, * indicate the significance at 5% and 1% probability, respectively

DAH : Days after hatching

MLP : Mean larval period

Table 11b: Genetic components of variation for life history parameters of *Spodoptera litura* (F.)

Source of variation	Larval number			Larval mortality (%)			Larval weight (g)			Gain in weight (g)		Pupae (%)	Mal formed pupae (%)	Pupal weight (g)	Moth emergence (%)	MLP (Days)	No. of eggs/mass
	5 DAH	10 DAH	15 DAH	5 DAH	10 DAH	15 DAH	5 DAH	10 DAH	15 DAH	10 DAH	15 DAH						
Minimum	106	91	61	53	59	67	0.02	0.21	2.55	0.19	2.22	27	3.17	0.15	32.5	20.58	205
Maximum	162	140	115	70	74	77	0.05	0.72	7.29	0.68	6.5	73	51.54	0.19	75.3	22.73	509
Mean	136	118	92	61	66	73	0.03	0.43	4.13	0.37	3.7	51.8	14.94	0.17	62.24	21.5	393
PCV	19.9	18.6	20.5	12.7	9.3	74	41.2	37.7	39.9	46.2	39.1	33.6	112.4	9.4	-	6.1	25.8
GCV	19.7	18.0	20.3	12.5	9.3	7.3	40.6	37.5	39.7	46.1	39	33.0	112.3	8.0	-	0.2	25.8
H	98.0	98.0	97.0	97.0	98.0	97.0	97.0	99.0	98.0	99.0	99.0	96.0	99.0	72.0	-	0.0	99.0
GAM	40.2	37.1	41.3	25.6	18.9	15.0	100	79.0	81.3	97.3	80.3	66.8	231.3	117.6	-	0.00	53.1

Table 12: Life history parameters of *Spodoptera litura* on selected groundnut genotypes

Genotypes	Larval number			Larval mortality (%)			Larval weight (g)			Gain in weight (g)		Pupae (%)	Mal formed pupae (%)	Pupal weight (g)	Moth emergence (%)	MLP (Days)	No. of eggs/mass
	5 DAH	10 DAH	15 DAH	5 DAH	10 DAH	15 DAH	5 DAH	10 DAH	15 DAH	10 DAH	15 DAH						
R 9227	110 a	98 b	84 b	68 b	72 b	76 c	0.02 a	0.42 d	3.37 b	0.24 b	2.73 c	39 b	11.78 d	0.17 bc	62.3	22.24 a	347 b
28-2	106 a	91 a	80 b	70 a	74 a	77 b	0.02 a	0.39 c	2.55 a	0.31 d	2.22 a	27 a	51.54 a	0.15 a	32.5	21.75 ab	205 a
NC Ac 343	107 a	100 b	61 a	69 ab	71 b	83 a	0.03 b	0.31 b	3.86 c	0.27 c	3.56 d	40 b	12.5 c	0.17 bc	60.7	21.59 ab	368 c
ICGV 86031	160 c	124 c	91 c	54 de	64 c	74 d	0.02 a	0.21 a	2.55 a	0.19 a	2.65 b	53 c	15.4 b	0.16 ab	56.5	22.73 a	396 d
TAG 24	150 b	137 d	101 d	57 c	61 d	71 e	0.05 d	0.5 e	4.49 d	0.49 f	3.95 e	61 d	5.43 e	0.19 d	75.1	21.1 ab	458 e
GPBD 4	162 c	138 d	111 e	53 e	60 de	68 f	0.04 c	0.51 e	4.81 e	0.47 e	4.3 f	70 e	4.62 e	0.18 cd	73.3	20.91 b	509 g
JL-24	157 c	140 d	115 e	55 d	59 e	67 f	0.05 d	0.72 f	7.29 f	0.68 g	6.5 g	73 e	3.17 f	0.19 d	75.3	20.58 b	471 f
GM	136	118	92	61	66	73	0.03	0.43	4.13	0.37	3.7	51.8	14.94	0.17	62.24	21.55	393
CV(%)	2.78	2	3.03	1.9	1.2	1.2	6.89	3.14	4.35	2.71	2.38	6.32	3.73	4.91	-	6.1	1.54
S.Em $\pm$	0.3	0.2	22	0.9	0.7	0.7	0.19	0.11	0.14	0.83	0.72	0.26	0.45	0.7	-	0.1	0.49

DAH : Days after hatching MLP : Mean larval period

Figure(s) with same alphabet (s) do not differ significantly at 1% level of probability

R 9227 recorded less larval weight at all the three stages compared to susceptible genotypes JL 24, TAG 24 and GPBD 4. A similar trend was evident for the parameter gain in weight by the larvae at both the intervals.

Pupal characters namely percentage of pupae formed (27%), moth emergence (32.5%) and pupal weight (0.15 g) were significantly less in mutant 28-2 compared to rest of the genotypes. Whereas, NC Ac 343 (40%, 60.7% and 0.17 g), R 9227 (39%, 62.3% and 0.17 g) and ICGV 86031 (53%, 56.5% and 0.16 g) recorded lower values for all these parameters compared to susceptible genotypes.

The mutant 28-2 showed significantly more malformed pupae (51.54 %) and less eggs per mass (205) compared to rest of the genotypes, whereas NC Ac 343, R 9227 and ICGV 86031 recorded high percentage of malformed pupae (12.5, 11.8 and 15.4 %) and less number of eggs/mass (368, 347 and 396) compared to susceptible genotypes. The entry R 9227 (22.24) and ICGV 86031 (22.73) recorded slightly higher mean larval period when compared to rest of the genotypes.

In general, mutant 28-2, NC Ac 343, and R 9227 recorded significantly lesser number of larvae and higher larval mortality at all the three stages compared to susceptible genotypes. Besides these genotypes, ICGV 86031 also recorded less larval weight and gain in weight at all the intervals compared to susceptible checks. The effect of 28-2, NC Ac 343, and R 9227 was also evident on pupal characteristics, mean larval period and number of eggs per mass.

4.5 ENHANCING RESISTANCE THROUGH HYBRIDIZATION

The resistant sources currently available are commercially unacceptable as they carry one or the other undesirable features. The available resistance in cultivated groundnut is not to an appreciable level. Hence, there is a strong need to enhance the resistance available in cultivars and combine it with good agronomic features through hybridization. Therefore, eight crosses involving resistant (Mutant

28-2, NC Ac 343 and R 9227) and susceptible (GPBD 4 and TAG 24) parents were effected. Four hundred and eighty one progenies in F₃ generation of eight crosses were evaluated for resistance to *Spodoptera*, thrips and late leaf spot, besides productivity traits during the rainy season 2001. Resistance to *Spodoptera* was assessed through field damage and gain in weight by starved larvae in laboratory. The productivity traits were evaluated through pod yield, shelling out turn and hundred seed mass. To know the potentiality of the crosses, genetic components of variation *viz.*, genotypic (GCV) and phenotypic coefficient of variation (PCV), heritability in broad sense (H) and genetic advance over mean (GAM) besides, mean and frequency of superior segregates were studied.

4.5.1 Pest, disease and productivity parameters

4.5.1.1 Damage due to *Spodoptera* (DS)

Spodoptera litura (F.) is one of the major defoliators in groundnut. Therefore, assessing the leaf area eaten is a good measure of resistance in plants. The per cent leaf area damage was visually assessed on top five leaflets on the main stem and average was computed. In parents, leaf area damage ranged from 18 to 44% (Appendix II). The least damage was noticed in NC Ac 343 (18%) followed by mutant 28-2 (21%) and R 9227 (29%). The maximum damage was noticed in GPBD 4 (44%) and TAG 24(34%). Hence, the progenies with less than 30% leaf area damage were considered as superior progenies.

Analysis of variance indicated significant variation in all the crosses except in 28-2 X NC Ac 343, TAG 24 X R 9227 and GPBD 4 X NC Ac 343 for damage due to *Spodoptera* (Table 13a). Phenotypic coefficient of variation was low to moderate (17.1-32.7%). Heritability was moderate to high (34-83%) in all the crosses except 28-2 X NC Ac 343 and TAG 24 X R 9227 (Table 13b). The crosses *viz.*, 28-2 X R 9227, R 9227 X NC Ac 343, TAG 24 X NC Ac 343 and GPBD 4 X NC Ac 343 exhibited higher genetic advance (30.1-56%) revealing scope for selection for the trait. The per cent superior progenies varied from 3 to 72%

Table 13: Components of variance for damage due to *Spodoptera* (%) in various crosses

a: ANOVA

Source of variation	28-2XR9227		28-2XNCAc343		28-2 X GPBD 4		R9227XNCAc 43		R9227XGPBD4		TAG24XNCAc 343		TAG24 X R 9227		GPBD4XNCAc343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	28	1	265	1	159	1	214	1	2	1	88	1	5	1	31
Genotypes	93	186**	37	202	33	193**	137	177**	55	40**	64	147**	48	54	21	154
Error	93	30	37	123	33	64	137	59	55	19	64	13	48	37	21	36

b : Genetic components of variation

Source of variation	28-2XR9227	28-2XNCAc343	28-2XGPBD4	R9227XNCAc343	R9227XGPBD4	TAG24XNCAc343	TAG24XR9227	GPBD4XNCAc343
Minimum	18.5	16.5	21.5	15.0	18.0	19.5	19.0	16.0
Maximum	63.0	55.0	73.5	58.0	38.0	61.5	47.5	46.0
Mean	37.7	38.9	50.2	36.7	31.9	27.4	28.0	33.9
PCV	27.6	32.7	22.6	29.6	17.1	32.6	24	28.7
GCV	23.4	16.1	15.9	20.9	10.1	29.8	10.2	22.6
H	72	24	50	50	35	83.4	18	61
GAM	40.9	16.3	23.3	30.5	12.4	55.9	8.9	36.6
No. of progenies selected	15 (16.3%)	04 (11.0%)	01 (3.0%)	32 (23.0%)	15 (27.0%)	43 (68.0%)	34 (72.0%)	05 (25.0%)
CV (%)	14.59	28.52	15.93	20.90	13.74	13.39	21.80	17.72

Values in parenthesis indicate the percentage of progeny selected

in all the crosses. Based on mean (27.4 and 28.0) and percent superior lines (68 and 72%), TAG 24 X NC Ac 343 and TAG 24 X R 9227 were more potential. Other crosses R 9227 X NC Ac 343, R 9227 X GPBD 4, and GPBD 4 X NC Ac 343 were also good for per cent super progenies (23-27%).

4.5.1.2 Gain in weight by *Spodoptera* larvae (GIW)

Screening for resistance to pest under laboratory condition is relatively simple. Twenty-four hours starved III instar larvae were allowed to feed on leaves of progenies for next 24 hours and the percent gain in weight of larvae over initial weight was computed. Parents differed significantly for gain in weight (Appendix II). Larvae fed on NC Ac 343 showed the least GIW (88%) followed by R 9227(114%) and mutant 28-2 (123%). The maximum GIW was noticed in susceptible parents TAG 24 (300%) and GPBD 4 (207%). Average GIW of the resistant parents was around 100%. Hence, the progenies showing less than 100% GIW was treated as resistant to *Spodoptera*.

Analysis of variance indicated significant genotypic variation in all the crosses (Table 14a). Genetic advance was high (57.2-143.2 %) in all the crosses, which was due to high heritability (58-95%) coupled with high magnitude of variation (PCV: 43.7-72.8%), indicating heritable nature of the variation and better scope for selection for resistance (Table 14b). Based on mean (73.2-125.8) and percent superior progenies (39-72.39%), six out of eight crosses *viz.*, 28-2 X R 9227, R 9227 X NC Ac 343, R 9227 x GPBD 4, TAG 24 X NC Ac 343, TAG 24 X R 9227 and GPBD 4 X NC Ac 343 were found potential for GIW. The remaining crosses 28-2 X NC Ac 343 and 28-2 X GPBD 4 (22 and 16%) also yielded few superior progenies.

4.5.1.3 Damage due to thrips

Thrips injure groundnut foliage at seedling stage but recently because of vector activity to viral diseases, it has attained the status of key pest. In progenies, thrips damage was scored using 1-5 scale, where 1 = highly resistant and 5 = highly susceptible. The progenies scoring 2 and

Table 14: Components of variance for gain in weight (%) by larvae of *Spodoptera* in various crosses

a: ANOVA

Source of variation	28-2XR9227		28-2XNCAC343		28-2 X D 39 d		R9227XNCAC 43		R9227XGPBD4		TAG24XNCAC 343		TAG24 X R 9227		GPBD4XNCAC343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	186	1	168	1	5967	1	27	1	11	1	88	1	183	1	918
Genotypes	93	5670**	37	8723**	33	21044**	137	5216**	55	6670**	64	5730**	48	5565**	21	3111**
Error	93	1486	37	1318	33	3276	137	514	55	744	64	619	48	128	21	576

b : Genetic components of variation

Source of variation	28-2XR9227	28-2XNCAC343	28-2XGPBD4	R9227XNCAC343	R9227XGPBD4	TAG24XNCAC343	TAG24XR9227	GPBD4XNC Ac343
Minimum	28.0	37.0	69.5	17.0	21.5	24.5	16.0	57.5
Maximum	227.5	271	564	238.5	156.0	300.0	168	207.0
Mean	125.8	162.1	179.6	101.5	110.7	124.3	73.2	94.0
PCV	47.5	43.7	61.4	52.7	54.9	45.3	72.8	45.6
GCV	36.3	37.5	52.5	47.8	49.1	40.6	71.1	37.8
H	58	73	73	82	79	80	95	68
GAM	57.2	66.4	92.4	89.1	90.5	75.1	143.2	64.6
No. of progenies selected	38 (41.3%)	08 (22.0%)	05 (16.0%)	78 (57.0%)	27 (49.0%)	24 (39.0%)	37 (72.4%)	11 (55.0%)
CV (%)	30.62	22.40	31.87	22.40	24.64	20.01	15.44	23.51

Values in parenthesis indicate the percentage of progeny selected

below were considered as resistant. Among the parents, mutant 28-2 was resistant to thrips (score of 2), while others (score of 4) were susceptible (Appendix II).

Significant variation for thrips damage existed in all the crosses, except TAG 24 X R 9227 as indicated by analysis of variance (Table 15a). Components of variance revealed scope for selection in crosses 28-2 X R 9227, 28-2 X NC Ac 343, R 9227 X NC Ac 343 and TAG 24 X NC Ac 343, as they showed moderate genetic advance (32-40%) which was due to moderate PCV (28-35%) and high heritability (44-68%). The same set of crosses showed low mean damage (2.82-3.34) and high frequency of superior progenies (5-24%). Other crosses *viz.*, 28-2 X GPBD 4, R 9227 X GPBD 4, TAG 24 X R 9227 and GPBD 4 X NC Ac 343, which recorded high mean damage did not yield any superior progenies (Table 15b).

4.5.1.4 Late leaf spot damage

Late leaf spot is one of the key diseases in groundnut, which causes yield loss up to 70 %. The modified 9 point scale for late leaf spot as given by Subb Rao *et al.* (1990) was used for scoring. The mutant 28-2 and GPBD 4 were resistant, while NC Ac 343 was moderately resistant. R 9227 and TAG 24 were susceptible to late leaf spot (Appendix II). The progenies scoring 5 and below were considered as resistant to LLS.

Analysis of variance indicated significant variation in all the crosses except in 28-2 X NC Ac 343 (Table 16a). Components of variation revealed scope for selection in 28-2 x GPBD 4, R 9227 X GPBD 4, TAG 24 X NC Ac 343 and GPBD 4 X NC Ac 343. Genetic advance in these crosses ranged from 24.30 to 26.87%. This response was mainly due to moderate level of variance (19-22%) and moderate to high heritability (55-62%). Crosses involving either mutant 28-2 and GPBD 4 as female parent showed low mean (4.34-5.25) and very high percentage of superior progenies (51-87%) revealing the superiority of the crosses. The cross TAG

Table 15: Components of variance for damage due to thrips in different crosses

a. ANOVA

Source of Variation	28-2XR9227		28-2XNCAC343		28-2XGPBD4		R9227XNCAC343		R9227XGPBD4		TAG24XNCAC343		TAG24XR9227		GPBD4XNCAC343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	4.78	1	9.59	1	0.94	1	1.44	1	0.08	1	1.11	1	0.16	1	0.09
Genotype	93	1.44**	37	1.38**	33	0.79**	137	1.47**	55	0.62**	64	1.43**	48	0.3	21	0.93**
Error	93	0.51	37	0.53	33	0.54	137	0.43	55	0.26	64	0.26	48	0.26	21	0.32

** indicate the significance at 1 % probability

b. Genetic components of variation

Source of Variation	28-2XR9227	28-2XNCAC343	28-2XGPBD4	R9227XNCAC343	R9227XGPBD4	TAG24XNCAC343	TAG24XR9227	GPBD4XNCAC343
Minimum	1.00	1.00	2.00	2.00	3.00	0.00	3.00	2.50
Maximum	4.00	4.50	5.00	5.00	5.00	4.00	4.50	5.00
Mean	2.96	2.82	4.26	3.34	4.02	3.30	3.59	3.59
PCV	33.42	34.67	19.21	29.16	16.55	27.85	14.90	22.14
GCV	22.99	23.01	8.25	21.51	10.59	23.11	3.85	15.33
H	47.00	44.00	18.00	54.00	41.00	68.00	6.00	47.00
GAM	32.77	31.56	7.27	32.62	13.93	39.69	0.94	22.00
No. of progenies superior to best parent	22 (23.91)	08 (22.22)	00 (00)	12 (8.82)	00 (00)	03 (4.76)	00 (00)	00 (00)

Figures in parenthesis indicate the percentage of superior segregants.

Table 16: Components of variance for damage due to late leaf spot in various crosses

a. ANOVA

Source of Variation	28-2XR9227		28-2XNCAc343		28-2XGPBD4		R9227XNCAc343		R9227XGPBD4		TAG24XNCAc343		TAG24XR9227		GPBD4XNCAc343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	9.38	1	6.36	1	1.13	1	0.43	1	0.22	1	3.08	1	4.5	1	0.2
Genotype	93	5.21**	37	1.92	33	1.53**	137	1.9**	55	2.83**	64	2.96**	48	0.85**	21	1.49**
Error	93	0.98	37	1.55	33	0.4	137	0.57	55	0.65	64	0.86	48	2.5	21	0.34

** Indicate the significance at 1% probability

b. Genetic components of variation

Source of Variation	28-2XR9227	28-2XNCAc343	28-2XGPBD4	R9227XNCAc343	R9227XGPBD4	TAG24XNCAc343	TAG24XR9227	GPBD4XNCAc343
Minimum	2.00	2.00	2.00	3.00	2.00	4.00	7.00	2.00
Maximum	7.50	6.00	6.00	8.00	7.00	8.00	9.00	6.00
Mean	5.25	4.34	4.39	6.58	6.38	6.46	8.19	4.39
PCV	33.50	30.38	22.42	16.94	20.71	21.38	9.05	19.44
GCV	27.66	9.83	17.12	12.40	16.34	15.85	6.69	15.33
H	68.00	10.00	58.00	53.00	62.00	55.00	54.00	62.00
GAM	2.47	6.45	26.87	18.69	26.64	24.30	10.13	24.94
No. of progenies Superior to best Parent	47 (51.08)	29 (80.55)	28 (87.50)	18 (13.23)	06 (10.90)	04 (1.58)	00 (00)	17 (85.00)

Figures in parenthesis indicate the percentage of superior segregants.

24 X R 9227 did not yield any superior progenies, while in other crosses low percentage of superior progenies were seen (Table 16b).

4.5.1.5 Pod yield.

Pod yield is the main indicator of productivity. In progenies, pod yield per plant was calculated by dividing total dry weight of pods of each line by number of plants in that line. In parents plant yield varied from 6.78 to 15.7g (Appendix II). The highest plant yield was recorded in GPBD 4 (15.7 g) followed by mutant 28-2 (14.3g). Therefore, the progenies yielding more than the best parent GPBD 4 were considered superior.

Analysis of variance revealed significant variation in all the crosses except 28-2 X NC Ac 343 (Table 17a). Low to moderate genetic advance (2-46%) was observed in all the crosses due to low to moderate heritability (13-59%) and moderate phenotypic coefficient of variation (24to 43%)(Table 17b). Among the crosses, 28-2 X GPBD 4 was markedly different and was superior to all the crosses, as it recorded higher mean (21.64g), moderate genetic advance (32%) and high percentage of superior progenies (91%). Other crosses did not differ markedly based on these criteria.

4.5.1.6 Shelling out turn

Shelling out turn is important in the recovery of kernels. Weight of kernels from 100g pods expressed in percentage gives shelling out turn. More than 60 percent shelling was found in parents (Appendix II). However, among the parents GPBD 4 was best, as it showed 73 percent shelling. Thus, the progenies showing more than 73% were treated as superior.

Significant variation existed in all the crosses except 28-2 X GPBD 4 as revealed by analysis of variance (Table 18a). In spite of high heritability (50-89%) in most of the crosses, genetic advance was low (2-18%), which was mainly due to low magnitude of variation (PCV 5-12%) indicating limited scope for selection of the trait. Based on mean, 28-2 X GPBD 4 (70.51g), R 9227 X GPBD 4 (73.28g), TAG 24 X R 9227 (71.63g), and GPBD

Table 17: Components of variance for pod yield in various crosses

a. ANOVA

Source of Variation	28-2XR9227		28-2XNCAc343		28-2XGPBD4		R9227XNCAc343		R9227XGPBD4		TAG24XNCAc343		TAG24XR9227		GPBD4XNCAc343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	19.31	1	99.5	1	10.07	1	152.5	1	18.32	1	4.41	1	6.21	1	0.07
Genotype	93	31.21**	37	50.8	33	65.35**	137	85.5**	55	57.11**	64	65.18**	48	30.28	21	74.67
Error	93	20.4	37	39	33	21.05	137	33.13	55	14.58	64	17.01	48	20.27	21	2.5

** indicate the significance at 1 % probability

b. Genetic components of variation

Source of Variation	28-2XR9227	28-2XNCAc343	28-2XGPBD4	R9227XNCAc343	R9227XGPBD4	TAG24XNCAc343	TAG24XR9227	GPBD4XNCAc343
Minimum	8.60	6.73	13.75	6.60	8.00	7.60	6.70	11.20
Maximum	25.10	27.00	21.64	34.00	36.30	34.40	27.30	21.00
Mean	16.38	18.21	21.64	17.90	17.16	16.95	16.20	17.14
PCV	31.10	36.82	30.36	43.06	34.88	38.64	31.01	24.43
GCV	14.19	13.36	21.74	28.54	26.87	29.58	13.17	42.25
H	20.00	13.00	51.00	43.00	59.00	58.00	19.00	33.00
GAM	2.19	9.99	32.07	38.93	42.65	45.66	12.59	30.04
No. of progenies Superior to best Parent	56 (60.86)	23 (68.88)	29 (90.62)	85 (62.50)	33 (60.00)	34 (53.96)	29 (61.70)	14 (70.00)

Figures in parenthesis indicate the percentage of superior segregants.

Table 18: Components of variance for shelling out turn in various crosses

a. ANOVA

Source of Variation	28-2XR9227		28-2XNCAc343		28-2XGPBD4		R9227XNCAc343		R9227XGPBD4		TAG24XNCAc343		TAG24XR9227		GPBD4XNCAc343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	1.25	1	10.15	1	2.46	1	43.75	1	0.93	1	0.81	1	107.71	1	0.23
Genotype	93	33.8**	37	82.06**	33	17.53	137	117.47**	55	31.56**	64	75.36**	48	87.21**	21	69.07**
Error	93	8.8	37	21.45	33	12.07	137	18.48	55	13.98	64	24.84	48	5.03	21	1.4

** indicate the significance at 1 % probability

b. Genetic components of variation

Source of Variation	28-2xR9227	28-2XNCAc343	28-2xGPBD4	R9227XNCAc343	R9227XGPBD4	TAG24XNCAc343	TAG24XR9227	GPBD4XNCAc343
Minimum	53.32	50.40	62.50	34.92	60.05	43.00	59.18	58.75
Maximum	75.50	73.55	77.10	74.85	80.30	78.60	77.44	82.00
Mean	68.76	67.18	70.51	68.85	73.28	67.08	71.63	72.23
PCV	6.72	10.71	5.46	11.97	6.51	10.65	9.48	7.11
GCV	5.14	8.19	2.34	10.22	4.05	7.49	8.95	9.04
H	58.00	58.00	18.00	72.00	38.00	50.00	89.00	62.00
GAM	5.58	12.92	2.08	17.96	5.17	9.12	17.39	11.50
No. of progenies Superior to best Parent	11 (11.95)	05 (13.88)	07 (21.87)	40 (29.41)	36 (65.45)	13 (20.63)	26 (55.31)	13 (65.00)

Figures in parenthesis indicate the percentage of superior segregants

4 X NC Ac 343 (72.23g) were marginally superior for the trait. Range of superior progenies varied from 12 to 65 percent and the same set of crosses viz., R 9227 X GPBD 4 (65%), TAG 24 X R 9227 (55%), and GPBD 4 X NC Ac 343 (65%) were superior in terms of number of progenies recovered (Table 18b).

4.5.1.7 Hundred seed mass (HSM)

In recent times, with increasing emphasis for confectionery types, high priority is given to bolder seeds. Weight of 100 kernels of a genotype is HSM. In parents, HSM ranged from 36 to 54g (Appendix II). Mutant 28-2 (54g) was the best parent. Therefore, progenies recording higher than 54 g were considered as superior.

Analysis of variance indicated significant variation in all the crosses (Table 19a). Though heritability was high (55-88%) in all the crosses (Table 19b), genetic advance was moderate to low (11-40%) because of low magnitude of variation (PCV 14-21%). Based on higher mean and percentage of superior lines recovered, 28-2 X NC Ac 343(58.26 g and 78%), R 9227 X NC Ac 343(56.09 g and 61%), 28-2 X R 9227(52.62 g and 40%) and TAG 24 X NC Ac 343(52.14 g and 39%) were found potential.

Correlation studies among *Spodoptera* resistance parameters, damage due to thrips, late leaf spot and productivity in different crosses did not indicate any association between the characters (Table 20).

4.5.2 Crosswise distribution of progenies for resistance

Progeny distribution in the crosses provides an insight into the nature of the character and its inheritance, which facilitates the designing of breeding programmes to enhance the development of resistant cultivars.

Table 19: Components of variance for hundred seed mass in various crosses

a. ANOVA

Source of Variation	28-2XR9227		28-2XNCAc343		28-2XGPBD4		R9227XNCAc343		R9227XGPBD4		TAG24XNCAc343		TAG24XR9227		GPBD4XNCAc343	
	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS	df	MSS
Replication	1	1.34	1	4.43	1	20.1	1	0.56	1	37.53	1	30.22	1	7.84	1	7.84
Genotype	93	95.02**	37	145.5**	33	160.8**	137	172.13**	55	93.07**	64	178.63**	48	114.26**	21	234.10**
Error	93	16.25	37	34.09	33	43.25	137	10.95	55	26.35	64	33.59	48	30.77	21	30.77

** indicate the significance at 1 % probability

b. Genetic components of variation

Source of Variation	28-2XR9227	28-2XNCAc343	28-2XGPBD4	R9227XNCAc343	R9227XGPBD4	TAG24XNCAc343	TAG24XR9227	GPBD4XNCAc343
Minimum	32.00	36.00	37.00	36.00	37.00	36.00	31.64	26.40
Maximum	76.70	77.00	65.35	78.00	74.40	75.95	70.50	75.70
Mean	52.62	58.26	50.55	56.09	42.75	52.14	45.50	48.00
PCV	14.17	16.27	19.98	17.06	18.08	19.76	18.71	21.45
GCV	11.92	12.81	15.16	16.00	13.51	16.33	14.20	23.56
H	70.00	62.00	57.00	88.00	55.00	68.00	57.00	83.00
GAM	10.88	20.80	23.71	30.93	20.71	27.80	22.19	40.22
No. of progenies Superior to best Parent	34 (36.95)	28 (77.78)	11 (34.37)	76 (55.88)	02 (03.63)	24 (38.90)	02 (04.25)	04 (20.00)

Figures in parenthesis indicate the percentage of superior segregants

Table 20: Correlation coefficients for pest and disease resistance parameters and plant yield in different crosses

Character combinations	28-2 x R 9227	28-2 x NC Ac 343	28-2 x GPBD 4	R 9227 x NC Ac 343	R 9227 x GPBD 4	TAG 24 x NC Ac 343	TAG 24 x R 9227	GPBD 4 x NC Ac 343
GIW & DS	-0.14	-0.04	0.19	-0.04	0.10	0.05	0.06	-0.05
GIW & THRIPS	0.07	0.16	0.12	0.12	0.02	-0.02	0.01	0.31
GIW & LLS	-0.11	-0.15	0.08	-0.16	-0.01	0.09	0.08	-0.39
GIW & PY	0.00	0.02	-0.08	0.04	0.10	0.03	0.04	-0.19
DS & THRIPS	-0.02	0.25	0.22	-0.07	0.00	0.20	0.29*	-0.12
DS & LLS	0.11	-0.05	0.26	0.04	-0.06	0.18	0.05	-0.32
DS & PY	0.26**	0.14	0.03	0.14	0.00	0.28*	0.11	0.56**
THRIPS & LLS	0.22*	-0.15	0.27	-0.11	-0.17	-0.04	-0.01	0.06
THRIPS & PY	-0.03	0.14	0.15	-0.04	0.24	0.01	-0.24	-0.05
LLS & PY	0.00	0.01	0.32	-0.05	-0.04	0.05	-0.10	-0.09

* & ** indicates significance at 1% and 5% probability respectively

DS : Damage due to *Spodoptera* GIW : Gain in weight by larvae

LLS : Late leaf spot

PY : Pod yield

4.5.2.1 Damage due to *Spodoptera*

Though the parents are resistant, majority of the progenies in 28-2 X R 9227, 28-2 X NC Ac 343 28-2 X GPBD 4 and R 9227 X NC Ac 343 were distributed away from the midparent towards susceptibility (Fig. 1a and b). In the crosses, R 9227 X GPBD 4, TAG 24 X NC Ac 343 and GPBD 4 X NC Ac 343 majority of the lines were distributed between the parental range. However, the progenies in all the crosses were normally distributed indicating the continuous nature of the trait.

4.5.2.2 Gain in weight by larvae

The progenies in all the crosses showed continuous variation with unimodal distribution (Fig. 2a and b). In crosses *viz.*, 28-2 X R 9227, 28-2 X NC Ac 343, 28-2 X GPBD 4 and R 9227 X NC Ac 343, the progenies were distributed normally on either side of the parents and majority of them were away from the mid parental value towards susceptibility. Even though one of the parents was susceptible, a majority of the progenies from R 9227 X GPBD 4, GPBD 4 X NC Ac 343, TAG 24 X R 9227, TAG 24 X NC Ac 343 and GPBD 4 X NC Ac 343 were better than best parent indicating transgression for resistance.

The progenies in all crosses were normally distributed for both the parameters of resistance (DS and GIW) indicating that the trait was quantitative in nature and not amenable for classical Mendelian analysis. Appearance of high frequency of susceptible segregates in resistant X resistant crosses (28-2 X R 9227, 28-2 X NC Ac 343, and R 9227 X NC Ac 343) and appearance of high frequency of resistant segregates in susceptible X resistant or resistant X susceptible crosses (R 9227 X GPBD 4, GPBD 4 X NC Ac 343, TAG 24 X R 9227 TAG 24 X NC Ac 343 and GPBD 4 X NC Ac 343) indicates the possibility of different type of resistant factors involved and gene interactions in governing the resistance.

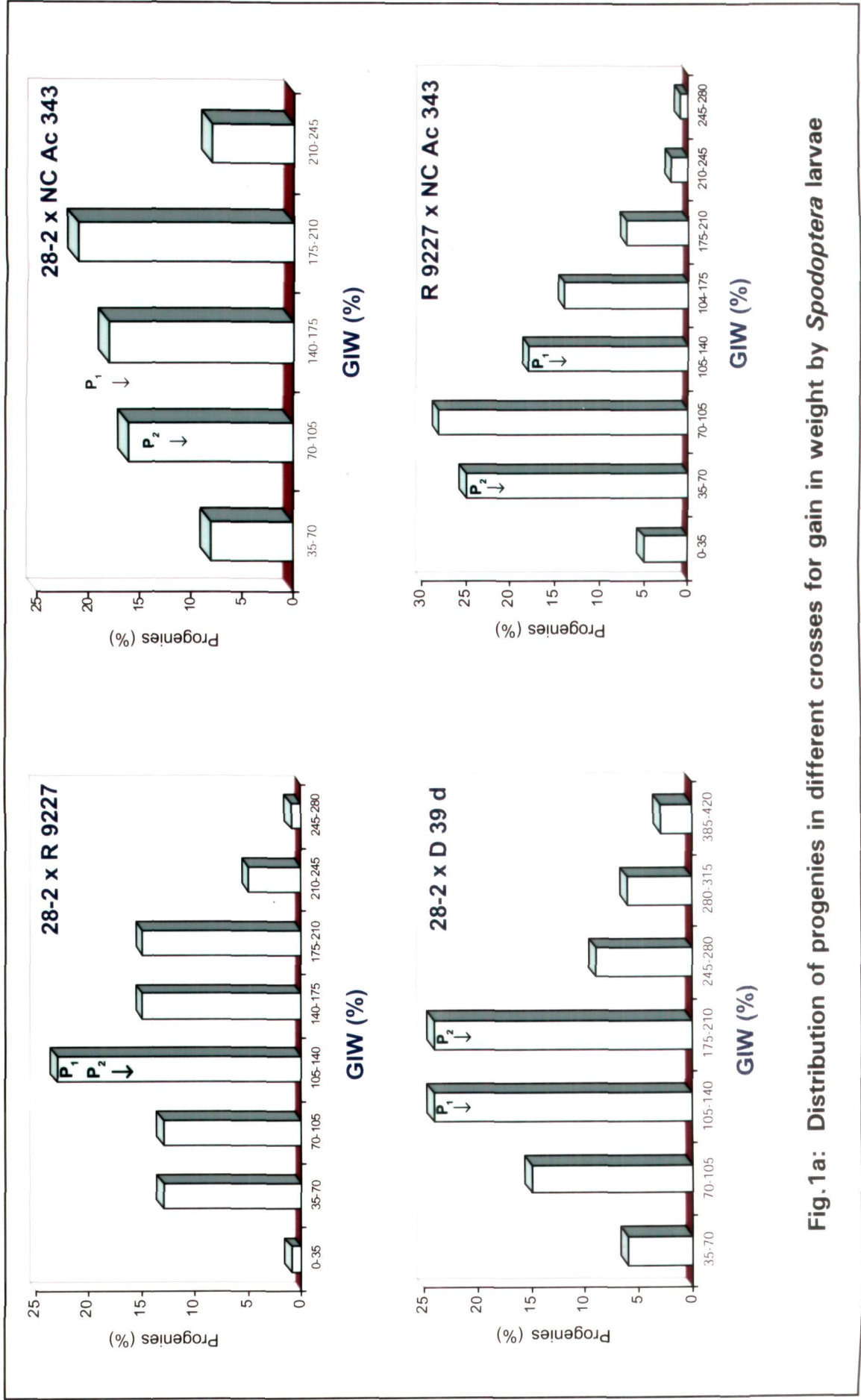


Fig.1a: Distribution of progenies in different crosses for gain in weight by *Spodoptera* larvae

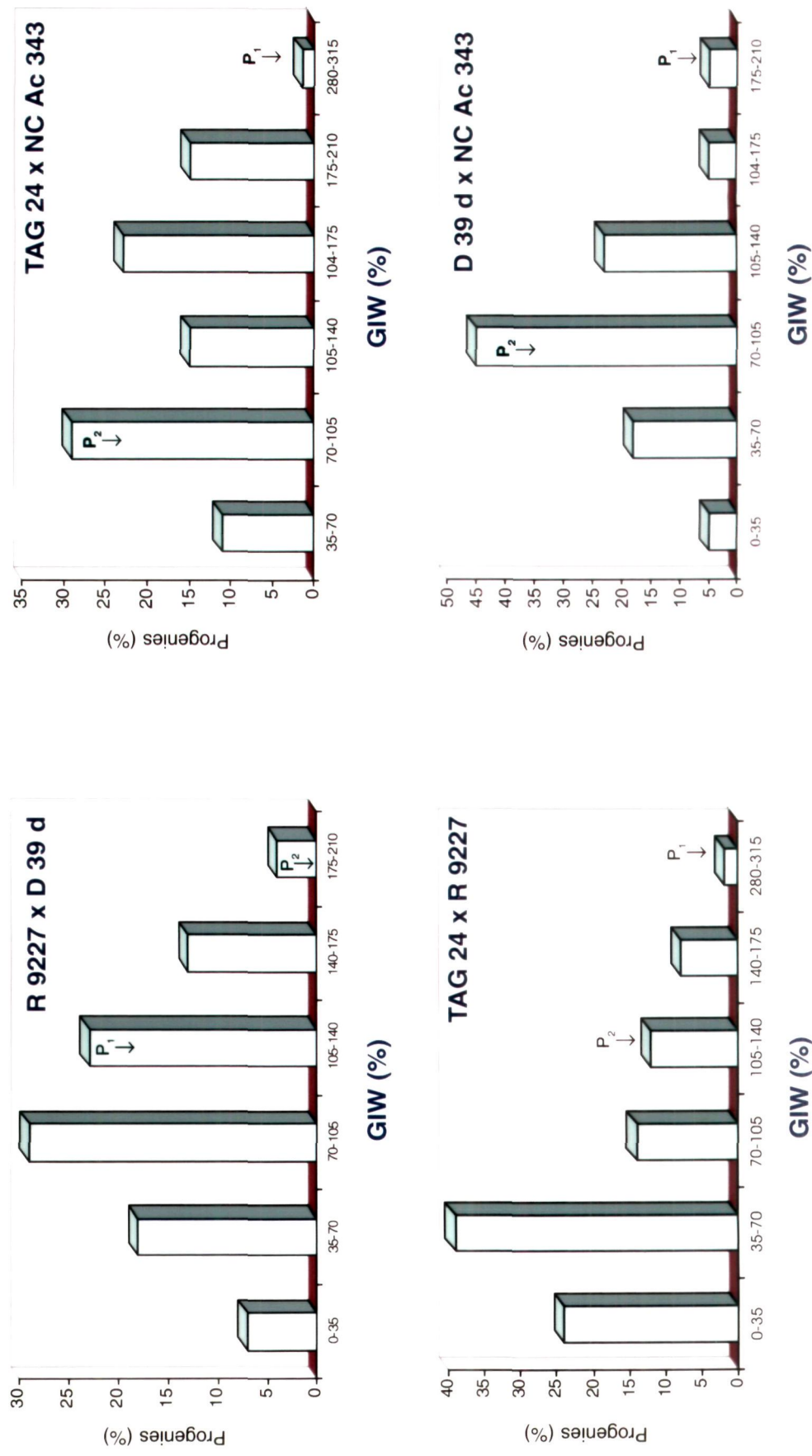


Fig.1b: Distribution of progenies in different crosses for gain in weight by *Spodoptera* larvae

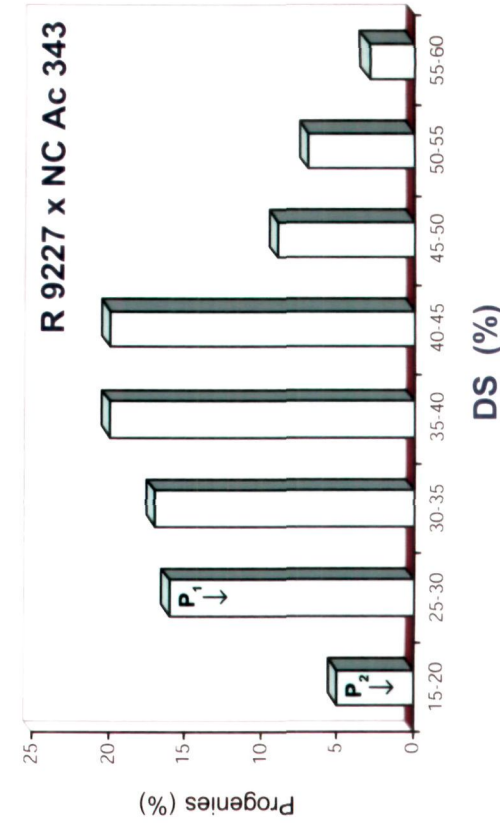
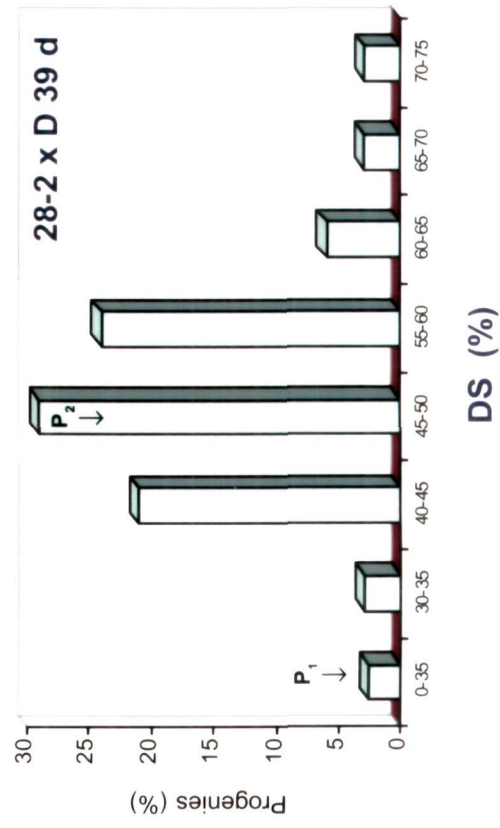
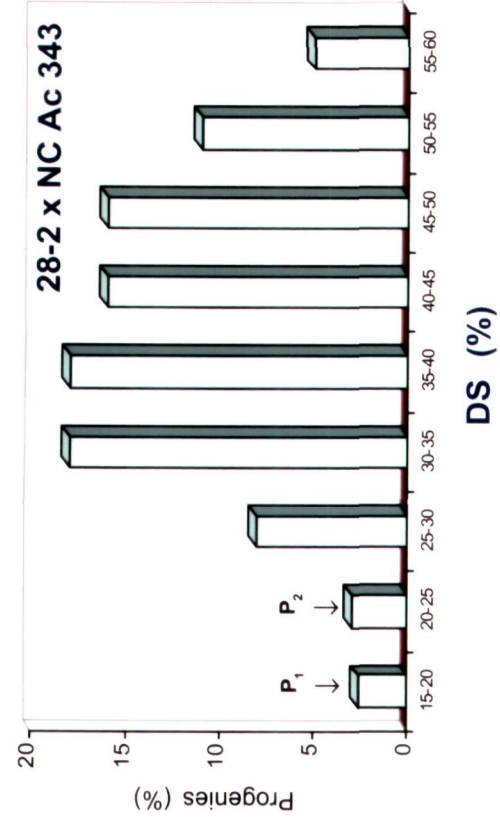
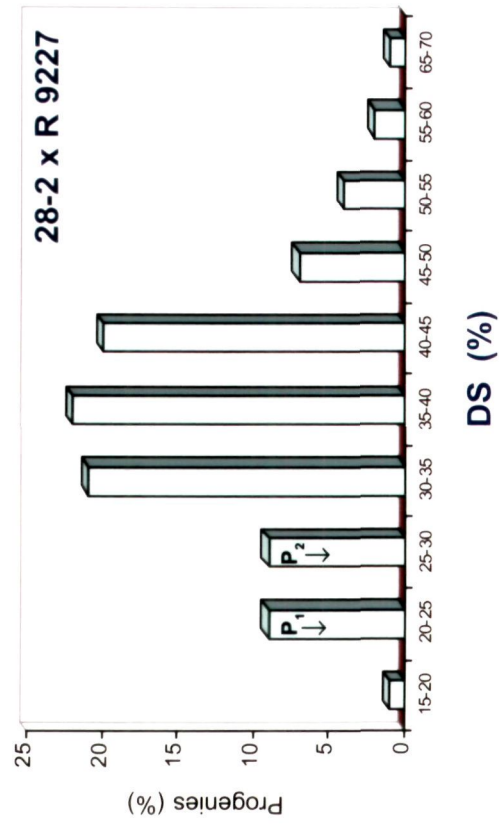


Fig.2a: Distribution of progenies in different crosses for damage due to *Spodoptera* larvae

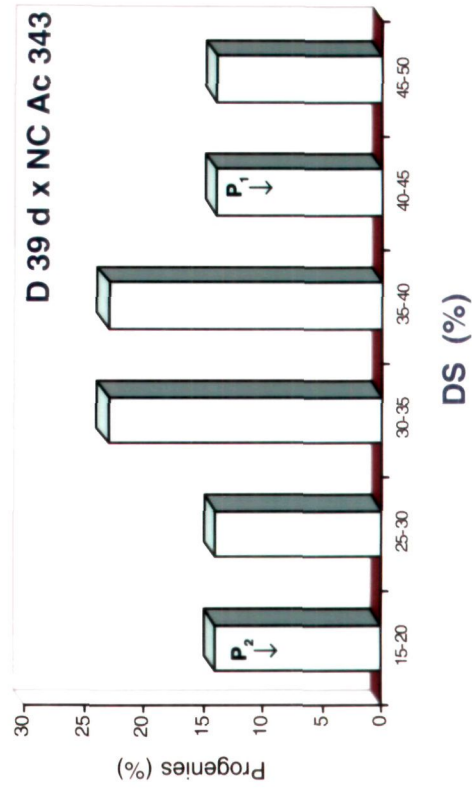
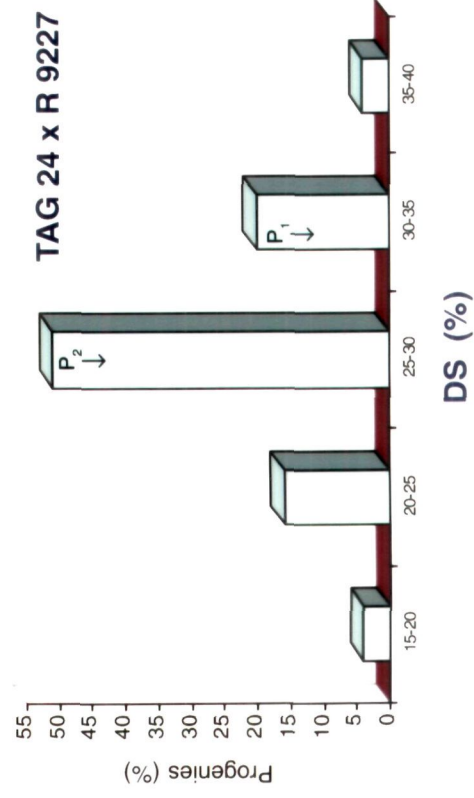
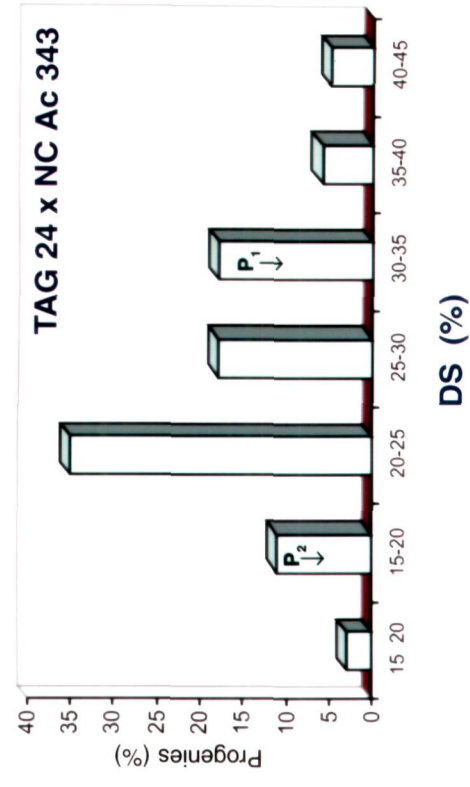
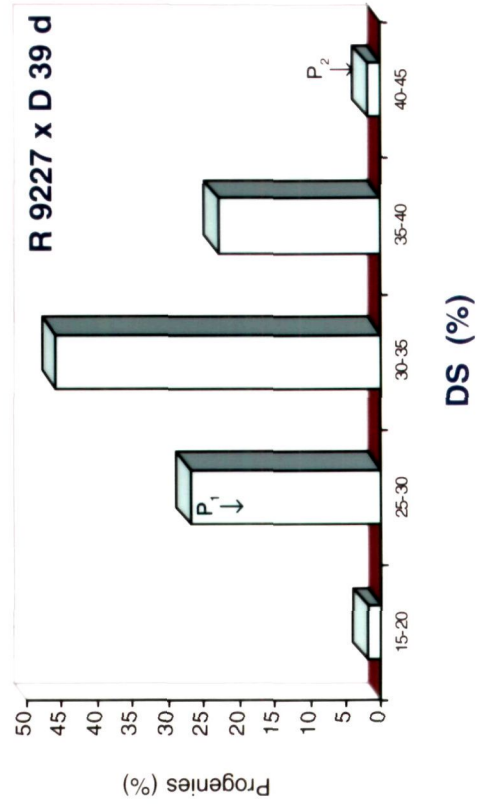


Fig.2b: Distribution of progenies in different crosses for damage due to *Spodoptera* larvae

4.5.3 Desirable segregates for multiple traits

Assessing the population of different crosses using means, variability and ability to release desirable segregates for individual characters will not give the full worth of each cross. It is worthwhile, to assess the ability of crosses for releasing superior segregates for combinations of different characters. The frequency of such lines is presented in Table 21.

Out of 481 lines, 149 lines were superior for field damage and majority of them were found in three crosses namely, TAG 24 X NC Ac 343(72%), TAG 24 X R 9227(72%) and R 9227 X NC Ac 343(23%). Two hundred and twenty eight lines were superior based on gain in weight and were mostly from TAG 24 X R 9227(79%), R9227 X NC Ac 343(57%), R 9227 X GPBD 4(49%) and GPBD 4 X NC Ac 343(55%). The correlation between field damage and gain in weight by larvae did not show any association hence, combination of both (DS and GIW) was used to judge superiority of segregates for resistance to *Spodoptera*. Based on this criterion, TAG 24 X R 9227(60%) yielded high percentage of desirable segregates, followed by TAG 24 X NC Ac 343(25%), R9227X GPBD 4 (19%), R9227 X NC Ac 343(12%) and GPBD 4 X NC Ac 343(10%). Based on both DS and GIW, 76 lines were superior for *Spodoptera* resistance, out of which, six lines viz., RN 3-17(18 and 48%), RN 3-26(16 and 44%), RN 4-4(18 and 65%), RG 1-8(18 and 50%), TN 1-20 (17 and 79%), and TN 1-23(18 and 85%), were superior to the best parent NC Ac 343(18 and 88%) for both DS and GIW (Table 22). When DS and GIW along with plant yield was considered, 43 lines were superior and many of the lines were produced by two crosses namely TAG 24 X R 9227(29.8%) and TAG 24 X NC Ac 343(19%). Progenies, RN 2-19, RN 1-2, RG 1-19, RG 1-25, TN 6-1, TN 7-8, and TR 2-1 were exceptionally superior for both resistance and pod yield (Table 22). Only 16 lines were found superior when shelling out turn was combined with *Spodoptera* resistance (DS and GIW) and plant yield. Majority of these lines were from crosses TAG 24 X R 9227(19.1%) and R9227X GPBD 4 (10.9%). Among these 16 lines, RN 1-2, RG 1-19, TN 7-6,

Table 21: Frequency of superior segregants for multiple traits in various crosses

Character combination	No. of Superior Progenies	28-2 x R 9227	28-2 X NC Ac 343	28-2 x GPBD 4	R 9227 X NC Ac 343	R 9227 X GPBD 4	TAG 24 X NC Ac 343	TAG 24 X R 9227	GPBD 4 X NC Ac 343
DS	149 (30.9)	15 (16.3)	04 (11.1)	01 (3.1)	32 (23.5)	15 (27.3)	43 (68.2)	34 (72.3)	05 (25.0)
GIW	228 (47.4)	38 (41.3)	08 (22.2)	05 (15.6)	78 (57.3)	27 (49.1)	24 (38.1)	37 (78.7)	11 (55.0)
DS+GIW	76 (15.8)	04 (4.3)	01 (2.8)	00 (00)	16 (11.7)	10 (18.2)	16 (25.4)	28 (59.6)	02 (10.0)
DS+GIW+PY	43 (8.7)	00 (00)	00 (00)	00 (00)	09 (6.6)	06 (10.9)	12 (19.0)	14 (29.8)	02 (10.0)
DS+GIW+PY+SP	16 (3.3)	00 (00)	00 (00)	00 (00)	01 (0.1)	06 (10.9)	00 (00)	09 (19.1)	01 (5.0)
DS+GIW+TH	06 (1.2)	01 (1.0)	00 (00)	00 (00)	04 (2.9)	00 (00)	01 (1.6)	00 (00)	00 (00)
DS+GIW+TH+PY	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
DS+GIW+LLS	11 (2.3)	02 (2.2)	01 (2.8)	00 (00)	02 (1.5)	03 (5.5)	02 (3.2)	00 (00)	01 (5.0)
DS+GIW+LLS+PY	06 (1.2)	00 (00)	00 (00)	00 (00)	02 (0.1)	01 (1.8)	02 (3.1)	00 (00)	01 (5.0)
DS+GIW+TH+LLS	01 (0.2)	01 (1.0)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
DS+GIW+TH+LLS+PY	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)

Values in parenthesis indicate percentages. DS : Damage due to *Spodoptera* GIW : Gain in weight by larvae LLS : Late leaf spot PY : Pod yield

Table 22: Performance of *Spodoptera* resistant lines for thrips, late leaf spot and productivity parameters

Lines	DS	GIW	Thrips	LLS	PY	SP	HSM
28-2 X R 9227(MR)							
MR 2-6	23	87	3	4 *	08.8	69	49
MR 2-7	23	96	2 *	5 *	09.5	69	45
MR 2-21	29	72	3	7	11.1	60	46
MR 2-53	22	38	3	8	07.5	69	32
28-2 X NC Ac 343 (MN)							
MN 1-22	26	90	3	4 *	15.3	65	55 *
R 9227XNC Ac 343 (RN)							
RN 2-2	27	97	3	7	14.4	37	65 *
RN 2-4	24	78	3	7	17.6 *	71	47
RN 2-19	28	61	3	7	25.1 *	71	55 *
RN 1-2	27	52	4	5 *	25.0 *	76 *	60 *
RN 2-25	25	57	4	6	19.6 *	48	47
RN 3-15	29	90	2 *	7	10.8	72	43
RN 3-17	18	48	4	7	17.4 *	72	45
RN 3-18	23	73	2 *	8	13.7	69	50
RN 3-24	26	44	2 *	7	15.3	75 *	38
RN 3-26	16	44	1 *	7	18.1 *	71	60 *
RN 3-27	27	21	3	5 *	21.4 *	67	68 *
RN 3-29	28	25	4	7	12.9	56	59 *
RN 4-3	27	88	4	6	19.0 *	75 *	42
RN 4-4	18	65	5	7	10.6	64	54
RN 4-25	25	96	3	6	15.8 *	71	49
RN 4-44	27	91	4	6	06.6	74 *	50
R 9227 X GPBD 4 (RG)							
RG 1-11	29	49	5	7	11.3	74 *	44
RG 1-8	18	50	4	4 *	12.0	66	56 *
RG 1-19	28	81	5	5 *	25.6 *	74 *	39
RG 1-22	25	84	3	4 *	11.3	63	34
RG 1-25	26	59	5	6	24.0 *	71	47
RG 2-12	28	90	4	7	13.4	76 *	42
RG 2-21	30	60	4	7	18.7 *	74 *	44
RG 2-22	26	55	5	7	16.3 *	73 *	30
RG 2-23	27	98	4	6	16.6 *	72	46
RG 2-27	27	84	4	6	16.0 *	79 *	36
TAG 24 X NC Ac 343 (TN)							
TN 1-16	28	89	3	8	11.0	65	47
TN 1-20	17	79	5	7	17.8 *	69	69 *
TN 1-23	18	85	5	6	10.5	73 *	58 *
TN 2-2	24	67	4	6	11.4	73 *	63 *
TN 2-4	23	81	2 *	6	12.7	64	49
TN 3-1	24	77	3	5 *	16.5 *	71	42
TN 5-2	21	96	4	7	07.6	61	52
TN 5-6	20	75	3	6	12.4	69	65 *
TN 6-1	19	95	4	4 *	25.0 *	43	25
TN 6-2	22	66	5	6	12.4	63	49
TN 6-4	23	30	5	6	11.5	58	47

Table 22. Contd...

Lines	DS	GIW	Thrips	LLS	PY	SP	TW
TN 7-5	29 *	68 *	4	7	17.3 *	69	53
TN 7-6	28 *	88 *	3	7	22.8 *	74 *	53
TN 7-7	29 *	32 *	4	6	08.5	77 *	54
TN 7-8	29 *	95 *	3	7	24.8 *	66	59 *
TN 7-12	20 *	25 *	4	7	16.0 *	70	57 *
TAG 24 X R 9227 (TR)							
TR 1-1	19 *	95 *	3	9	15.0	67	31
TR 1-4	24 *	63 *	3	9	09.4	69	45
TR 1-6	29 *	40 *	4	9	19.5 *	74 *	43
TR 1-7	29 *	33 *	3	8	20.0 *	73 *	45
TR 1-8	23 *	69 *	4	8	14.6	74 *	54
TR 1-10	26 *	79 *	3	9	14.3	77 *	38
TR 1-13	27 *	98 *	4	8	14.2	76 *	44
TR 1-15	26 *	54 *	4	8	16.4 *	72	43
TR 1-16	25 *	37 *	4	9	11.8	75 *	42
TR 1-20	27 *	62 *	4	8	17.6 *	76 *	47
TR 1-22	24 *	16 *	4	8	15.6	70	54
TR 1-23	27 *	66 *	4	9	18.0 *	75 *	43
TR 1-24	28 *	42 *	4	9	09.1	72	52
TR 1-25	23 *	68 *	3	9	16.0 *	69	40
TR 1-26	26 *	80 *	3	9	15.3	73 *	40
TR 1-32	25 *	90 *	4	9	21.6 *	70	41
TR 1-34	26 *	32 *	4	9	15.4	72	40
TR 1-37	24 *	41 *	4	9	16.1 *	75 *	46
TR 1-38	28 *	51 *	4	9	15.8 *	73 *	51
TR 2-1	28 *	39 *	4	7	27.3 *	73 *	50
TR 2-4	27 *	26 *	4	8	18.5 *	72	48
TR 2-5	27 *	37 *	4	8	17.3 *	74 *	45
TR 2-9	25 *	68 *	4	7	17.1 *	32	18
TR 1-2	25 *	40 *	4	8	06.7	72	45
TR 1-3	19 *	34 *	3	8	14.2	74 *	58 *
TR 1-11	21 *	29 *	4	7	13.0	59	44
TR 1-19	20 *	18 *	3	7	18.0 *	71	53
TR 1-36	28 *	22 *	4	9	09.8	72	35
GPBD 4 X NC Ac 343 (GN)							
GN 1-2	29 *	57 *	4	5 *	16.4 *	62	51
GN 2-5	27 *	70 *	4	6	16.0 *	74 *	47
Parents							
28-2	22	124	2	3	14.4	68	54
R 9227	29	117	4	7	11.8	64	43
NC Ac 343	18	88	4	5	06.7	59	36
GPBD 4	44	207	4	2	15.7	72	37
TAG 24	34	300	4	8	12.9	66	49

*Superior progeny

DS : Damage due to *Spodoptera*

GIW : Gain in weight by larvae

LLS : Late leaf spot

PY : Pod yield SP: Shelling percentage HSM : Hundred seed mass

and TR 2-1 combined good shelling percentage with high yield besides being resistant to *Spodoptera*.

The crosses *viz.*, R 9227 X NC Ac 343(4), TAG24 X NC Ac 343(1) and 28-2 X R 9227(1) produced six lines (MR 2-7, RN 3-15, RN 3-8, RN 3-24, RN 3-26 and TN 2-4) superior to both *Spodoptera* and thrips resistance parameters. But, none of the crosses yielded progenies combining high yield with *Spodoptera* and thrips resistance along with plant yield. Eleven progenies from R 9227 X GPBD 4 (3), TAG 24 X NC Ac 343(2), 28-2 X R 9227(2) and R 9227 X NC Ac 343(2) combined *Spodoptera* resistance with late leaf spot resistance. Out of which six lines *viz.*, RN 1-2, RN 3-27, RG 1-19, TN 3-1, TN 6-1 and GN 1-2 combined high yield with *Spodoptera* and late leaf spot resistance. while only one line MR 2-7, of the cross 28-2 X R 9227 combined resistance to *Spodoptera*, thrips as well as late leaf spot, none of the progenies combined high yield, with resistance to all the three biotic stresses *viz.*, *Spodoptera*, thrips and late leaf spot.

4.5.4 Evaluation of superior segregates

Genetic components of variation

In order to reconfirm the resistance, superior segregates based on both DS and GIW (evaluated during 2001 rainy season) were again evaluated for gain in weight by larvae (GIW II) in laboratory during 2002 post rainy season. These lines were also evaluated for thrips and rust during 2002 rainy season, and for productivity during 2001 post rainy, 2002 rainy and post rainy seasons.

Genetic components of variance indicated genetic variation for all the characters (Table 23). The extent of variability was high for GIW II as indicated by range, phenotypic and genotypic co-efficient of variation (55%), whereas it was low for the remaining characters (16-31%). Heritability and genetic advance was very high for GIW II (80 and 111%) indicating the heritable nature of the variation and better scope for selection. Genetic

Table 23: Genetic components of variation for insect resistance, disease resistance and productivity parameters in selected progenies of different crosses

Sources of Variations	GIW II	Thrips	LLS	Rust	Mean of four seasons		
					PY	SP	HSM
Minimum	20	03	03	03	07.5	49	35
Maximum	262	05	09	07	16.7	73	63
Mean	73.3	3.6	6.6	5.8	13.5	64.8	46.4
PCV	55.07	20.56	23.58	15.92	36.12	11.68	24.07
GCV	54.48	11.85	21.25	12.43	13.96	6.84	16.34
H	97.9	33.2	81.2	60.9	14.9	34.3	46.1
GAM	111.1	14.16	39.69	20.17	12.44	8.25	24.33

DS : Damage due to Spodoptera GIW II : Gain in weight by larvae(II) LLS : Late leaf spot
 PY : Pod yield SP: Shelling percentage HSM : Hundred seed mass

advance was low for remaining characters due to low heritability and magnitude of variation.

Analysis of variance for stability revealed genetic variability among genotypes and diversity of environments for the productivity traits (Table 24). Interaction between genotypes and environment was significant implying differential behavior of genotypes under various environments. Significant environment (linear) for all the traits suggests linear change in environmental index as per change in environmental conditions. Significant G X E (linear) as well as deviation from mean sum of squares for shelling percentage and hundred seed mass indicated that G X E interactions were shared by predictable and unpredictable components. But, non significant G X E (linear) component for pod yield indicated unpredictable nature that is expression was not in accordance with changes in environmental conditions.

Mean performance

Mean performance of the progenies is presented in Table 25. Compared to the best resistant parent NC Ac 343(18, 88 and 60%) progenies, RN 3-17(18, 48 and 22), RN 3-26(16, 44 and 53%), RN 4-4(18, 65 and 32%) and RG 1-8(18, 56 and 63), were significantly superior to *Spodoptera* resistance, based on DS, GIW I and GIW II. Segregates, MR 2-53, RG 1-8 and TN 1-20, TN1-23 and TN 7-12 were on par with best resistant parent NC Ac 343 for resistance to *Spodoptera*. For thrips, RN 3-26 was superior to best parent mutant 28-2 as it recorded less damage in both the seasons, while MR 2-7, RN 3-24 and TN 2-4 were on par with resistant parent mutant 28-2. None of the lines were superior to best parent GPBD 4 for late leaf spot. However, MR 2-6, MR 2-7, MN 1-22, RG1-8 and TN 6-2 were moderately resistant to late leaf spot. Among them MR 2-7 combined resistance for both the insects *Spodoptera* and thrips and was on par with respective parents.

Table 24: Analysis of variance for stability of productivity parameters in *Spodoptera* resistant groundnut genotypes

Source of variation	Mean Sum of Squares			
	df	Pod yield per plant	Shelling per cent	Hundred seed mass
Genotype (G)	51	20.11**	113.99**	182.35**
Environment (E)	03	308.37**	440.36**	971.62**
G × E	153	14.25**	36.85**	51.33**
E (linear)	01	925.15**	1320.52**	2914.79**
G × E (linear)	51	6.40	54.21**	66.52**
Pooled deviation	104	17.82**	27.63**	42.89**
Pooled error	204	9.36	18.60	27.03

** indicate significance at 1 % probability

Table 25: Performance of *Spodoptera* resistant progenies for various biotic stresses

Lines	DS (%)	GIW I	GIW II	Thrips I	Thrips II	LLS	Rust
28-2 X R 9227(MR)							
MR 2-6	23	87	93	3	4	4	6
MR 2-7	23	96	64	2	3	5	7
MR 2-21	29	73	79	3	4	7	6
MR 2-53	22	38	45	3	4	8	6
28-2 X NC Ac 343 (MN)							
MN 1-22	26	90	71	3	3	4	6
R 9227 X NC Ac 343 (RN)							
RN 2-4	24	78	79	3	3	7	6
RN 2-19	29	61	69	3	3	7	6
RN 2-25	25	57	45	4	4	6	6
RN 3-17	18	48	22	4	5	7	7
RN 3-24	44	26	20	2	4	7	6
RN 3-26	16	44	53	1	3	7	7
RN 3-29	28	25	38	4	4	7	6
RN 4-4	18	65	32	5	5	7	7
R 9227 X GPBD 4 (RG)							
RG 1-11	30	49	68	5	4	7	6
RG 1-8	18	56	63	4	4	4	4
RG 2-21	30	60	69	4	3	7	4
RG 2-22	26	56	52	5	5	7	3
TAG 24 X NC Ac 343 (TN)							
TN 1-16	28	89	73	3	3	8	7
TN 1-20	17	79	63	5	4	7	6
TN 1-23	18	85	79	5	4	6	7
TN 2-2	24	67	56	4	4	6	6
TN 2-4	23	81	74	2	3	6	6
TN 3-1	24	77	62	3	4	5	7
TN 6-2	22	66	42	5	4	6	6
TN 6-4	23	30	89	5	3	6	6
TN 7-5	29	68	94	4	4	7	7
TN 7-6	28	88	77	3	5	7	7
TN 7-7	29	32	35	4	4	6	7
TN 7-12	20	25	35	4	5	7	6

Table 25: Contd..

Lines	DS (%)	GIW I	GIW II	Thrips I	Thrips II	LLS	Rust
TAG 24 X R 9227 (TR)							
TR 1-1	19	95	97	3	4	9	7
TR 1-4	24	63	73	3	4	9	7
TR 1-6	29	40	51	4	4	9	6
TR 1-16	25	37	47	4	4	9	6
TR 1-22	24	16	40	4	4	8	6
TR 1-37	24	41	70	4	4	9	6
TR 2-4	27	26	38	4	4	8	6
TR 2-5	27	37	67	4	4	8	5
TR 1-36	28	22	89	4	4	9	6
GPBD 4 X NC Ac 343 (GN)							
GN 2-5	27	70	83	4	4	6	5
Parents							
Mutant 28-2	21	122	101	2	3	3	6
R 9227	29	116	106	4	4	7	6
NC Ac 343	18	88	60	3	4	6	6
GPBD 4	44	207	188	4	4	2	4
TAG 24	34	300	262	4	5	8	7
GM	24.6	68.6	73.4	3.3	3.6	6.6	5.9
CV(%)	21.9	9.4	8.0	19.6	16.8	10.2	9.9
CD @ 5%	12.5	15.9	13.6	1.5	1.4	1.6	1.3

DS : Damage due to *Spodoptera* (Rainy season 2001)
 LLS : Late leaf spot (Rainy season 2001)
 GIW I : Gain in weight by larvae (Rainy season 2001)
 GIW II : Gain in weight by larvae (Rainy season 2002)
 Thrips I : Damage due to thrips (Rainy season 2001)
 Thrips II : Damage due to thrips (Post rainy season 2001)
 Rust : Rust (Post rainy season 2001)

All the three superior segregates for resistant to *Spodoptera* namely RN 3-17, RN 3-26, and RN 4-4 yielded around 12 g of pod per plant (Table 26), which was less than the best parent but significantly more than NC Ac 343. However, the moderately resistant progenies for *Spodoptera* MR 2-53(13.8), RG 1-8(14.3) and TN 7-12(13.6) were on par with the best parent GPBD 4 for pod yield and were stable across seasons. The line TR 1-22(16.7) showed highest yield and was numerically superior to the best parent and was also stable across seasons. This line was also significantly superior for hundred seed mass compared to best parent, mutant 28-2. Among the other progenies, RG 2-21(15.9), TR 2-4(15.4) and TR 2-5(16) were on par with best parent for pod yield and the performance was stable across seasons. None of the lines were superior to the best parent GPBD 4 for shelling out turn. The progenies RN 3-26(56), RG 1-8(52), TN 1-20(60) TN 2-2 (63) and TN 7-12 (49) were significantly superior to the best parent mutant 28-2 for hundred seed mass and they were also moderately resistant of *Spodoptera*.

Correlation studies among pest and disease resistance parameters with productivity parameters revealed highly significant positive association between GIWI and GIW II and DS (Table 27) indicating scope for using GIW as a criteria of indirect selection for *Spodoptera* resistance in lieu of field damage.

4.5.5 Single plant selections for yield and agronomic features

Single plant selections were made visually for higher pod yield and good agronomic features *viz.*, plant habit, early maturity, pod features etc., in F₃ segregates. Subsequently, these selections were evaluated over four seasons for productivity (during 2001 rainy and post rainy season and 2002 rainy and post rainy season) and rust during post rainy season of 2001 apart from screening for resistance to *Spodoptera* (field damage and gain in weight by larvae), thrips and late leaf spot during 2001 rainy season.

Table 26: Stability parameters for various productivity traits in *Spodoptera* resistant lines

Genotypes	Plant Yield			Shelling percentage			Hundred Seed Mass		
	Mean	bi	S ² di	Mean	bi	S ² di	Mean	bi	S ² di
28-2 X R 9227 (MR)									
MR 2-6	15.0	1.45	65.15*	67	0.47	-7.09	45	0.84	8.61*
MR 2-7	15.0	-0.1	39.93**	67	0.5	-7.67	48	-0.78	-7.99
MR 2-21	11.9	1.27	7.3**	60	1.36	12.49**	41	1.61	-7.78
MR 2-53	13.8	0.57	0.84	68	-0.21	-0.79	38	1.67	113.78**
28-2 X NC Ac 343 (MN)									
MN 1-22	7.9	0.86	31.2**	49	0.49	146.69**	52	1.14	140.44**
R 9227 X NC Ac 343 (RN)									
RN 2-4	11.6	0.52	27.03**	65	1.84	-3.78	48	2.24	115.44**
RN 2-19	14.6	0.24	10.85**	63	1.96	-0.72	46	1.62	8.82*
RN 2-25	14.2	0.56	16.73**	58	-3.07	3.57	47	1.21	26.79**
RN 3-17	10.8	0.13	27.88**	60	2.39	44.59**	39	1.49	-7.66
RN 3-24	11.8	0.61	0.98	68	1.67	25.91**	38	0.3	5.33
RN 3-26	12.3	0.76	9.53**	64	2.94	14.31**	56	0.67	64.23**
RN 3-29	11.4	0.18	15.21**	62	-1.72	16.46**	46	2.37	5.87
RN 4-4	11.5	0.85	13.94**	69	-1.46	8.84*	43	-1.14	-7.14
R 9227 X GPBD 4 (RG)									
RG 1-11	12.3	1.39	100.03**	70	1.08	-7.43	37	2.99	102.43**
RG 1-8	14.3	0.16	1.51	64	0.07	0.77	52	1.55	119.6**
RG 2-21	15.9	1.49	-0.19	67	0.66	26.73**	40	0.28	1.18
RG 2-22	15.4	1.61	12.53**	63	2.26	21.83**	34	0.22	29.36**
TAG 24 X NC Ac 343 (TN)									
TN 1-16	10.5	1.48	20.07**	63	1.11	-7.12	46	2.38	69.71**
TN 1-20	11.0	0.93	21.56**	65	1.49	51.16**	60	0.48	25.37**
TN 1-23	13.2	0.57	5.24**	68	2.59	6.31*	52	0.51	10.03**
TN 2-2	14.5	0.82	7.76**	66	2.17	2.39	63	1.02	6.59
TN 2-4	11.7	1.58	-2.18	55	3.48	59.14**	44	3.63	56.76**
TN 3-1	13.6	1.51	19.5**	55	-2.48	149.08**	51	2.59	103.11**
TN 6-2	11.0	1.8	2.57**	55	2.58	-1.33	48	0.41	-5.77
TN 6-4	12.5	1.04	0.29	54	1.79	62.31**	44	0.99	14.21*
TN 7-5	15.5	1.72	52.04**	64	2.66	29.84**	51	2.03	22.56**
TN 7-6	15.9	1.94	10.36**	64	2.86	3.57	56	0.14	19.19**
TN 7-7	14.4	1.28	37.24**	70	2.73	-3.69	60	-0.57	28.74**
TN 7-12	13.6	1.27	-2.47	68	0.69	-7.07	49	1.3	-2.64

Table 26: Contd...

Genotypes	Plant Yield			Shelling percentage			Hundred Seed Mass		
	Mean	bi	S ² di	Mean	bi	S ² di	Mean	bi	S ² di
TAG 24 X R 9227 (TR)									
TR 1-1	13.9	1.57	-3.69	72	-1.2	2.14	37	-0.22	9.85**
TR 1-4	8.4	0.56	1.79	66	0.85	-2.68	43	0.79	0.73
TR 1-6	14.8	1.12	4.1*	72	-0.39	7.65*	41	0.56	-10.06
TR 1-16	13.4	0.6	0.5	71	1.18	-1.23	41	1.03	8.35*
TR 1-22	16.7	0.86	1.96	65	1.84	13.84**	54	1.59	14.32**
TR 1-37	14.0	1.55	-1.64	70	0.84	16.26**	46	1.65	24.68**
TR 2-4	15.4	1.54	-3.92	71	1.63	4.68*	46	0.43	-9.39
TR 2-5	16.0	0.87	-3.83	70	1.18	36.16**	45	1.42	13.4*
TR 1-36	10.7	0.35	-1.88	64	0.46	81.54**	43	-0.23	26.67**
GPBD 4 X NC Ac 343 (GN)									
GN 2-5	13.1	0.68	2.58*	75	-0.75	8.09*	49	-1.2	1.14
Parents									
Mutant 28-2	16.4	0.14	4.74**	58	2.81	-4.22	48	1.16	3.24
R 9227	14.9	0.74	15.43**	64	-0.14	25.58**	42	0.4	-6.62
NC Ac 343	7.5	0.34	2.19	59	-0.25	-7.11	35	1.01	86.29**
GPBD 4	15.1	0.09	-2.84	73	0.05*	-8.88	39	0.11	-8.15
TAG 24	14.5	1.54	6.9**	67	-0.92	11.02	46	-1.16	55.87*
Mean	13.30	1.00		64.86	1.00		46.40	1.00	
S.Em±	2.43	1.00		3.30	1.04		3.78	0.87	

* & ** - Significantly deviating from zero and unity in case of mean square deviation and regression coefficient, respectively

bi: Regression coefficient,

S²d: Mean square deviation

Table 27: Correlation coefficients for insect resistance, disease resistance and productivity parameters in selected progenies of different crosses

	GIW II	GIW I	DS	Thrips	LLS	Rust	PY	SP	HSM
GIW II	1	0.742 **	0.480 **	-0.019	-0.101	-0.068	0.096	0.106	-0.031
GIW I		1	0.347 *	-0.068	-0.321 *	0.065	-0.057	-0.025	0.055
DS			1	-0.020	-0.029	-0.341*	0.232	0.215	-0.217
Thrips				1	0.372 **	0.002	0.124	0.410 **	-0.086
LLS					1	0.316 *	-0.071	0.328*	-0.182
Rust						1	-0.312 *	-0.103	0.278 *
PY							1	0.370 **	0.132
SP								1	0.123
HSM									1

DS : Damage due to *Spodoptera* GIW I : Gain in weight by larvae(I) GIW II : Gain in weight by larvae(II) LLS : Late leaf spot

PY : Pod Yield SP ; Shelling percentage HSM : Hundred seed mass

Genetic variability among genotypes for all the traits and diversity among environments was significant as revealed by analysis of variance for stability (Table 28). Differential behavior of genotypes to various environments was evident by significant G X E interactions. Significant environment (linear) component for all the traits suggests linear change in environmental index as per change in environmental conditions. Predominance of unpredictable component was evident as revealed by non significance of G X E (linear) and significance of only mean deviation from regression.

All the selections except RD 1-8a were superior for pod yield (Table 29 and 30) compared to resistant parents (NC Ac 343, R 9227 and mutant 28-2). However, the same line RG 1-8a showed significantly higher yield (12 g) and higher resistance to *Spodoptera* (DS:18 and GIW: 50%) compared to the best resistant check NC Ac 343 (DS: 18 and GIW: 88%). Based on both DS and GIW, MR 1-7-1, and TR 1-19-1 were superior for *Spodoptera* resistance and comparable for productivity (16.8 and 20.8 g) to other two resistant checks viz., mutant 28-2 and R 9227, besides being resistant / moderately resistant to thrips (2 and 3) and rust (5 and 5). All the selections were superior for resistance to *Spodoptera* compared to popular varieties TAG 24 (34 and 300%) and GPBD 4 (44 and 193%) based on either DS or GIW or both.

Majority of the selections were superior for pod yield and stable across seasons. The segregates, MR 1-18-1 (23.5 g), TR 1-7-1a (22.8 g), MG 1-31-1 (22.5 g), TR 1-7-1b (20 g), TR 1-19-1 (20.8 g), TR 1-33-1 (20.6 g), TR 1-6-1 (20.9 g) and TR 2-1-2 (20.3 g) were the top performers for pod yield and the performance was also stable across the seasons compared to the best check GPBD 4 (Table 30). All these lines were also resistant to rust and comparable to checks GPBD 4, mutant 28-2 and R 9227, while three lines namely TR 1-7-1a, TR 1-7-1b, and TR 1-19-1 also combined resistance to thrips and rust along with high yield. Some of the high yielding lines viz., MR 1-18-1 (52 g), TR 1-7-1a (51 g) and TR 1-7-1b (52 g) had higher HSM compared to TAG 24 (50 g).

Table 28: Analysis of variance for stability of productivity parameters in single plant selections in groundnut

Source of variation	Mean Sum of Squares			
	df	Pod yield per plant	Shelling per cent	Hundred seed mass
Genotype (G)	41	45.94 **	71.12 **	121.59**
Environment (E)	.03	246.11**	1686.88**	991.81**
G × E	123	32.19 **	82.99 **	62.71 **
E (linear)	01	738.35 **	5060.91 **	2975.33 **
G ×E (linear)	41	35.34	58.97	49.66
Pooled deviation	84	29.88 **	48.81 **	67.59 **
Pooled error	164	14.23	40.58	44.39

** indicate significance at 1 % probability

Table 29: Performance of single plant selections for pest, disease and productivity parameters

LINE NO.	DS (%)	GIW (%)	THRIPS	LLS	RUST
MR 1-7-1	23	96	2	5	5
MG 1-2a	49	72	5	4	2
RN 1-13-1	39	46	4	7	4
RG 1-8a	18	50	4	4	3
MR 1-18-1	21	129	4	7	5
MR 1-10-1	63	74	1	2	5
MR 1-51-1a	20	133	2	6	3
MR 2-37-1	47	77	3	7	4
MR 1-51-1b	19	133	2	6	3
MN 1-31-1	45	148	1	4	6
RG 2-25-1	25	157	4	6	2
TN 2-3-1	31	55	3	5	4
TN 5-5-1	42	50	4	6	5
TR 1-7-1a	30	33	3	8	2
MR 1-49-1	41	66	4	6	4
MR 1-51-1c	19	133	2	6	3
MG 1-31-1	50	53	4	6	5
MN 1-28-1	34	77	3	5	4
TR 1-7-1b	30	33	3	8	2
TR 1-19-1	20	18	3	7	5
TR 1-33-1	30	49	4	9	5
TR 1-6-1	29	40	4	9	5
TR 2-1-2	29	39	4	7	4
TR 2-4-1	27	26	4	8	5
TR 2-7-1	48	23	4	8	2
Parents					
28-2	22	124	2	3	4
R 9227	29	117	4	7	5
NC Ac 343	18	70	4	5	6
GPBD 4	44	193	5	2	2
TAG 24	34	300	4	8	7
Grand Mean	34.4	83.0	3.3	5.9	4.1
CV (%)	16.4	10.1	18.9	15.0	23.9
CD (5%)	11.17	15.87	1.176	1.76	1.96

MR=28-2 X R 9227, MN=28-2 X NC Ac 343, RN=R 9227 X NC Ac 343, RG=R 9227 X GPBD 4
 TN=TAG 24 X NC Ac 343, TR=TAG 24 X R 9227, GN=GPBD 4 X NC Ac 343

DS : Damage due to *Spodoptera* GIW : Gain in weight by larvae LLS : Late leaf spot

Table 30: Stability parameters for various productivity traits in single plant selections

Genotypes	Plant Yield			Shelling percentage			HSM		
	Mean	bi	S ² di	Mean	bi	S ² di	Mean	bi	S ² di
MR 1-7-1	16.8	1.01	35.71	64	0.85	-16.71	44	0.67	7.09
MG 1-2a	16.4	0.25	76.61	63	0.5	-8.81	42	0.64	74.97
RN 1-13-1	16.8	0.80	59.92	66	1.63	-7.22	58	2.91	15.49
RG 1-8a	12.4	0.43	3.42	62	1.69	92.93	56	0.74	29.32
MR 1-18-1	23.5	0.68	99.71	66	1.59	10.06	52	1.45	-15.82
MR 1-10-1	15.0	2.28	22.04	62	0.98	83.74	49	0.94	302.89
MR 1-51-1a	15.7	-1.45	5.73	68	1.06	48.93	48	1.18	15.52
MR 2-37-1	16.5	0.86	-2.65	64	1.63	-14.7	51	1.35	1.12
MR 1-51-1b	17.1	3.28	18.14	57	2.56	-5.63	50	0.82	126.31
MN 1-31-1	17.6	1.77	-6.09*	67	0.95	64.54	45	0.65	-16.08
RG 2-25-1	17.9	2.43	36.57	65	1.71	-9.01	47	0.82	24.83
TN 2-3-1	19.5	3.02	32.27	55	2.28	-18.65**	52	1.26	109.02
TN 5-5-1	18.9	-1.03	45.23	69	0.65	-12.69	46	0.45	58.02
TR 1-7-1a	22.8	-0.95	22.68	68	0.81	-19.95**	51	1.27	130.35
MR 1-49-1	19.8	-0.99	3.27	65	0.98	-20.27**	50	1.02	29.06
MR 1-51-1c	18.7	3.59	25.18	60	2.08	128.74	49	1.77	107.77
MG 1-31-1	22.5	-0.38	45.76	64	2.35	168.01	47	1.73	-6.7
MN 1-28-1	17.6	3.56	39.20	58	1.64	21.6	48	2.46	137.54
TR 1-7-1b	20.0	0.48	-2.39	60	-0.47	122.78	52	2.95	46.36
TR 1-19-1	20.8	2.88	5.11	63	1.05	22.06	41	2.55	37.03
TR 1-33-1	20.6	0.65	90.69	63	1.55	-8.47	50	0.07	20.42
TR 1-6-1	20.9	0.93	32.36	67	0.9	3.81	44	-0.01	-3.51
TR 2-1-2	20.3	1.85	72.07	67	1.12	-18.41**	47	0.51	-20.11**
TR 2-4-1	18.0	1.92	-3.33	69	0.24	20.51	47	0.18	-20.43**
TR 2-7-1	16.9	2.20	0.63	68	0.45	-13.96	44	0.28	24.21
Parents									
28-2	14.9	-0.14	-5.95*	57	0.98	23.25	49	0.47	3.51
R 9227	16.1	1.72	9.59	63	0.66	-12.01	41	0.29	-19.75*
NC Ac 343	7.9	0.82	-5.81	61	0.23	4.45	32	0.37	-11.5
GPBD 4	16.9	-0.12	1.25	70	-0.03	-1.36	34	0.22	-3.55
TAG 24	14.6	1.67	-0.69	70	0.14	-19.77**	50	0.97	133.26
Mean	16.60	1.00		64	1.00		47	1.00	
S.E.m±	3.15	1.30		4.03	0.63		4.74	0.97	

MR=28-2 X R 9227, MN=28-2 X NC Ac 343, RN=R 9227 X NC Ac 343, RG=R 9227 X GPBD 4
 TN=TAG 24 X NC Ac 343, TR=TAG 24 X R 9227, GN=GPBD 4 X NC Ac 343

* & ** - Significantly deviating from zero and unity in case of mean square deviation and regression coefficient, respectively

bi: Regression coefficient,

S²d: Mean square deviation

Few lines namely MR 1-10-1(1), MR 1-51-1a(2), MR 1-15-1b(2), MN 1-31-1(1) and MR1-51-1c (2) were superior for thrips resistance. These lines were also superior / on par for yield when compared to the best parent, mutant 28-2.

For late leaf spot resistance MR 1-7-1(5), MG 1-2a(4), RG 1-8a(4), MR1-10-1 (2), MN 1-31-1(4), TN 2-3-1(5) and MN 1-28-1(5) showed moderate / high resistance. Among these lines MN 1-31-1(17.6), TN 2-3-1(19.5 g) and MN 1-38-1(17.6 g) were also superior for pod yield compared to the best parent GPBD 4 (16.9g). All the segregates except MN 1-31-1 were either resistant / moderately resistant to rust.

In general, one line MR 1-7-1 was resistant to *Spodoptera*, thrips, late leaf spot and rust, indicating the multiple pest and disease resistant nature of the line, besides it was on par with GPBD 4 for pod yield (16.6 g). However, all the selections have to be reevaluated for resistance to *Spodoptera* and other biotic stresses before exploiting them further.

4.6 BIOCHEMICAL BASIS OF RESISTANCE

Plant resistance to insects depends on different mechanisms of which, biochemical basis of resistance is one. The types of chemicals responsible for insect resistance are numerous but most commonly known groups are secondary metabolites. The potential benefits from identifying a resistance mechanism are enormous. When a resistant trait has been identified, the screening process becomes quick and efficient. This also helps in screening in the absence of pest and it could hasten the breeding programme, allowing large amount of germplasm to be screened, as well as reducing the area and number of plants grown and crossed.

Study of life history parameters of *Spodoptera litura* on selected groundnut genotypes revealed antibiosis as the principal mechanism operating in resistant genotypes. Antibiosis is characterized by presence of chemicals that interfere with growth and development of the insect. Hence, thirteen selected genotypes including three resistant (NC Ac 343, mutant

28-2 and R 9227) four moderately resistant (ICGV 86031, ICGV 86699, ICGV 87165 and ICGV 86590) and seven susceptible genotypes (JL 24, TMV 2, TAG 24, GPBD 4, TG 26 and TG 49) were assessed for presence of trypsin inhibitors and lectins. Estimation of trypsin inhibitors was done according to Kakade *et al* .(1969), while lectin activity was determined by hemagglutination assay using trypsinized neuraminidase treated human O-group erythrocytes.

The trypsin inhibitor activity could not be detected in any of the groundnut genotypes as they recorded higher absorbance than the enzyme reference (Table 31). This indicates that, the protease inhibitors may not have a role in conferring plants resistant to *Spodoptera*. However, lectin activity was detected in all the genotypes and quantitative differences were observed among the genotypes. But, correlation between lectin activity and pest damage parameters (DS and GIW) did not show any association indicating that, the lectins may not be associated with resistance in these genotypes.

However, maximum lectin activity was found in a resistant genotype R 9227(910) and the least was in a susceptible genotype TAG 24(141) indicating possibility of role of lectins in conferring resistance in some genotypes. This provided an opportunity to study the variation in lectin activity *vis a vis* resistance in progenies of these two parents. Progenies showed wide range of activity comparable to parents revealing the variability (Table 32). But, the correlation of lectin activity with pest parameters did not indicate any association, reconfirming that lectins are not the basis for *Spodoptera* resistance in these groundnut genotypes.

4.7 ASSESSMENT OF MOLECULAR DIVERSITY IN SELECTED GROUNDNUT GENOTYPES

Variability in groundnut genotypes has been examined using molecular markers by different researchers. Lanham *et al* (1992) demonstrated that RAPDs are a convenient and effective marker system for *Arachis* species. The RAPD assay was chosen for the present study since

Table 31: Biochemical parameters in selected groundnut genotypes

Genotypes	Protein (μl/ ml of extract)	Lipid (%)	Trypsin Inhibitor	Lectin specific activity
NC Ac 343	252	49.6	ND	243.8
Mutant 28-2	150	53.2	ND	288.4
R 9227	270	48.8	ND	910.2
ICGV 86031	194	50.4	ND	303.9
ICGV 86590	264	39.0	ND	029.1
ICGV 86699	179	49.8	ND	156.8
ICGV 87165	161	46.8	ND	350.5
TAG 24	217	47.2	ND	141.6
GPBD 4	205	55.0	ND	299.7
JL- 24	133	45.0	ND	211.5
TMV 2	150	38.8	ND	187.6
TG 26	265	45.8	ND	231.8
TG 49	191	45.6	ND	206.1

ND : Not detected

**Table 32: Lectin specific activity in the progenies of the cross
TAG 24 X R 9227 vis-a-vis Spodoptera resistance**

Genotypes	DS (%)	GIW (%)	Fat content (%)	Protein (µl/ ml of extract)	Lectin specific activity
TR 1-1	19	95	52	204	289
TR 1-6	29	40	52	169	348
TR 1-11	21	29	50	179	328
TR 1-19	20	18	49	152	388
TR 1-22	24	16	58	140	420
TR 1-24	28	42	47	168	701
TR 1-36	28	22	60	191	309
TR 1-37	24	41	51	214	551
TR 1-38	28	51	51	197	1198
TR 2-1	28	39	50	167	706
TR 2-4	27	26	43	184	640
TR 2-5	27	37	53	189	624
TR 2-7-1	48	23	45	194	606
TR 2-4-1	27	26	61	193	1219
TR 2-1-2	29	39	51	169	697
TR 1-33-2	30	49	60	100	1234
TR 1-33-1	30	49	51	169	1393
TR 1-19-1	20	18	45	206	1144
TR 1-16-1	29	40	49	169	697
TR 1-7-1b	30	33	50	192	307
TR 1-7-1a	30	33	50	187	628
TR 1-16-2	29	40	47	181	328
TR 1-6-1	29	40	54	192	613
R 9227	29	117	48	233	1009
TAG 24	34	300	48	176	168

DS :Damage due to *Spodoptera*

GIW : Gain in weight by larvae

the procedure involved was simple, not requiring probes or southern-blot hybridization as in RFLP.

Thirty five decamers obtained from Operon technologies Inc., U.S.A., were used for analyzing thirteen genotypes showing varying levels of resistance to *Spodoptera*.

A total of 262 bands were amplified, of which 75 bands were polymorphic (28.63 %) giving a ratio of 2.41 polymorphic bands per primer (Table 33). Number of bands varied with the primer used. Minimum and maximum number of bands obtained with different primers were in the range of 2 to 21. Number of polymorphic bands identified also varied with primer used. The primer OPJ 09 and OPJ 10 yielded high polymorphism, while seven primers (OPJ 16, OPJ 20, OPF 03, OPF 10, OPF 11, OPF 16 and OPF 19) did not show polymorphism.

The genetic similarity matrix generated from pooled data from thirty five primers revealed the highest genetic similarity of 99 per cent between TG 26 and TG 49 (Table 34) and the lowest of 88 per cent between 28-2 and NC Ac 343 and 28-2 and R 9227. This indicated that the diversity present among the genotypes is rather limited. The dendrogram constructed from the pooled data revealed distinct clustering of *Spodoptera* resistant mutant 28-2 away from the rest at S_{ij} 0.91 (Fig. 3). However, all the three resistant genotypes (NC Ac 343, mutant 28-2 and R 9227) clustered as one group at 0.95 similarity coefficient. The genotypes TG 26, TG 49, ICGV 87165 and GPBD 4 (0.97); ICGV 86699, ICGV 86031, TMV 2, and JL 24 (0.98); ICGV 86590 and TAG 24 (0.98) and NC Ac 343 and R 9227 (0.95) formed four clusters at different similarity coefficients.

Table 33: Analysis of RAPD patterns in selected groundnut genotypes

Sl. No.	Primer Code	Total number of bands	Number of bands polymorphic	Percentage of polymorphism
1	J 04	06	00	00.00
2	J 05	11	02	18.18
3	J 06	07	00	00.00
4	J 07	09	07	77.78
5	J 08	06	05	83.33
6	J 09	13	10	76.92
7	J 10	21	10	47.62
8	J 11	07	02	28.57
9	J 12	07	04	57.14
10	J 13	04	01	25.00
11	J 14	07	04	57.14
12	J 15	07	01	14.29
13	J 16	05	00	00.00
14	J 18	07	03	42.86
15	J 19	04	01	25.00
16	J 20	04	00	00.00
17	F 02	02	01	50.00
18	F 03	07	00	00.00
19	F 04	07	02	28.57
20	F 05	06	01	16.67
21	F 06	05	01	20.00
22	F 07	13	02	15.38
23	F 08	11	03	27.27
24	F 09	08	02	25.00
25	F 10	05	00	00.00
26	F 11	05	00	00.00
27	F 12	08	02	25.00
28	F 13	04	01	25.00
29	F 14	13	03	23.08
30	F 15	06	02	33.33
31	F 16	04	00	00.00
32	F 17	12	02	16.67
33	F 18	08	01	12.50
34	F 19	04	00	00.00
35	F 20	09	02	22.22
	Total	262	75	28.63

Table 34: Genetic similarity as DICE co efficient based on pooled data of RAPD profiles of selected groundnut genotypes

	TG26	TG49	ICGV 87165	ICGV 86699	TMV2	ICGV 86590	TAG24	28-2	JL24	ICGV 86031	NC Ac 343	GPBD4	R9227
TG26	1.00												
TG49	0.99	1.00											
ICGV87165	0.98	0.98	1.00										
ICGV86699	0.97	0.97	0.98	1.00									
TMV2	0.97	0.97	0.96	0.98	1.00								
ICGV86590	0.95	0.95	0.96	0.97	0.96	1.00							
TAG24	0.95	0.96	0.97	0.97	0.96	0.98	1.00						
28-2	0.91	0.93	0.91	0.91	0.91	0.91	0.92	1.00					
JL24	0.96	0.96	0.96	0.97	0.97	0.96	0.96	0.91	1.00				
ICGV86031	0.96	0.96	0.97	0.98	0.97	0.96	0.97	0.92	0.98	1.00			
NC Ac343	0.94	0.94	0.95	0.95	0.95	0.94	0.94	0.88	0.95	0.95	1.00		
GPBD4	0.97	0.96	0.98	0.97	0.97	0.97	0.96	0.90	0.95	0.97	0.96	1.00	
R9227	0.94	0.94	0.94	0.95	0.96	0.95	0.95	0.88	0.94	0.94	0.94	0.95	1.00

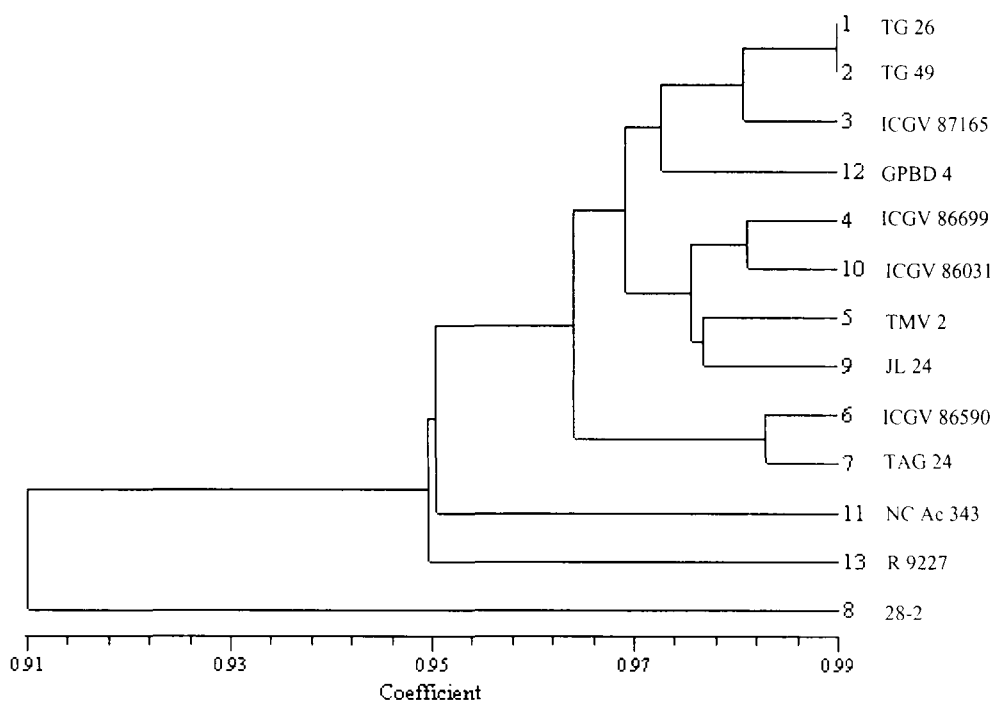


Figure 3: Dendrogram obtained from pooled data of RAPD profiles of Selected groundnut genotypes

Discussion

V. DISCUSSION

World wide there is an increased awareness that agricultural practices must be sustainable. Greater emphasis is laid on integrated management of pests of which resistant cultivars form an important component. Incorporation of resistance to cultivars has assumed a greater importance and is an integral part of most of the crop breeding programmes.

In groundnut, the Spanish bunch cultivars are most popular in India but they are highly susceptible to pests and diseases in general and *Spodoptera* in particular. The research efforts have been successful in identifying resistant germplasm (Leuk and Skimmer, 1971, Anon, 1984, Anon, 1985, Xie Ji Lhi, 1987, Wightman *et al.*, 1990, Patil *et al.*, 1991, Todd *et al.*, 1991, Dwivedi *et al.*, 1993, Singh *et al.*, 1993, Nadaf *et al.*, 1995, Reddy *et al.*, 1996, Moss *et al.*, 1997, Tiwari, *et al.*, 1989, Prasad *et al.*, 1998, Rajgopal *et al.*, 1988, Dhare and Patel, 2000 and Gowda *et al.*, 2002), but the level of resistance is rather low and often they suffer from undesirable agronomic features. Hence, development of early maturing germplasm combining high level of resistance to *Spodoptera* with desirable pod and kernel features besides productivity is a priority for the groundnut breeders.

The present investigation was undertaken to screen and evaluate groundnut genotypes against *Spodoptera*, enhance resistance through hybridization, discern the mechanisms of resistance and to assess molecular diversity in groundnut genotypes with varying levels of resistance.

5.1 Status of germplasm for resistance to *Spodoptera litura*

A set of thirty nine groundnut germplasm comprising interspecific derivatives, advanced breeding lines, mutants and released cultivars along with known sources of resistance were screened for *Spodoptera* damage in

field during 1998 rainy season. Damage was assessed at 70 days after sowing (DAS) when the crop suffered maximum defoliation. High incidence of *Spodoptera* resulting in heavy damage (57 %) in the season provided excellent conditions for meaningful screening. The coefficient of variation (12.49 %) revealed that the screening was reliable.

The range (34.3 to 80.7%) and phenotypic (25.7%) and genotypic (22.4%) coefficient of variation indicated the existence of significant variation in the germplasm. High heritability (76.3 %) resulted in high genetic advance (40.4 %) indicating substantial scope for selection in the germplasm (Table 6a and Fig. 4).

The mutants and advanced breeding lines constituted the good source of resistance as revealed by proportion of resistant lines (Fig.5). Most of the released cultivars (except ICGV 86590 and TAG 24) suffered very high damage (> 60%). Interspecific derivatives sustained moderate to high (> 50%) damage. Though many wild species have shown very high resistance to *Spodoptera* (Table 2), lack of focussed work in targeting improvement for resistance to *Spodoptera* in cultivated species could be the reason for low to moderate level of resistance in interspecific derivatives.

The genotypes ICGV 91180, NC Ac 343, mutants (28-2 and 45) suffered least damage (<40 %) revealing resistance to the defoliator (Table 8 and Fig.5). Other genotypes namely, ICGV 86031(41%), R 9214(41%), TAG 24(41%), ICGV 86590(42 %), mutant 110(45%), ICGV 93021(46%) and NC Ac 17090 (49%) recorded significantly lesser damage compared to highly susceptible genotypes (>70%).

The resistance nature of mutants 28-2 and 45(Prasad *et al.*, 1998 and 2000), NC Ac 343 and ICGV 86031 (Sreenivasa *et al.*, 1997 Singh and Sachan, 1992, Tiwari *et al.*, 1991, Patil *et al.*, 1995 and Dwivedi *et al.*, 1993) and ICGV 86590 (Patil *et al.*, 1991 and Reddy *et al.*, 1996) reported earlier has been confirmed in the present study. The genotypes ICGV 86031 and ICGV 91180 shared a common parent NC Ac 2214, which

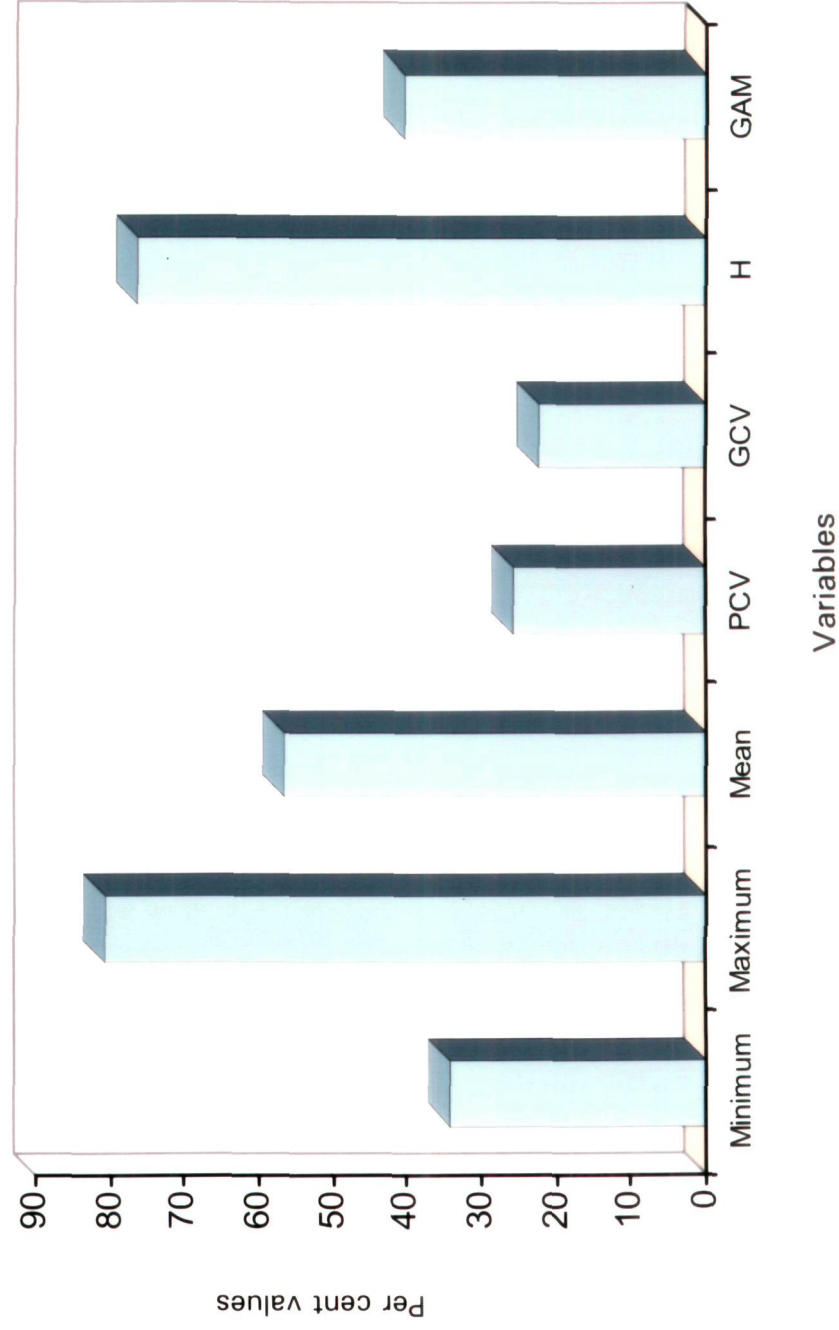
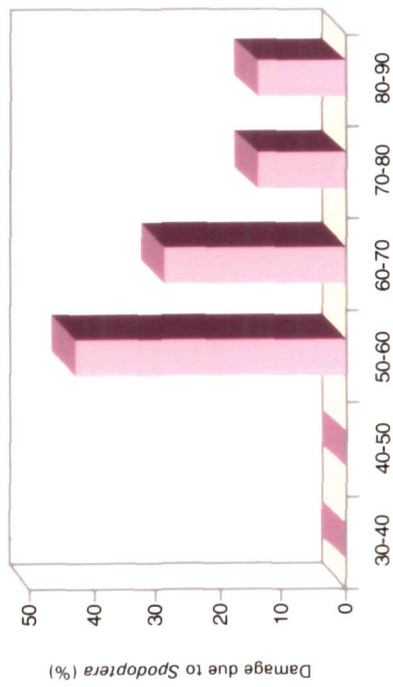
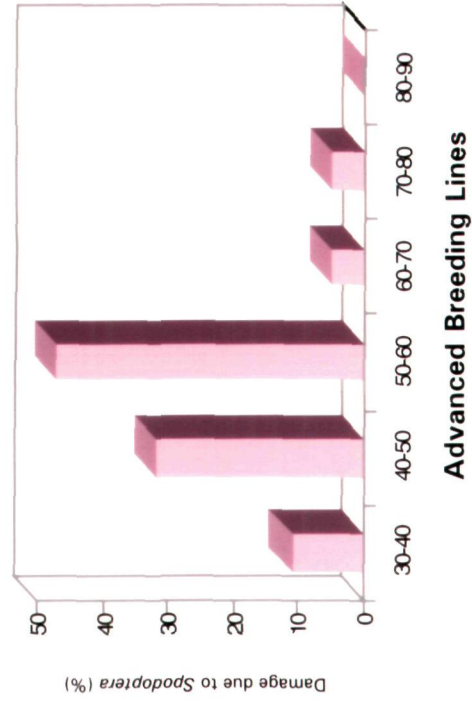


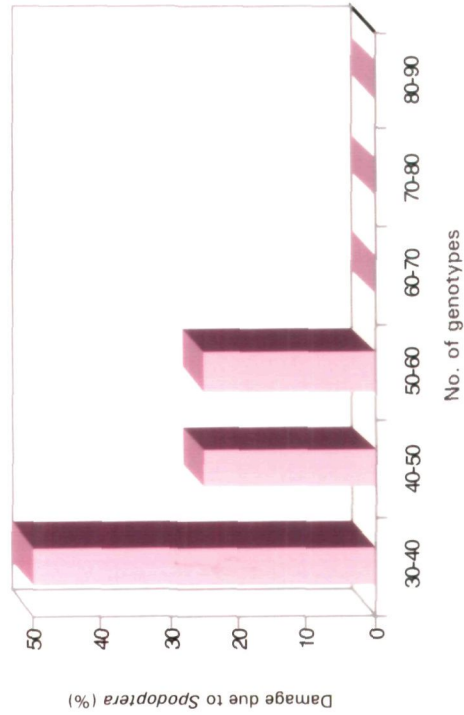
Fig. 4: Genetic components of variance for damage due to *Spodoptera* in groundnut germplasm



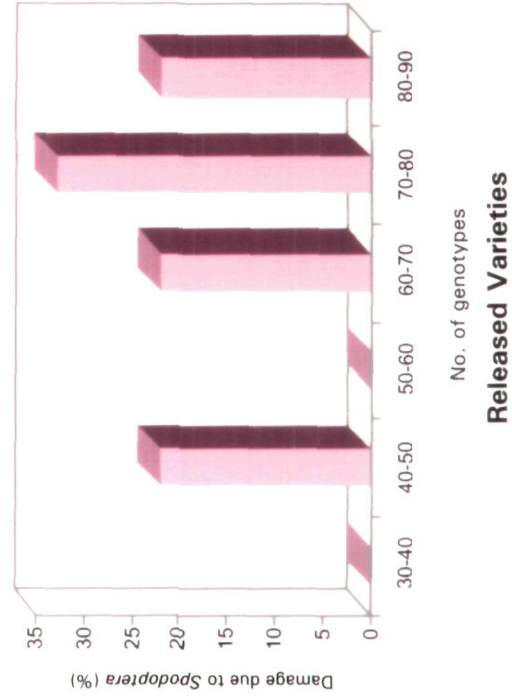
Interspecific derivatives



Advanced Breeding Lines



Mutants



Released Varieties

Fig. 5: *Spodoptera* damage in groundnut germplasm

is a known multiple pest resistant genotype (Lynch, 1990), while R 9214 is a derivative of a three way cross involving ICGV 86031 and NC Ac 2214 as parents.

The mutants were the best source of resistance as they mature early and are resistant to late leaf spot and thrips besides possessing desirable pod and kernel features with high productivity (Motagi *et al*, 1996 and Prasad *et al.*, 1998). NC Ac 343 and ICGV 91180 matured late (120 days) while, ICGV 86031 and R 9214 matured early (101 days), but all were poor yielders and susceptible to foliar diseases (Naidu, 2003). The rust resistant genotype NC Ac 17090 had low shelling out turn with undesirable pod and kernel features (Naidu, 2003).

All the released varieties (except ICGV 86590 and TAG 24) were susceptible to *Spodoptera*. Though released for cultivation, ICGV 86590 is not popular among farmers due to low shelling out turn with undesirable pod and kernel features. TAG 24 is early maturing and determinate in habit because of which the vegetative growth ceases at 50 days after sowing (DAS). The low damage due to *Spodoptera* scored at 70 DAS, could be due to seasonal avoidance, as insect prefers to feed only on young leaves.

5.2 Standardization of a new criterion for large scale screening of germplasm

Spodoptera litura is a major defoliator in groundnut. The damage is confined to top young leaves, as it prefers to feed on freshly emerging foliage. The early instars feed by scraping leaves while the later instars are voracious defoliators and cause maximum leaf damage.

Screening plants for resistance under natural infestation is a convenient method and can be effective if the desired growth stage is properly synchronized with the peak activity of the pest. However, it is difficult to achieve uniformity in the distribution of the infestation, or control the level of infestation among the screening materials. Escapes, as well as excessive infestation levels are likely to occur, which will reduce the

precision of field screening. Hence an alternative method for large scale screening of germplasm in laboratory is desirable to overcome the inherent problems of field screening.

In soybean, researchers have used mean weight of surviving larvae after 10 days of feeding on detached leaves as a measure of resistance for Mexican bean beetle (Rufener *et al.*, 1989). A modified version of this technique was evaluated for its suitability. Fully opened leaves from the top (I, II and III) were used separately for feeding pre-starved (24 hours) larvae of III, IV and V instars. The larvae were allowed to feed for 24 hours and the percent gain over initial weight of larvae and leaf area damaged (cm²) were recorded. Two resistant (NC Ac 343 and mutant 28-2) and susceptible (TAG 24 and GPBD 4) groundnut genotypes were employed to standardize the technique.

The analysis of variance revealed significant variation only for the gain in weight by the III instar larvae (Table 8a). Significant genotypic differences were observed when III instar larvae were fed with different leaves (Table 8b and Fig.6), which could be due to the uniform feeding and size of the III instar larvae. The later stage larvae are voracious feeders and ensuring uniformity in feeding is difficult. Besides uniformity, the use of early stage larvae has an additional advantage of increased sensitivity to the resistant factors (Isman and Duffey, 1982).

Among the leaves used for feeding, II leaf differentiated the susceptible and the resistant genotypes clearly, which could be due to optimum combination of resistant factors and age of the leaf. The resistant factor in the first leaf may not be sufficient to differentiate resistant and susceptible genotypes, whereas the third leaf could be over aged.

The parameter, gain in weight by larvae differentiated the genotypes well as compared to leaf area damaged. The coefficient of variation was least in III instar with II leaf combination for gain in weight (9.6%) indicating its reliability and superiority. As the stage of the larvae

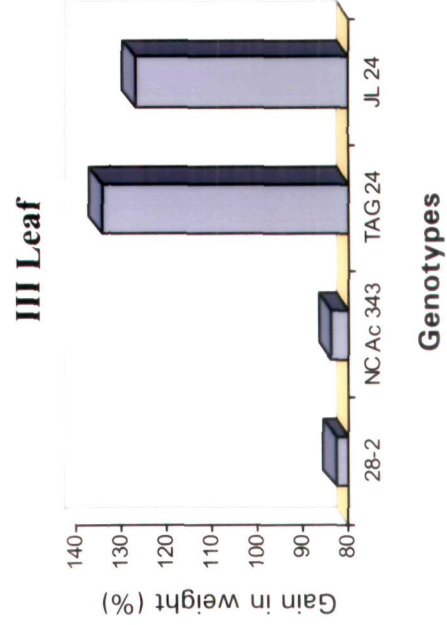
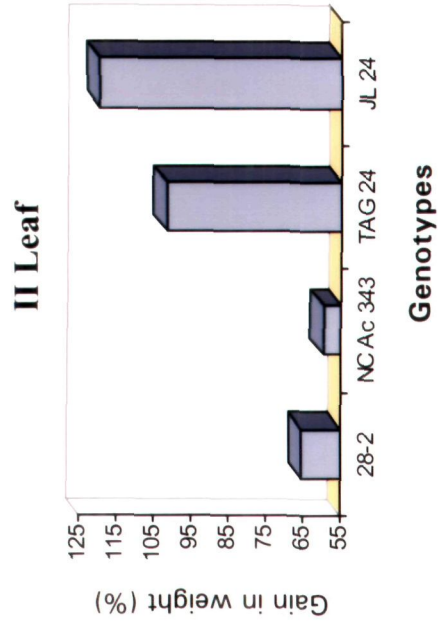
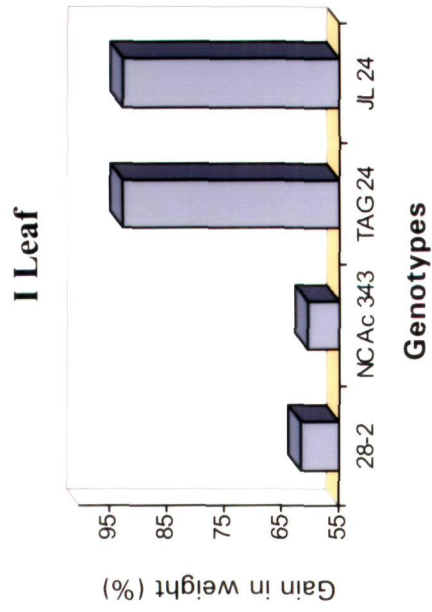


Fig.6: Performance of groundnut genotypes for gain in weight by III instar larvae of *Spodoptera litura*

increases, even a slight change in the age and size of the larvae greatly affects the leaf area damaged. Further, gain in weight by larvae is easy to measure as compared to leaf area damaged, which requires use of sophisticated instruments.

The laboratory screening will enable rapid screening of germplasm with less labour and resources compared to field screening. The problem of non-uniform feeding and non-synchrony of stage of the crop with peak activity of the insect as observed in the field experiments could also be avoided resulting in greater precision of screening programmes.

This technique can be employed where the resources for rearing are inadequate to produce sufficient number of insects for artificial infestation, when standard resistant and susceptible entries do not show proven and repeatable reactions over locations and vagaries in normal weather patterns occur (Ampofo and Saxena, 1987).

5.3 Evaluation of Selected groundnut genotypes for resistance

Based on full range of variation for resistance to *Spodoptera* besides desirable agronomic features, resistance to other pests and diseases thirteen genotypes namely NC Ac 343, mutant 28-2, R 9227, ICGV 86031, ICGV 86699, ICGV 87165, ICGV 86590, JL 24, TMV 2, GPBD 4, TG 26 and TG 49 were selected for detailed evaluation during 1999 rainy season.

Significant variation was found for both the parameters of resistance namely GIW and DS (Table 9a) and it was of heritable (Table 9b). However, genetic advance (68%) was high for GIW due to high heritability (94%) and moderate level of variation revealing better scope for selection. The error coefficient of variation was less for GIW (8%) as compared to DS (16%) revealing better control over experimental error leading to reproducibility of results based on GIW. Gain in weight by larvae showed gradation of resistance and better differentiation among the genotypes compared to field damage. However, significant correlation between GIW and DS (0.58) indicated the possibility of using GIW in lieu of DS (Table 10).

The cultivars viz., JL 24, TMV 2, TG 49 and GPBD 4 were the most susceptible to *Spodoptera* as they recorded high field damage and gain in weight. Based on both the DS and GIW, NC Ac 343 and mutant 28-2 were superior confirming the resistant nature of these genotypes to *Spodoptera* (Table 10). The genotypes R 9227 and ICGV 86031 were resistant based on only GIW where as TAG 24 was resistant solely on DS. The genotypes viz., ICGV 86590, ICGV 86699 and ICGV 87165 were moderately resistant to *Spodoptera* based on both the parameters.

The genotype TAG 24 showed less foliage damage in field in spite of recording high gain in weight of the larvae in laboratory, revealing seasonal avoidance or non-preference as the possible mechanisms for low damage by the pest in this genotype.

5.4 Life history parameters of *Spodoptera litura* (F.) on selected groundnut genotypes

Knowledge of an insect biology on the host plant is imperative in host plant resistance investigations. Investigating developmental and life history parameters of an insect has become an integral part of the process of identifying and studying pest resistance in groundnut (Prasad, 2000). It is a key element in developing efficient techniques for screening plants for resistance to insects and for determining and elucidation of complex resistance mechanisms. Hence, *Spodoptera litura* (F.) was reared on resistant (NC Ac 343, Mutant 28-2, R 9227 and ICGV 86031) and susceptible (JL 24, TAG 24, and GPBD 4) genotypes. The effects of these genotypes on growth and developmental parameters of *S. litura* were ascertained in the laboratory.

The study demonstrated the existence of substantial amount of variability in the host affecting the life history parameters (Table 11a). The parameters, larval number and larval mortality at all the stages and number of eggs per mass showed low to moderate genetic advance due to low magnitude of variation in spite of high heritability, while all the larval

weight parameters and pupal characters showed high genetic advance due to high heritability and moderate to high level of variation (Table 11b).

The resistant genotypes NC Ac 343, mutant 28-2 and R 9227 recorded lesser number of larvae and high mortality throughout the life cycle (Table 12 and Fig. 7). The early mortality in 28-2 and NC Ac 343 has already been reported (Prasad, 2000 and Singh and Sachan, 1992). The high mortality at early stages indicated vulnerability of neonate larvae. In pest control strategies, neonate larvae should be a primary target in host plant resistance because plant damage can be minimized if the pest is eliminated as early in the life cycle as possible. Mortality at early stages has also been observed in *Heliothis zea* when reared on maize plant. The development of first stadium larvae of *H. zea* was retarded by the presence of chlorogenic acid and rutin in artificial diet. (Isman and Duffy, 1982).

The larvae reared on NC Ac 343, mutant 28-2 and R 9227 also showed significantly low gain in weight compared to susceptible checks (Fig. 8 and Plate 4). But ICGV 86031 recorded significantly low larval weight and gain in weight but not larval mortality and these features have been reported earlier (Sreenivasa *et al.*, 1997 and Tiwari *et al.*, 1989). The differential response of the genotypes on larval parameters indicates the possibility of different mechanisms of resistance operating in them.

The effects of resistant genotypes on larval survival and growth of the larvae could obviously be due to chemical factors, i.e., antibiosis as elucidated by Painter (1951). The chemicals *viz.*, queretin glycosides, chlorogenic acid and rutin were found responsible for resistance in wild *Arachis* species (Stevenson, 1993a) could be the cause for antibiosis. However, resistance in wild *Arachis* species was also shown to be due to leaf toughness (Stevenson *et al.*, 1993b) requiring greater biting effort and it could be a possible reason for the differential response of ICGV 86031.

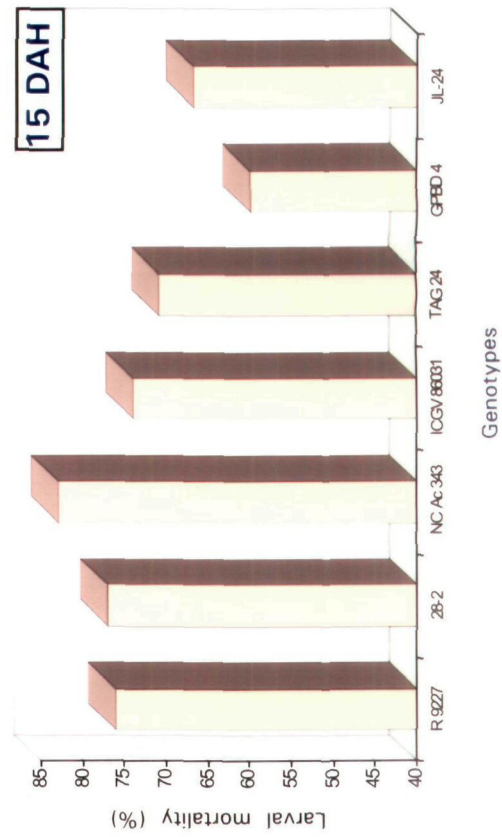
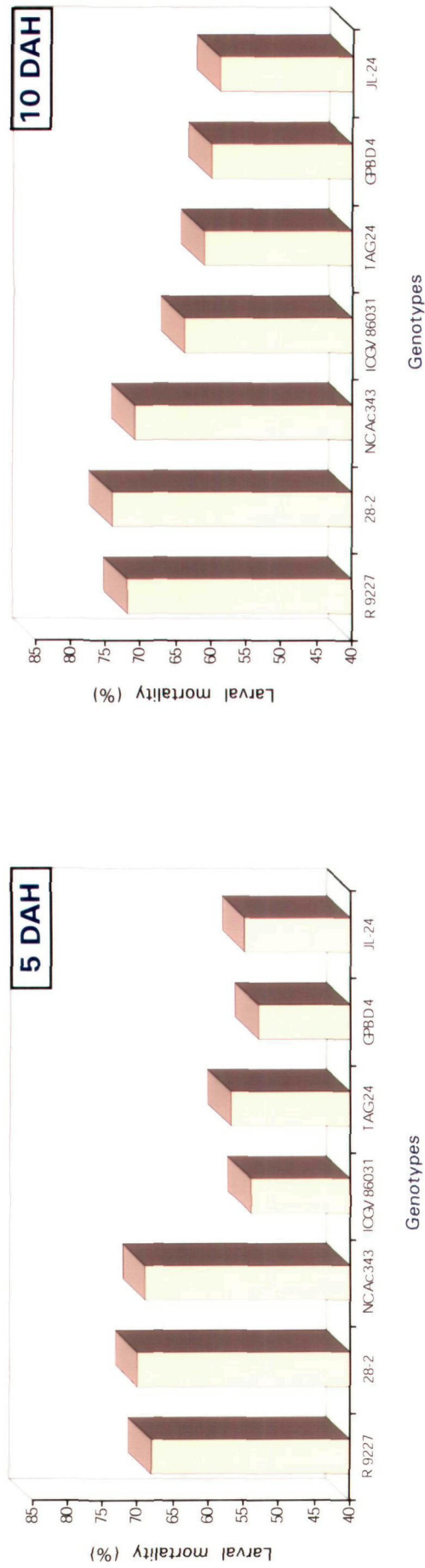


Fig. 7: Effect of groundnut genotypes on mortality of *Spodoptera litura* at different stages

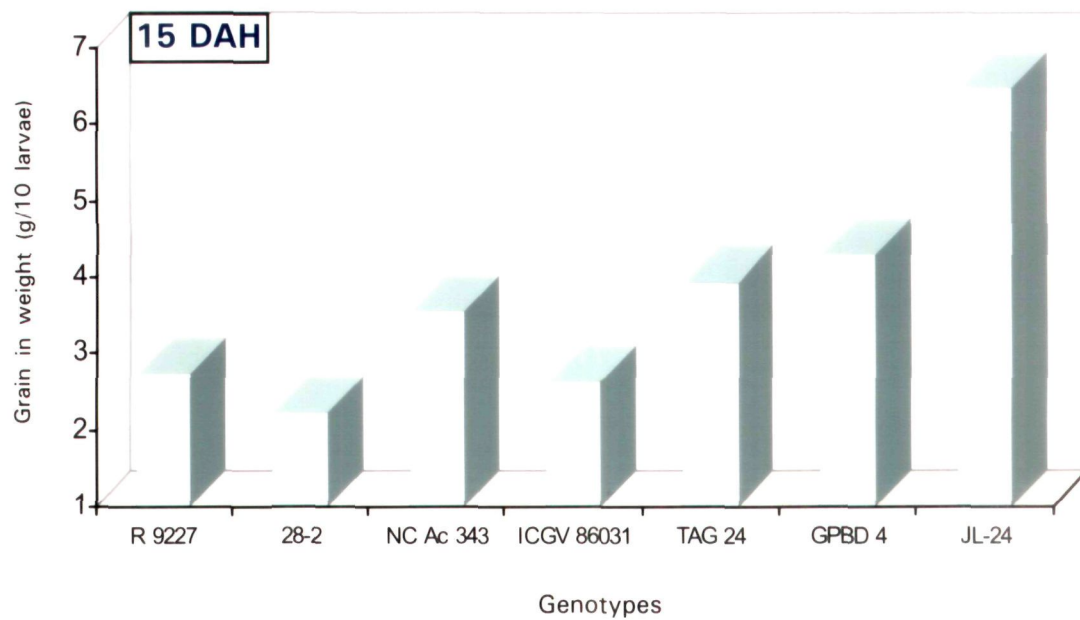
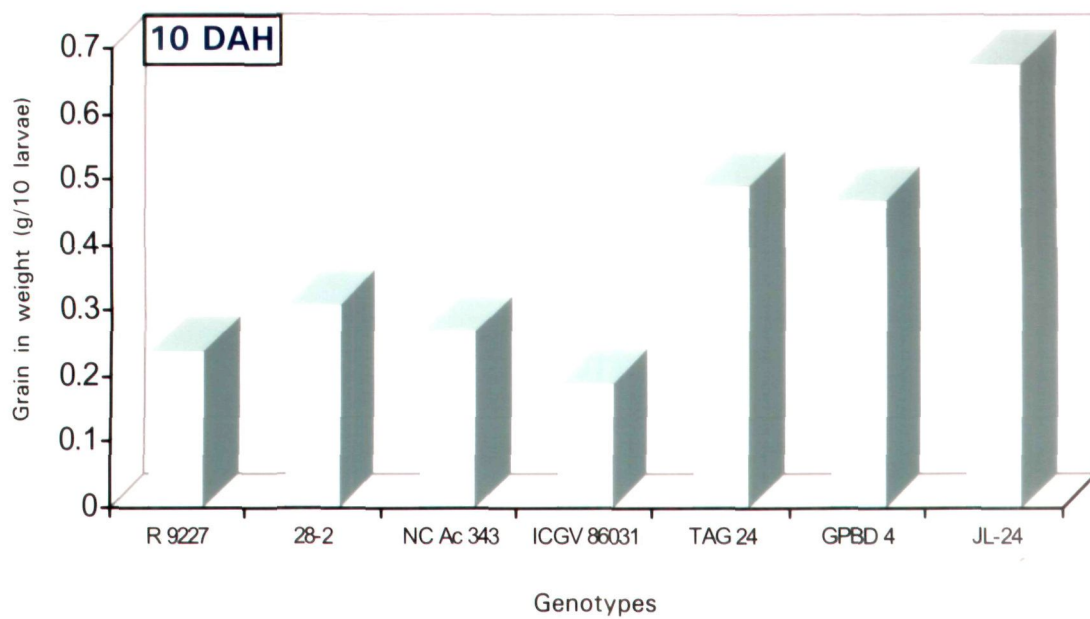


Fig. 8: Effect of groundnut genotypes on gain in weight of *Spodoptera* larvae at different stages

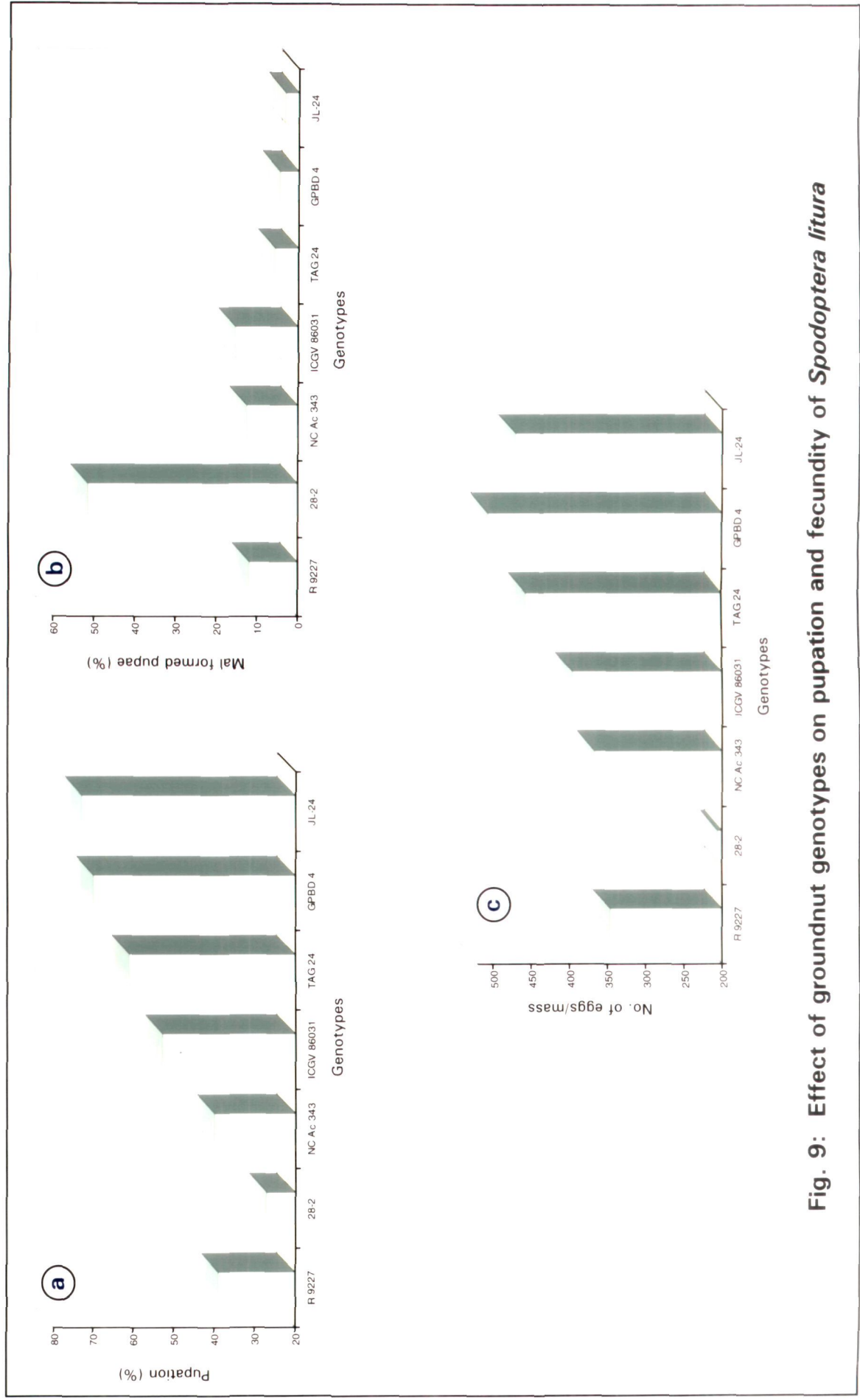


Fig. 9: Effect of groundnut genotypes on pupation and fecundity of *Spodoptera litura*

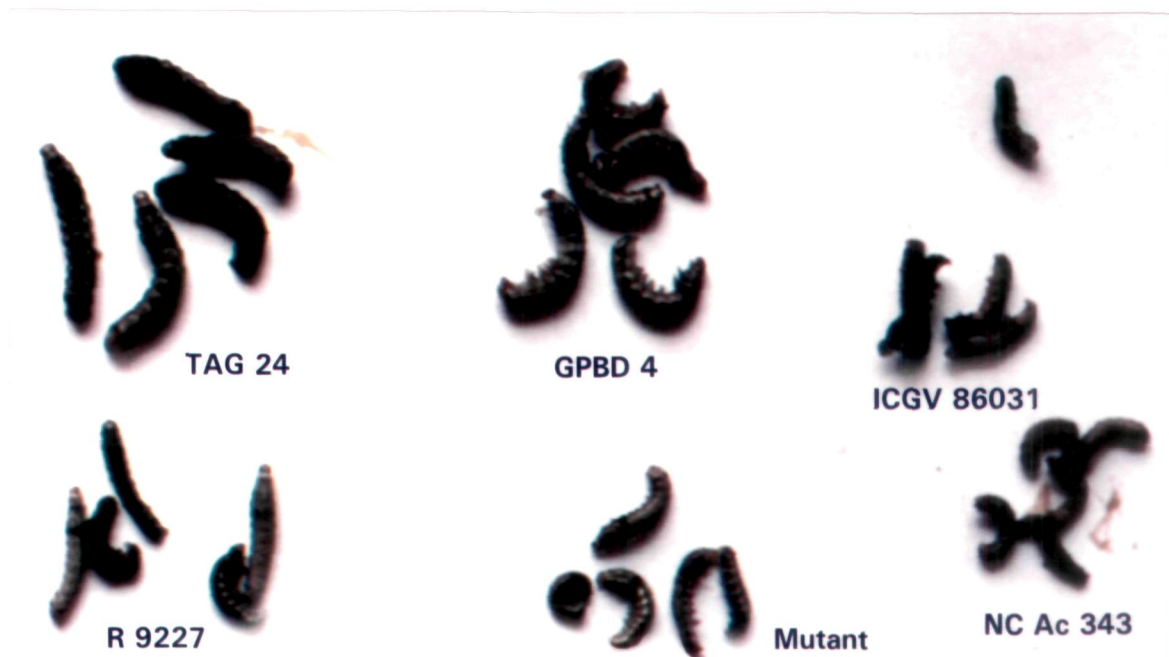


Plate 4: Genotypic effect on larval development at 13 days after hatching



Plate 5: Genotypic differences for the occurrence of malformed pupae

Pupal deformities namely larval head capsule retention, incomplete sclerotization, size reduction etc., was noticed in very high frequency (52%) on mutant 28-2 (Fig.9a & b and Plate 5). Similar symptoms were displayed by the pupae of *Spodoptera* raised on *Mucuna* seeds based artificial diet (Rehr *et al.* , 1973) and it was traced to L- dopa a constituent of the seeds that interfered with an enzyme (tyrosinase) involved in cuticle formation. Similar pupal disfiguration was also observed by Dahlman and Rosenthal (1976) in *Manduca Sexta* fed artificial diet containing L- Canavanine. Thus, it is worthwhile to investigate in detail the chemical responsible for resistant nature of mutant 28-2, which could be exploited later, as a chemical marker in resistance breeding.

The resistant effect of mutant 28-2 was also markedly observed on fecundity, which could be an important criterion for selecting resistant genotypes (Fig. 9c). The cumulative effect of such resistance on fecundity over generations will be enormous and such resistance is considered to be of high value as control measure (Painter, 1951).

The resistant effect of NC Ac 343, ICGV 86031 and R 9227 was also observed on pupal characters and moth emergence but not to similar intensity that of Mutant 28-2. Similar observations were also made by Leuk and Skimmer, (1971) and Garner and lynch, (1981), where mean percentage of pupae and moths were significantly less from larvae fed on foliages of the resistant than susceptible groundnut cultivars. The effect of resistance on pupal development confirms the antibiosis mechanisms of resistance existing in the resistant genotypes (Painter, 1951).

Though TAG 24 recorded lesser foliage damage in field (Table 10), but it showed normal growth and development of the insect in the laboratory and was comparable to the susceptible checks. It is evident that the cultivar TAG 24 does not carry any inherent factor for resistance to *Spodoptera*. Therefore, seasonal avoidance or non-preference could be the main mechanisms for low damage observed in TAG 24.

In conclusion, the death of the insect on resistant genotypes viz., mutant 28-2, NC Ac 343 and R 9227 during neonate stage is considered as an easily observable characteristic of antibiosis. The other characteristics viz., low gain in weight, low pupation, high percentage of mall formed pupae, low weight of pupae and extension of mean larval period and low fecundity by moths of larvae fed on the foliages of these genotypes confirm the role of antibiosis as a main mechanism of resistance in these genotypes (Painter, 1951).

5.5 Enhancement of resistance to *Spodoptera* in groundnut through hybridization

Among the resistant genotypes identified (Plate 6), mutant 28-2 and R 9227 possessed good pod and kernel features. These genotypes matured early and were on par with released cultivars. The mutant 28-2 is resistant to late leaf spot and thrips and R 9227 is resistant to rust and *Sclerotium* (Motagi *et al.*, 1998, Prasad, 2000 and Naidu 2002). Where as NC Ac 343 matured late but combined high level of resistance to *Spodoptera* among the available germplasm and was also reported to be multiple pest resistant (Sreenivasa *et al.*, 1997 Singh and Sachan, 1992, Tiwari *et al.*, 1989, Patil *et al.*, 1995 and Dwivedi *et al.*, 1986). Therefore, they were considered as good donors of resistance to *Spodoptera*.

Among the released cultivars GPBD 4 is early maturing, resistant to late leaf spot and rust besides possessing good agronomic features with high productivity but is susceptible to *Spodoptera*. Though susceptible TAG 24 suffered less damage in the field due to early maturity and determinate habit besides being tolerant to bud necrosis. Hence, they can be good parents for incorporation of *Spodoptera* resistance.

Resistant (mutant 28-2, NC Ac 343 and R 9227) and susceptible (GPBD 4 and TAG 24) genotypes were hybridized to produce eight crosses. Three crosses namely 28-2 X R 9227, 28-2 X NC Ac 343 and R 9227 X NC Ac 343 were effected in order to increase the level of



Plate 6: Variation in parents for feeding by starved larvae of *Spodoptera litura*

resistance to *Spodoptera*. While 28-2 X GPBD 4, R 9227 X GPBD 4, TAG 24 X NC Ac 343, TAG 24 X R 9227 and GPBD 4 X NC Ac 343 were effected to combine *Spodoptera* resistance in good agronomic background. Four hundred and eighty one F_{2:3} progenies along with parents were evaluated for resistance to *Spodoptera* during 2001 rainy season. Resistance to *Spodoptera* was evaluated both in the field as well as in the laboratory. Defoliation of leaves was visually assessed in the field and gain in weight of pre-starved larvae was utilized in laboratory. The lines were also assessed for resistance to thrips and late leaf spot besides productivity.

Among parents, the least defoliation (DS) and gain in weight of larvae (GIW) were shown by NC Ac 343, followed by mutant 28-2 and R 9227. Maximum DS was evident in GPBD 4 while TAG 24 recorded the maximum GIW, revealing distinct variation for resistance to *Spodoptera* (Fig. 10).

Variability in the progeny existed for both DS (Plate 7) and GIW in all the crosses as revealed by range and ANOVA. Genetic components of variation namely phenotypic co efficient of variation (PCV), genotypic co efficient of variation (GCV), heritability in broad sense (H) and genetic advance as per cent of mean (GAM) revealed moderate variability in turn moderate genetic advance for DS, where as high magnitude of variation and higher heritability in turn high genetic advance was apparent for GIW. Low heritable nature of variation for DS could be due to scoring procedure (visual), uneven distribution of infestation, variation in feeding habits of larvae in field due to preference and their migratory nature and vagaries of environment. The environment in laboratory was more controlled and the larvae were forced to feed on the foliage without any scope for preference in feeding and migration.

The progenies in all the crosses exhibited continuous and unimodal distribution for both the parameters of resistance revealing that it is quantitative in nature and may not be amenable for classical Mendelian analysis. It also implies that resistance to *Spodoptera* is more difficult to manipulate in breeding programmes. Occurrence of susceptible segregates

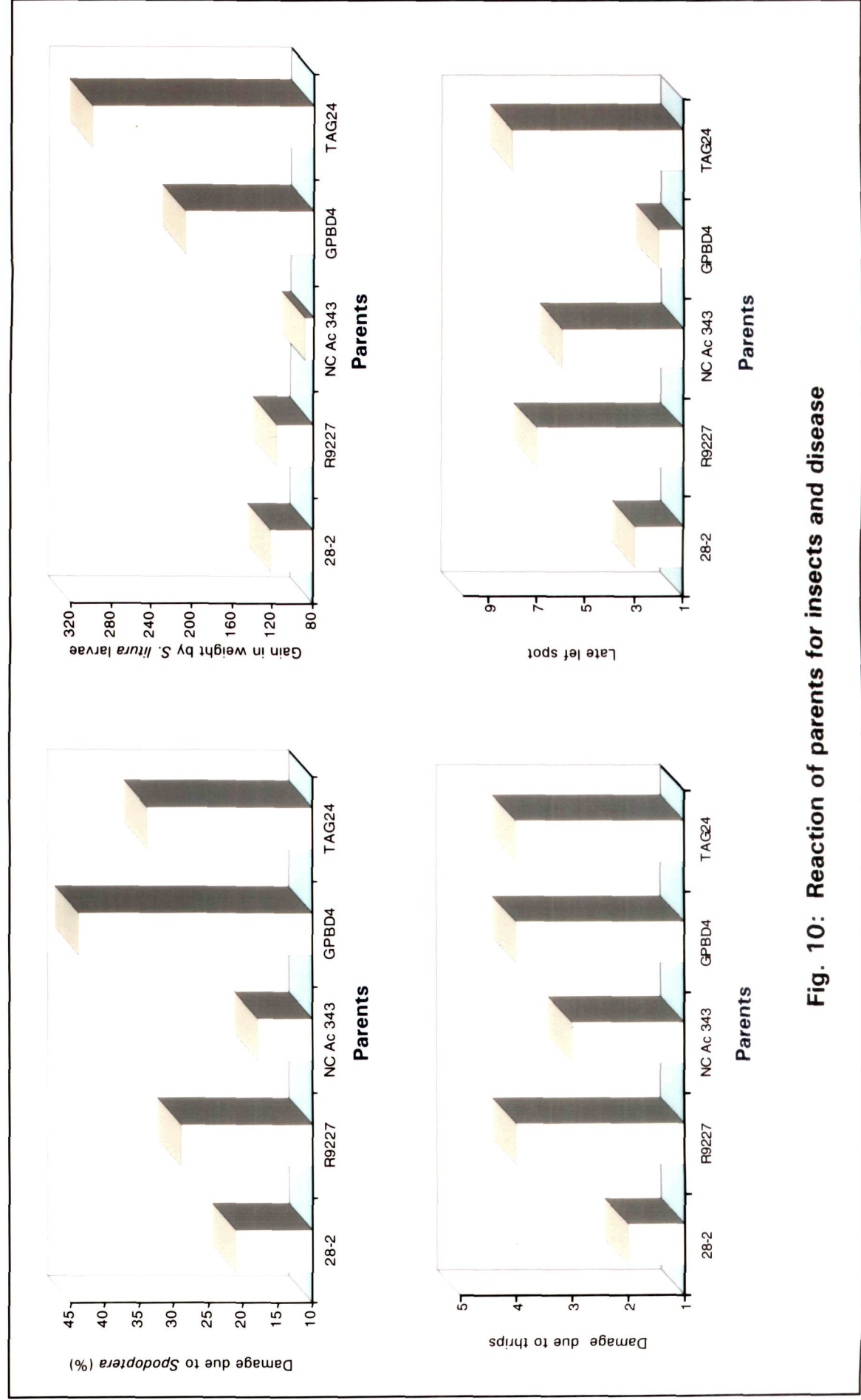


Fig. 10: Reaction of parents for insects and disease



Plate 7: Variation for *S. litura* damage in progenies

in resistant X resistant crosses (28-2 X R 9227, 28-2 X NC Ac 343 and R 9227 X NC Ac 343) and higher frequency of resistant types in susceptible X resistant crosses (TAG X R 9227 and TAG X NC Ac 343) indicated the existence of different factors and predominance of gene interactions in determining resistance to *S. litura*

Based on the parental performance, progenies recording less than 30 per cent of defoliation were considered as superior for DS, while the progenies showing less than 100 per cent gain in weight by larvae as superior for GIW. Based on DS, 30.9 percent of the lines were superior while as many as 47.4 per cent of lines were superior on GIW (Table 21). The low mean and high frequency of superior segregates revealed the potential of 28-2 X R 9227, R 9227 X NC Ac 343, R 9227 X GPBD 4 and GPBD 4 X NC Ac 343 for GIW, while two crosses TAG 24 X NC AC 343 and TAG 24 X R 9227 were good for both DS and GIW indicating that resistant X susceptible crosses are also useful in combining resistance besides resistant X resistant crosses. It also revealed susceptible parent TAG 24 as a good combiner. All the crosses with resistant parent mutant 28-2 produced low frequency of resistant segregates indicating its poor combining ability. Thus, phenotype of the parents cannot be a sole criterion while selecting parents for hybridization and needs a detailed evaluation of the progeny.

The correlation co-efficients between DS and GIW in all the crosses did not show association possibly indicating the role of different aspects in the performance in field and laboratory. Therefore, both DS and GIW were used in combination for selecting superior progenies for resistance. Based on this criterion only 15.8 per cent lines were superior for *Spodoptera* resistance (Table 21 and Fig 11).

Out of 76 superior segregates for *Spodoptera* resistance based on both the parameter DS and GIW, 43 progenies combined superior yield. Majority of these progenies were from the crosses R 9227 X GPBD 4, TAG 24 X NC AC 343 and TAG 24 X R 9227 revealing the importance of

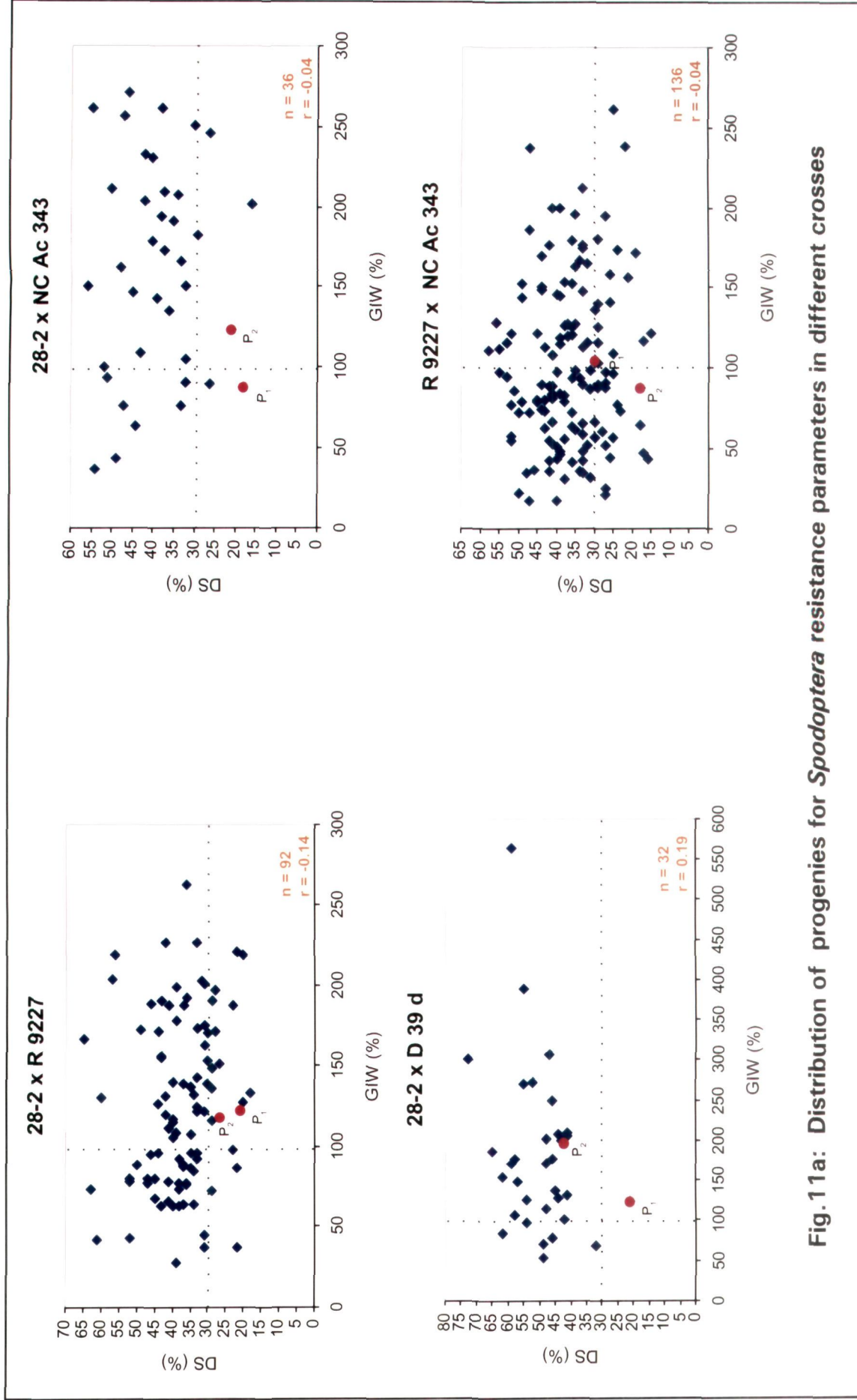


Fig.11a: Distribution of progenies for *Spodoptera* resistance parameters in different crosses

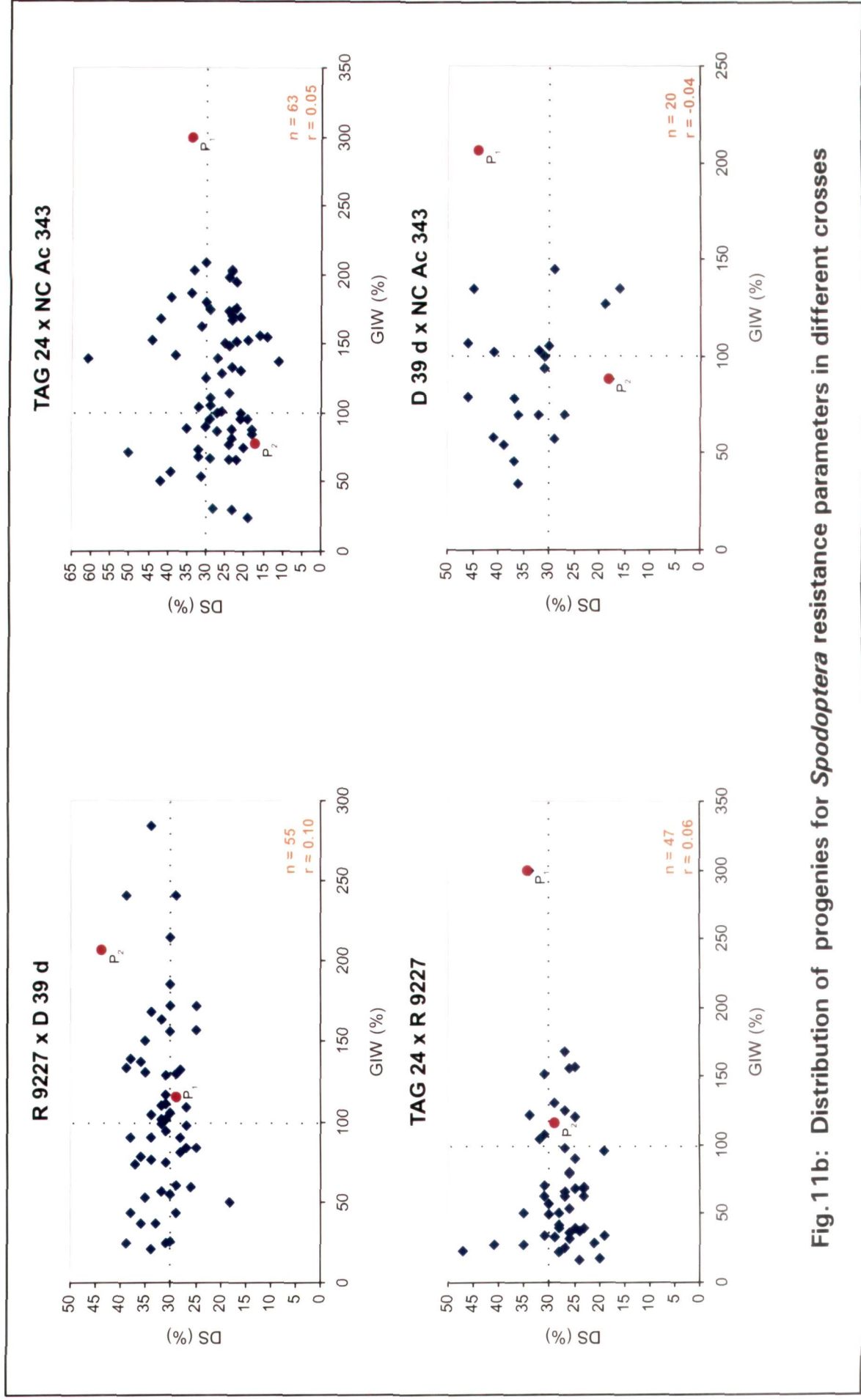


Fig.11b: Distribution of progenies for *Spodoptera* resistance parameters in different crosses

agronomically superior genotypes (TAG 24 or GPBD 4) in hybridization (Table 21 and 22). Six lines combined resistance to thrips and were mostly from 9227 X NC AC 343 and TAG 24 X NC Ac 343 but none were high yielding. Eleven progenies predominately from the crosses involving either 28-2 or GPBD 4 combined resistance to late leaf spot. Out of them, six lines were high yielding. Only one progeny of the cross 28-2 X NC Ac 343 combined resistance to *Spodoptera*, thrips and late leaf spot but was poor yielding. This indicated that the resistance for different stresses and productivity were distributed among different crosses and progenies. However, the correlations among *Spodoptera* resistance parameters, damage due to thrips, late leaf spot and productivity did not show any significant association in majority of the crosses indicating independent nature of the traits and thus revealing the possibility of combining all the desirable traits. Hence, multiple crossing and intermating among the resistant progenies could be attempted to derive multiple pest and disease resistant lines with high productivity.

5.5.1 Evaluation of superior segregates

After the first cycle of screening, it is generally desirable to test the selected lines for confirmation of resistance. Based on the yield performance, agronomic attributes and availability of seeds, forty-three progenies were again assessed for *Spodoptera* resistance using gain in weight by the larvae in laboratory (GIW II). Majority of the lines recorded a similar response as in the first assessment thus reconfirming their resistance (Table 25). This was also evident from the correlations between GIW I with GIW II (0.742), GIW I with DS (0.347) and GIWII with DS (0.480) indicating positive association and scope for selection through GIW in lieu of DS in the selected material (Table 27). It is interesting to note the change from lack of association in the crosses to positive association between DS and GIW in selected material. This supports the importance of employing both DS and GIW as criteria for effective selection of resistance to *Spodoptera*.



Plate 8: Differential response of progenies to thrips



Plate 9: Variation for late leaf spot resistance in Segregating Progenies



Plate 10: Variation for rust resistance in Segregating Progenies

Five superior progenies (RN 3-17, RN 3-26, RN 4-4, RG 1-8 and TN 1-20) with enhanced resistance to *Spodoptera* based on both DS and GIW I again recorded lesser gain in weight compared to NC Ac 343 (DS: 18% and GIW: 88%) reconfirming their superiority over the best resistant parent (Table 35 and Fig. 12) This indicated the possibility of enhancing the resistance through hybridization. However, the increased resistance in these lines has to be confirmed ultimately by a detailed analysis of growth and development of the insect before exploiting them further in breeding programme or registering them as resistant germplasm.

Among the five lines, RG 1-8 also combined late leaf spot and rust resistance thus, revealing its insect and disease resistant nature. The yield level was comparable to the best parent GPBD 4 and was stable across four seasons (bi:0.16 and S²d:1.51) indicating its potential for large scale trials for further exploitation. RN 3-26 was resistant to thrips and combined high test weight which could be the good source material for multiple pest resistance

Two segregates MR 2-7 and MN 1-22 combined resistance to *Spodoptera*, thrips and late leaf spot revealing their multiple pest and disease resistance. MR 2-7 was comparable to the highest yielding parent GPBD 4 for yield, indicating its potential for further exploitation.

Many of the lines were comparable to either mutant 28-2 or R 9227 for *Spodoptera* resistance. Of them two progenies MR 2-6 and TN 3-1 were resistant to late leaf spot, three progenies RG 2-21, RG 2-22 and GN 2-5 were resistant to rust, and two lines RN 3-26 and TN 1-20 were superior for test weight. Four progenies namely TR 1-22, TR 2-4 and TR 2-5 were comparable to 28-2 and GPBD 4 for plant yield and their performance was stable across four seasons indicating the improvement in other features besides retaining *Spodoptera* resistance (Table 25 and 26).

But, majority of the lines were inferior for shelling out turn compared to GPBD 4 (73%) necessitating a need for improvement of the trait. The line

Table 35: Performance of progenies of different crosses for various biotic stresses and productivity parameters

Lines	DS (%)	GIW		Th		Th II	LLS	Rust	PY			SP			HSM		
		I	II	I	II				Mean	bi	S ² d	Mean	bi	S ² d	Mean	bi	S ² d
RG 1-8	18	56	63	4	4	4	4	4	14.3	0.16	1.51	64	0.07	0.77	52	1.55	119.6**
RN 3-17	18	48	22	4	5	5	7	7	10.8	0.13	27.88**	60	2.39	44.59**	39	1.49	-7.66
RN 3-26	16	44	53	1	3	3	7	7	12.3	0.76	9.53**	64	2.94	14.31**	56	0.67	64.23**
RN 4-4	9	65	32	5	5	5	7	7	11.5	0.85	13.94**	69	-1.46	8.84*	43	-1.14	-7.14
MR 2-7	23	96	64	2	3	3	5	7	15.0	-0.1	39.93**	67	0.5	-7.67	48	-0.78	-7.99
MN 1-22	26	90	71	3	3	3	4	6	7.9	0.86	31.2**	49	0.49	146.69**	52	1.14	140.44**
MR 1-6	23	87	93	3	4	4	4	6	15.0	1.54	65.15*	67	0.47	-7.09	45	0.84	8.61*
TN 3-1	24	77	62	3	4	4	5	7	13.6	1.51	19.5**	55	-2.48	149.08**	51	2.59	103.11**
RG 2-21	30	60	69	4	3	3	7	4	15.9	1.49	-0.19	67	0.66	26.73**	40	0.28	1.18
RG 2-22	26	56	52	5	5	5	7	3	15.3	1.61	12.53**	63	2.26	21.83**	34	0.22	29.36**
TN 1-20	17	79	63	3	4	4	7	6	11.0	0.93	21.56**	65	1.49	51.16**	60	0.48	25.37**
TN 2-4	23	81	74	2	3	3	6	6	11.7	1.58	-2.18	55	3.48	59.14**	44	3.63	56.76**
GN 2-5	27	70	83	4	4	4	6	5	13.1	0.68	2.58*	75	-0.75	8.09*	49	-1.2	1.14
TR 1-22	24	16	40	4	4	4	8	6	16.7	0.86	1.96	65	1.84	13.84**	54	1.59	14.32**
TR 2-4	27	26	37	4	4	4	8	6	15.4	1.54	-3.92	71	1.63	4.68*	46	0.43	-9.39
TR 2-5	27	22	89	4	4	4	8	5	16.0	0.87	-3.83	70	1.18	36.16**	45	1.42	13.4*
MR 1-18-1	21	129	-	4	-	-	7	5	23.5	0.68	99.71	66	1.59	10.06	52	1.45	-15.82
TR 1-19-1	20	18	-	3	-	-	7	5	20.8	2.88	5.11	63	1.05	22.06	41	2.55	37.03
Parents																	
Mutant 28-2	21	122	101	2	3	3	3	6	16.4	0.14	4.74**	58	2.81	-4.22	48	1.16	3.25
R 9227	29	116	106	4	4	4	7	6	14.9	0.74	15.43**	64	-0.14	25.58**	42	0.04	-6.62
NC Ac 343	18	88	60	3	4	4	6	6	7.5	0.34	2.19	59	-0.25	-7.11	35	1.01	86.29**
GPBD 4	44	207	188	4	4	4	2	4	15.1	0.09	-2.84	73	0.05	-8.88	39	0.11	-8.15
TAG 24	34	300	262	4	5	5	8	7	14.5	1.54	6.9**	67	-0.92	11.02	46	-1.16	55.87*
Mean	24.6	68.6	73.4	3.3	3.6	3.6	6.6	5.9	13.30	1		64.86	1.00		46.40	1.00	

DS: Damage due to *Spodoptera* GIW: Gain in weight by *Spodoptera* larvae Th: Thrips LLS: Late leaf spot PY: Pod yield SP: Shelling percentage
HSM: Hundred seed mass bi: Regression coefficient S²d: Mean square deviation

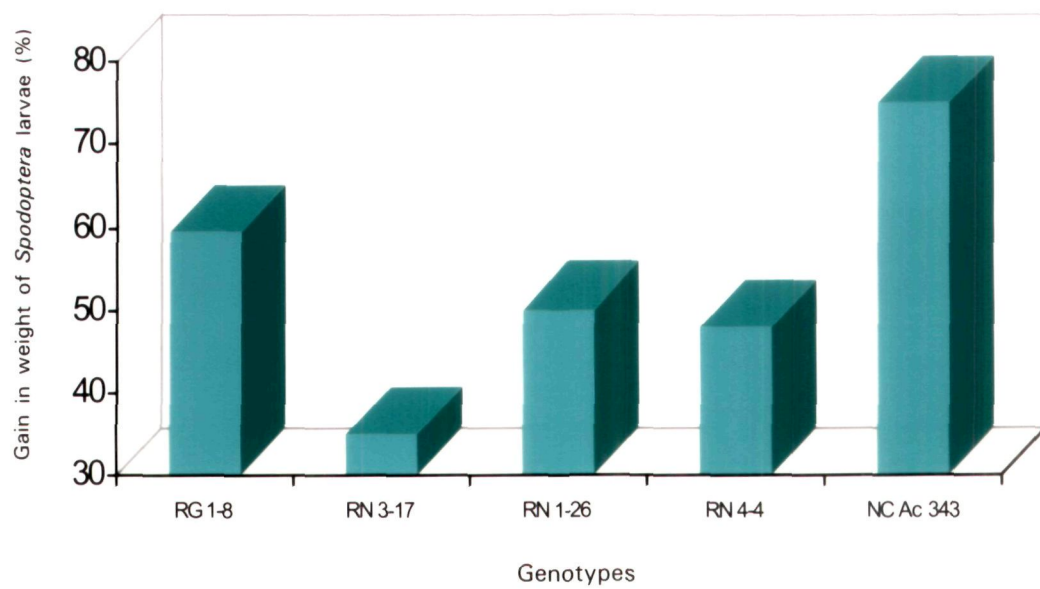
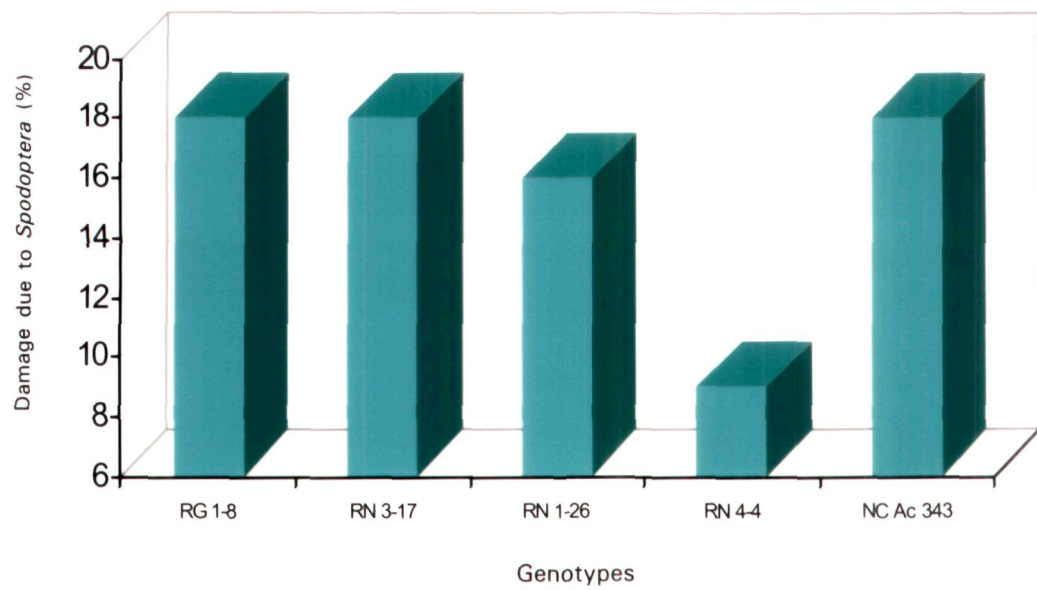


Fig. 12: Enhancement of resistance to *Spodoptera* in superior segregates

DN 2-5 has resistance to *Spodoptera* comparable to 28-2 and R 9227 with moderate yield but very good for shelling out turn (75%) and could be a good source to improve shelling out turn of the *Spodoptera* resistant material.

Twenty-six single plant selections selected visually for higher pod yield and good agronomic attributes in **F_{2:3}** progeny were evaluated for three subsequent seasons for productivity.

Nine lines were distinctly superior for yield compared to GPBD 4 and the performance was stable across the seasons. Of them two lines namely MR 1-18-1 and TR 1-19-1 were comparable to R 9227 and mutant 28-2 for *Spodoptera* resistance besides being resistant to rust. MR 1-18-1 was superior for hundred seed mass while TR 1-19-1 was moderately superior for thrips resistance. After reconfirming resistance to *Spodoptera*, these lines can be tested in large scale trial for further exploitation. The remaining progenies showed marginal improvement over susceptible parents for *Spodoptera* resistance.

Thus from the foregoing discussion it is evident that most of the resistant progenies still carried one / more deficiencies hence, there is a need to intermate these progenies in a complimentary fashion to generate better resistant material for future exploitation.

5.6 Biochemical basis of resistance

Greater understanding of mechanism of resistance is essential to approach the goal of developing insect resistant plants rapidly. Knowledge of biochemical basis of resistance makes it easier to select a plant that confers resistance. Such knowledge may make it possible to use biochemicals, as screening tool even in the absence of pest infestation more rapidly. Although enormous literature dealing with host plant resistance exists in many crops, very little is known about the *Spodoptera* resistance at biochemical level in groundnut. The chemistry of insect resistance in

groundnut is at preliminary level compared to other crops like maize and soybean.

Rearing *Spodoptera litura* on resistant and susceptible genotypes revealed possible existence of antibiosis, indicating the role of chemical factors in imparting resistance. Hence, thirteen selected groundnut genotypes with varying levels of resistance to *Spodoptera* were assayed for the presence of two anti-nutritional compounds namely, protease inhibitors (Jongsma *et al.*, 1995) and lectins (Boulter *et al.*, 1990) that are known to confer resistance to this pest in other crops.

Trypsin inhibitors of plant origin are proteinaceous in nature and inhibit proteinases of animal and microbial origin and not of plant origin. Ingestion of these inhibitors will interfere with digestive enzymes of the insect. The biochemical assay in selected groundnut genotypes did not indicate the presence of trypsin inhibitor activity in seeds. However, the presence of trypsin inhibitor in the leaves of groundnut has been reported (Nandagopal *et al.*, 1996). Hence, the above genotypes can be explored further for the presence of trypsin inhibitor in leaf to deduce the mechanism of resistance.

Plants produce a diverse set of chemicals that are toxic, in various degrees to insects. High molecular weight compounds such as phytohemagglutinins (lectins) inhibit growth and development of insect species and play a defensive role against insects. The selected groundnut genotypes with varying levels of resistance to *Spodoptera* were analyzed for lectin activity. Hemagglutination assay using trypsinized neuraminidase treated human O-group erythrocytes indicated wide variation in lectin activity among the genotypes. The correlation between lectin activity and pest resistant parameters did not show any association. However R 9227(1009) a resistant genotype and a susceptible genotype, TAG 24(168) showed maximum and minimum activity respectively (Table 31 and 32). Hence, the resistant and susceptible progenies derived from these genotypes constituted the best material to study the association of lectin

activity with resistance. Though, the progenies showed a wide range of variation as in parents, a definite pattern was not evident *vis a vis* resistance. This was also reflected by the lack of correlation between the lectin activity and pest resistance parameters in the progeny, confirming the lack of any role of lectins in imparting resistance to *Spodoptera* in these genotypes.

Allelochemicals such as L-Canavanine is known to exhibit high insecticidal activity. When the chemical was administered in artificial diet to *Spodoptera eridania*, the frequency of pupal deformities was very high (Rehr *et al.*, 1973). The life history parameters of *Spodoptera litura* reared on resistant groundnut genotype mutant 28-2 precisely displayed these symptoms. It is also reported that sugars namely melibiose and β -lactose also affect growth and development of the *Spodoptera littoralis* (Ishaaya and Meisner, 1973). Chemicals like quertin glycosides, coffeoylquinic acid, rutin and chlorogenic acids in wild *Arachis* species are known to inhibit growth and development of *Spodoptera litura* (Stevenson *et al.*, 1993). Therefore, these specific chemicals affecting the growth and development of *Spodoptera* have to be explored in order to deduce the mechanism of resistance operating in the resistant genotypes.

5.7 Assessment of molecular diversity in selected groundnut genotypes

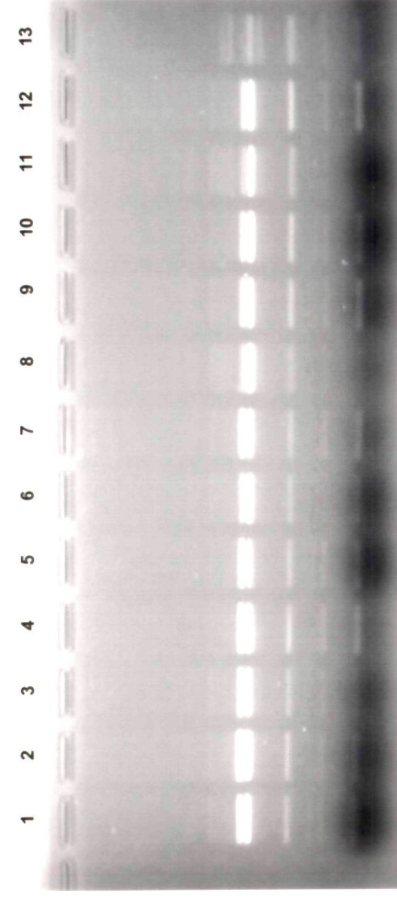
The molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programs but, their application in genetic enhancement of groundnut is lagging behind due to limited knowledge of the genome. Extensive variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al.*, 1992), but low DNA polymorphism has been observed in cultivated species (Kochert *et al.*, 1991 and Halward *et al.*, 1993), though a diverse variation for phenotypic characteristics exists. Of late, studies using RAPDs, SSRs and AFLPs techniques have revealed the presence of DNA polymorphism in cultivated groundnuts (Dwivedi and Gurtu, 2002,

Hopkins *et al.*, 1999 and He and Prakash, 1997). The observed polymorphism may be useful for developing molecular markers helpful for screening various traits in groundnut improvement programs.

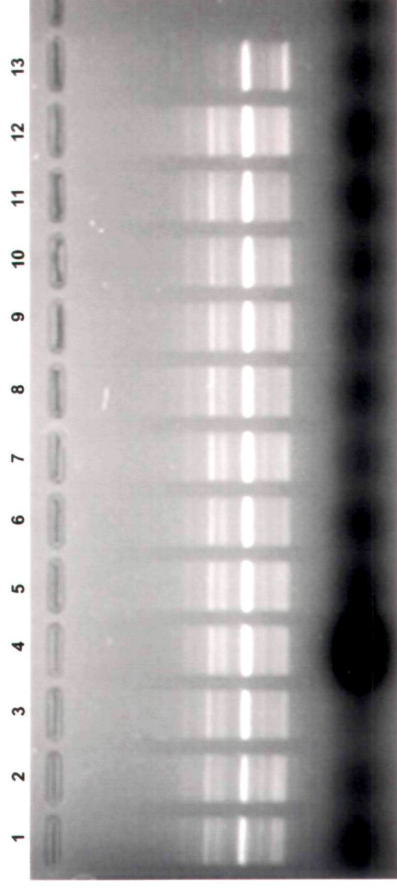
Knowledge of the genetic diversity in a crop species is fundamental to its improvement. At present the variability for *Spodoptera* resistance is of low magnitude. There is a need to identify diverse germplasm that differ in mechanism and factors of resistance and intercross them to enhance the level of resistance. However, information on the diversity for *Spodoptera* resistance is lacking. Further, resistant material should be genetically diverse to prevent crop vulnerability due to narrow genetic base. Variation in DNA profiles in lieu of phenotypic diversity may be used to identify diverse germplasm. Molecular diversity analysis by RAPD assay was carried out among resistant (NC Ac 343, mutant 28-2 and R 9227), moderately resistant (ICGV 86031, ICGV 86699 ICGV 87165, and ICGV 86590) and susceptible (JL 24, TMV 2, TAG 24, GPBD 4, TG 26 and TG 49) genotypes for better scientific exploitation of genetic resources.

Out of 35 primers used, twenty-eight primers produced polymorphism for two to ten bands (Plate 11a and b) with an average of two bands per primer (28.63 %). The primers J07, J09, J 08, J10, J12, and J14 produced greater polymorphic differences than others (Table 33). This study again confirms the earlier reports of limited variation in cultivated groundnut (Bhagwat *et al.*, 1997, Subramanian *et al.*, 2000, Dwivedi *et al.*, 2001 and Dwivedi *et al.*, 2001). The low level of polymorphism in cultivated groundnut has been attributed to recent origin of the crop by single event of polyploidization (Young *et al.*, 1996). Later, due to the ploidy barrier between the tetraploid groundnut and the diploid wild species further gene flow was prevented resulting in a highly conserved genome. The existing variation in morphological traits observed among the varieties of domesticated groundnut and different landraces are perhaps due to mutation, selection and recombination in those small numbers of genes selected in the beginning.

OPF 8

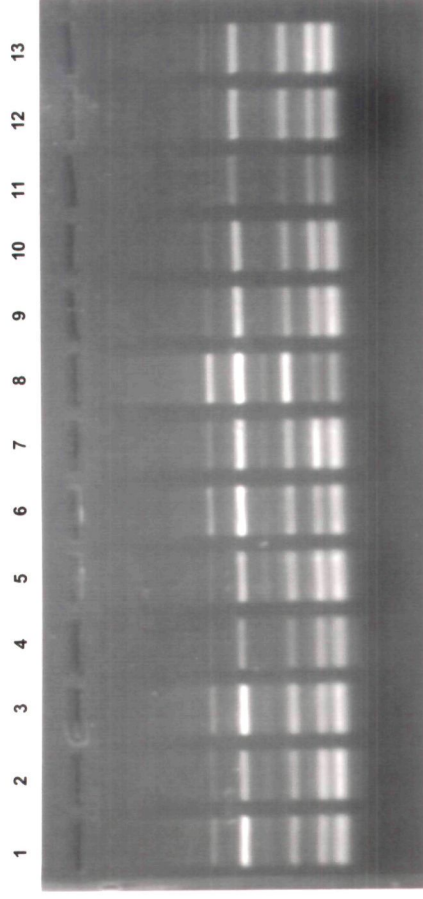


OPF 9

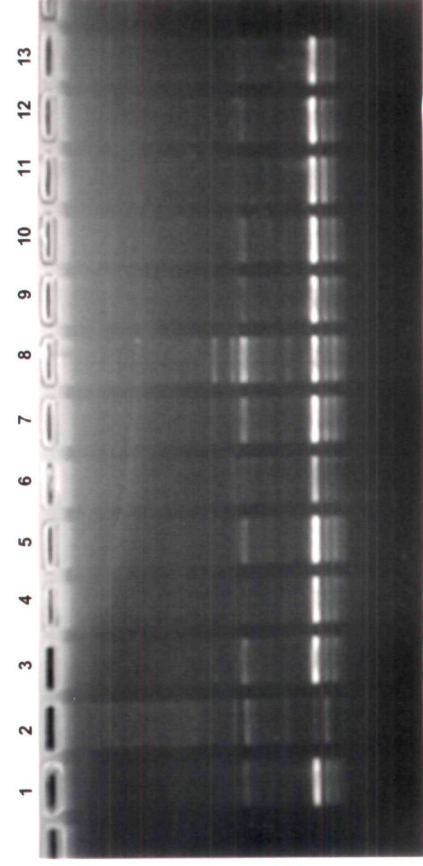


1. TG 26
2. TG 49
3. ICGV 87165
4. ICGV 86699
5. TMV 2
6. ICGV 86590

OPF 15



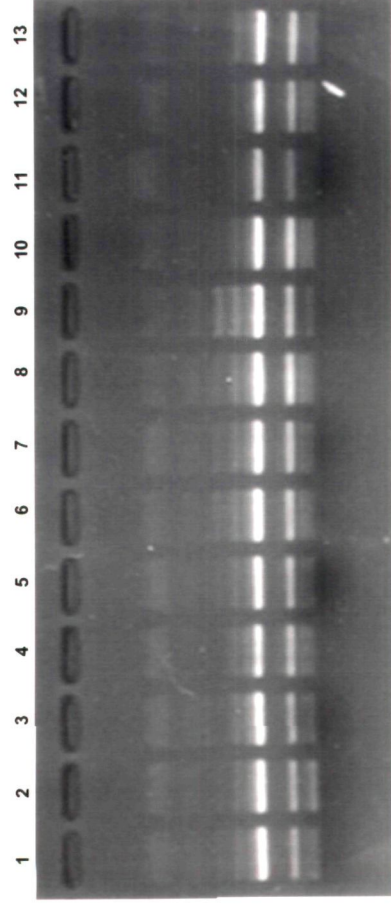
OPF 17



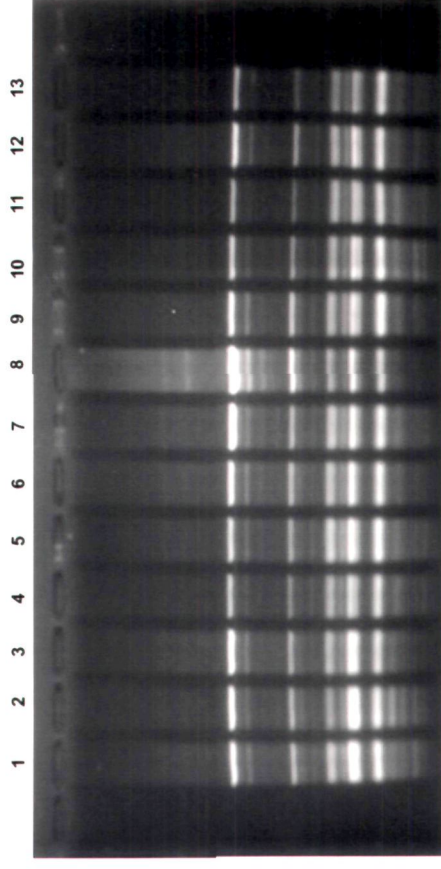
7. TAG 24
8. 28-2
9. JL 24
10. ICGV 86031
11. NC Ac 343
12. GPBD 4
13. R 9227

Plate 11a: RAPD pattern in selected groundnut genotypes

OPJ7

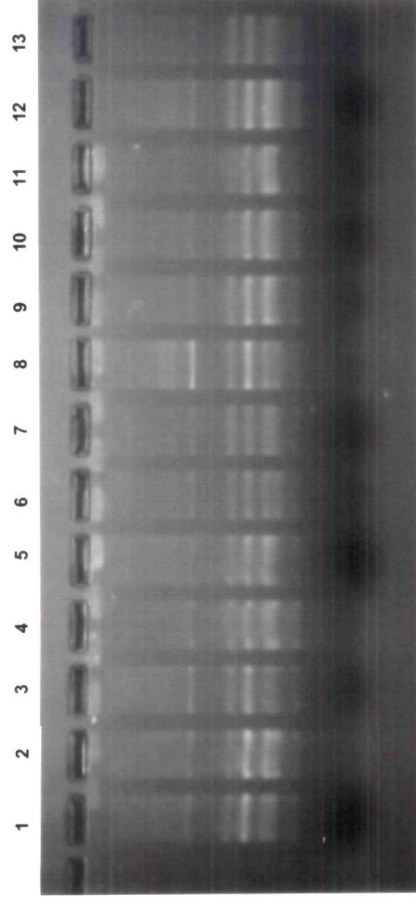


OPJ10

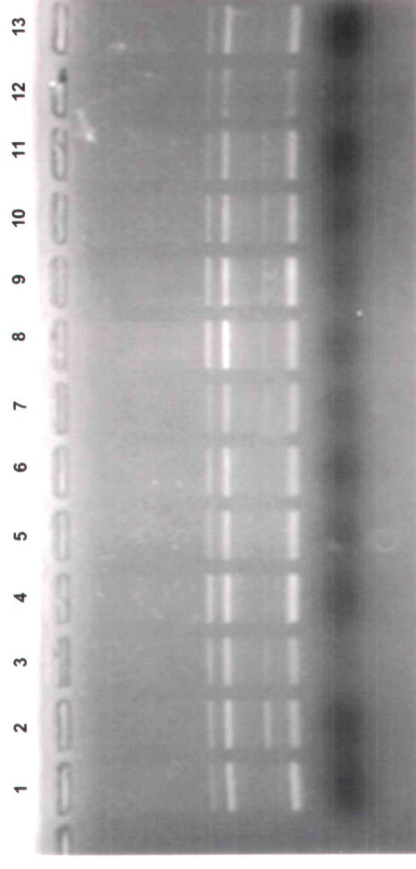


1. TG 26
2. TG 49
3. ICGV 87165
4. ICGV 86699
5. TMV 2
6. ICGV 86590

OPJ14



OPJ19



7. TAG 24
8. 28-2
9. JL 24
10. ICGV 86031
11. NC Ac 343
12. GPBD 4
13. R 9227

Plate 11b: RAPD pattern in selected groundnut genotypes

The Dice similarity coefficients (S_{ij}) ranged from 0.88 to 0.99 indicating less diverse nature of the selected genotypes. The S_{ij} value was minimum between 28-2 and NC Ac and 28-2 and R 9227 indicating low diversity among them. The resistant genotypes (NC Ac 343, mutant 28-2 and R 9227) were relatively distinct from the rest of the genotypes at S_{ij} value of 0.95. However, the mutant 28-2 was distinctly clustered from the other two at similarity coefficient of 0.91. The maximum S_{ij} (0.99) between TG 26 and TG 49 indicated high similarity. Incidentally, TG 26 is one of the parents of TG 49 and both were bred at BARC, Trombay. Similarly, ICGV 86699 and ICGV 86031 clustered together at S_{ij} value of 0.98 and were bred at North Carolina, USA (Table 34 and Fig.3). The resistant genotypes (28-2 or R 9227 or NC Ac 343) may be crossed with any of the susceptible genotypes in the above clusters in order to develop mapping populations for identification of DNA markers linked with *Spodoptera* resistance. However, more primers have to be screened in future for the better understanding of the molecular diversity.

One of the main problem in insect resistance breeding is the requirement of an accurate phenotypic assay for selecting resistant types in successive generations of segregating progenies. Evaluation of natural infestations in breeding nurseries has proved to be time consuming and labor intensive. The consistency is complicated by the effects of local environment and plant maturity besides migratory nature of the insects results in non-uniform infestation. Additionally, insect resistance is complex, inherited quantitatively and associated with poor agronomic features making full introgression difficult due to linkage drag. Identification of quantitative trait loci (QTLs) contributing to insect resistance can overcome these problems and enhance the efficiency and precision of resistance breeding. But the development of the QTLs demand large number of polymorphic markers. The large variation detected by SSRs and AFLPs in cultivated groundnut (He and Prakash, 1997 and Hopkins *et al.*, 1999) could be exploited for this purpose. The identification of genetic markers linked to pest resistance in groundnut will eventually improve the speed and precision of gene introgression.

Based on information available and results of the present study, following future line of work is be proposed to develop agronomically preferred cultivars with resistance to pest.

1. To facilitate the design of breeding programs to develop cultivars resistant to *Spodoptera* it would be beneficial to understand more completely the mode of inheritance of resistance.
2. Molecular markers associated with pest resistant QTLs should be identified so that the breeding for resistance to insect pest becomes easy, quick and efficient.
3. The superior lines with the enhanced resistance need to be analyzed in detail through growth and developmental assay of the insect to know the exact component responsible for increased resistance.
4. As revealed by growth and development of *Spodoptera*, antibiosis is the mechanism of resistance operating in the resistant cultivars. The role of the specific chemicals has to be discerned for better understanding of resistance and increased control in future breeding.
5. The promising multiple pest and disease resistant lines with superior and stable yield have to evaluated in large scale trials for further exploitation.
6. The identified superior segregates possessing resistance to different biotic stresses could be crossed in a complimentary fashion to generate improved multiple pest and disease resistant germplasm.
7. Systematic efforts should be made to exploit the high level of resistance found in wild species for enhancing resistance to *Spodoptera* in *Arachis hypogaea*.

Summary

VI. SUMMARY

The cultivated groundnut (*Arachis hypogaea*) is one of the most important vegetable oil seed crops in the world. Though India is a leading producer, the productivity is very low as compared to other major producers viz., USA and China. The low productivity has been mainly attributed to biotic and abiotic stresses affecting the crop. Among biotic stresses, *Spodoptera litura* is known to cause yield loss up to 70 per cent. Insecticides can control these pests, but the much safer, effective and economical strategy would be to cultivate resistant varieties.

Spanish bunch cultivars are most popular in India, as they mature early and facilitate double cropping under rainfed conditions besides possessing desirable pod and kernel features. However they are highly susceptible to *Spodoptera litura*. Research efforts have been successful in identifying resistant germplasm but the level of resistance is not very high. Most of the resistant germplasm possess undesirable attributes like low yield, low shelling outturn, other poor pod and kernel attributes and susceptibility to diseases.

Therefore, the present investigation was envisaged to screen and evaluate groundnut genotypes against *Spodoptera*, enhance resistance and combine it with other desirable attributes through hybridization and understand the biochemical basis of resistance besides assessing the molecular diversity in selected groundnut genotypes.

Status of germplasm for resistance to *Spodoptera litura*

Groundnut germplasm comprising inter-specific derivatives, advanced breeding lines and mutants along with popular cultivars were screened for *Spodoptera* resistance under conditions of high pest incidence.

Significant variability existed in the germplasm for *Spodoptera* resistance. High heritability combined with moderate genetic advance

indicated scope for effective selection. All the popular cultivars except TAG 24 and ICGV 86590 suffered heavy defoliation. The mutants 28-2, 45 and 110 were the good source of resistance as they matured early and recorded least damage. Among the breeding lines, ICGV 91180, NC Ac 343, ICGV 86031 and R 9214 were highly resistant but possessed undesirable agronomic features. The low damage in highly determinant TAG 24 could be due to non-availability of fresh foliage at the period of peak pest activity.

Standardization of a new criterion for large-scale screening

To overcome the inherent problems of field screening, a modified version of the technique used in soybean for screening against Mexican bean beetle was evaluated for its suitability to groundnut against *Spodoptera*. The technique was based on the gain in weight of the larvae fed on different genotypes.

Fully opened leaves from the top to bottom (I, II and III) were used separately for feeding pre starved (24 hours) *Spodoptera* larvae of III, IV and V instars. The combination of II leaf with III instar larvae differentiated the genotypes more clearly as compared to other combinations. The new technique could be conveniently used for rapid screening of germplasm with less labour and resources. The problem of non-uniform feeding often observed in the field experiments could also be avoided leading to greater precision in screening.

Evaluation of Selected groundnut genotypes

To evaluate the laboratory technique, 13 selected genotypes were assessed for resistance to *Spodoptera* vis-à-vis their performance in the field.

The gain in weight by larvae showed finer gradation of resistance among the genotypes compared to field damage revealing its usefulness in differentiating the genotypes. The strong positive correlation between gain in weight (GIW) by larvae and damage due to *Spodoptera* (DS) indicated the

possibility of using GIW in lieu of DS. All the popular cultivars viz., JL 24, TMV 2, TG 26, TG 49 and GPBD 4 were susceptible to *Spodoptera*. Based on both DS and GIW, the genotypes NC Ac 343 and mutant 28-2 were found superior. The genotypes R 9227 and ICGV 86031 were resistant based on only GIW. The genotype TAG 24 showed less damage in the field in spite of recording high gain in weight in laboratory, indicating that its superiority was not due to true resistance.

Effect of groundnut genotypes on growth and development of *Spodoptera litura* (F.)

To study the mechanism of resistance in detail, *Spodoptera litura* (F.) was reared on the selected resistant (NC Ac 343, Mutant 28-2, R 9227 and ICGV 86031) and susceptible (JL 24, TAG 24 and GPBD 4) genotypes.

The resistant genotypes NC Ac 343, mutant 28-2 and R 9227 recorded high larval mortality and reduced larval weight at different stages. The mortality was high in early stages indicating vulnerability of neonate larvae. Despite low mortality, the larval weight was significantly affected by ICGV 86031 at all the stages. Though pupal development, moth emergence and fecundity were affected in all the resistant genotypes, the effect was most severe in mutant 28-2. The effects of these genotypes on the growth and development of *S. litura* could obviously be due to chemical factors (i.e. antibiosis). The differential response of resistant genotypes on various components of insect growth and development could be due to different resistance factors present in these genotypes.

Though TAG 24 consistently suffered low damage in the field but the insect growth and development in the laboratory was normal and comparable to the susceptible cultivars. This response could be due factors other than inherent resistance i.e., seasonal avoidance.

Enhancement of resistance through hybridization

The cultivated varieties are susceptible to *Spodoptera* and suffer yield loss under infestation. The identified resistant germplasm either possess undesirable agronomic attributes or the extent of resistance is low. To combine high level of resistance with desirable agronomic features, hybridization was attempted between the resistant (mutant 28-2, NC Ac 343 and R 9227) and susceptible (GPBD 4 and TAG 24) genotypes. Four eighty one F_{2:3} progenies from eight crosses were evaluated along with parents for resistance to *Spodoptera*, thrips and late leaf spot, besides productivity.

Significant variation existed for either DS or GIW or both in all the crosses. The components of variation revealed its heritable nature and scope for selection. However, GIW exhibited higher genetic advance due to high heritability and magnitude of variation. Weak association between GIW and DS observed in different crosses could be due to different components/aspects of resistance operating in field and laboratory. Hence, both the parameters (DS and GIW) could be used together for assessing resistance.

Based on the parental performance, progenies recording less than 30 per cent of defoliation in the field and showing less than 100 per cent gain in weight in laboratory were considered superior for resistance. Only 15.8 per cent progenies were superior and most of them were from the crosses involving either R 9227 or TAG 24 as ovule parents. Among the crosses TAG 24 X NC Ac 343 and TAG 24 X R 9227 were found most potential for both DS and GIW and were even superior to the resistant X resistant (28-2 X R 9227, 28-2 X NC Ac 343 and R 9227 X NC Ac 343) crosses. The crosses involving resistant parent, mutant 28-2, produced very low frequency of resistant segregates indicating its poor combining ability. These responses indicate a need for progeny evaluation rather than parental phenotype as a sole criterion in selecting the parents for hybridization.

The progenies in all the crosses exhibited continuous and unimodal distribution for resistance revealing that it is quantitative in nature and may not be amenable for classical Mendelian analysis. Occurrence of susceptible segregates in resistant X resistant crosses and higher frequency of resistant types in susceptible X resistant crosses indicated the existence of different factors and predominance of gene interactions in determining resistance to *S. litura*.

When productivity of resistant segregates was considered, 43 progenies combined superior yield (comparable to the best parent, GPBD 4) and majority of them were from the crosses R 9227 X GPBD 4, TAG 24 X NC Ac 343 and TAG 24 X R 9227. Few lines combined resistance to thrips and they were mostly from R 9227 X NC Ac 343 and TAG 24 X R 9227. Some lines from the crosses involving either 28-2 or GPBD 4 as one of the parents, were also resistant to late leaf spot. Only one progeny combined resistance to all the three biotic stresses but was low yielding. This indicated that the resistance for different stresses and productivity were distributed among different crosses and progenies, which could be combined in future through intermating and multiple crossing.

Evaluation of superior segregates

Based on the yield performance, agronomic attributes and the availability of seeds, forty-three progenies were again assessed for *Spodoptera* resistance using gain in weight by the larvae in laboratory. Majority of the lines recorded a similar response as in the first assessment, reconfirming their resistance.

Five progenies, RN 3-17, RN 3-26, RN 4-4, RD 1-8 and TN 1-20 were superior to the best resistant parent, NC Ac 343 in resistance to *Spodoptera* based on both the parameters. The progeny RD 1-8 also combined resistance to late leaf spot with yield level comparable to the highest yielding parent, GPBD-4 and stable over seasons. The segregate MR 1-7 combined resistance to *Spodoptera*, thrips and late leaf spot along

with high yield indicating the multiple pest and disease resistant nature. The progenies TR 1-22, TR 2-4, TR 2-5 MR 1-18-1 and TR 1-19-1 were comparable to either mutant 28-2 or R 9227 for *Spodoptera* resistance and their performance was stable across seasons for yield and comparable to best parent GPBD 4 indicating the improvement in other features besides retaining *Spodoptera* resistance.

However, all these lines suffered from low shelling out turn, hence they have to be improved further and subjected to detailed analysis before exploiting them in breeding programmes or for registering them as resistant germplasm.

Biochemical basis of *Spodoptera* resistance

Biology of *S. litura* on selected genotypes confirmed the mechanisms of resistance as antibiosis. Hence, thirteen groundnut genotypes possessing various levels of resistance to *Spodoptera* were assayed for trypsin inhibitors and lectin activity.

The trypsin inhibitor activity could not be detected in these genotypes indicating that it does not have any role to play in imparting resistance among these genotypes. However, wide range of lectin activity was detected among genotypes, but the activity could not be associated with resistance. The maximum activity was found in resistant genotype R 9227 and minimum was found in TAG 24. The progenies of this cross showed lack of association with resistance. Hence, there is a need to explore other groups of specific chemicals to understand the mechanism of resistance.

Molecular diversity in groundnut genotypes

Knowledge of genetic diversity in a crop species is fundamental to its improvement. In groundnut, the variability present for resistance to *Spodoptera* is low, and it is continuous in nature and difficult to manipulate necessitating the application of modern biotechnological tools

to enhance the efficiency of resistance breeding. Therefore, RAPD assay was carried out using 35 primers to determine genetic diversity among thirteen selected groundnut genotypes with varying levels of resistance for better utilization of genetic resources.

RAPD assay indicated polymorphic difference of two to ten bands with an average of two bands per primer (28.63 %). The S_{ij} values ranged from 0.88 to 0.99 indicating the limited variability present among these genotypes. The two genotypes TG 26 and TG 49 formed one cluster, while ICGV 86699 and ICGV 86031 formed another cluster. The resistant genotypes (NC Ac 343, mutant 28-2 and R 9227) were relatively distinct from rest of the genotypes at S_{ij} value of 0.95. However, 28-2 was distinctly clustered from the other two at similarity coefficient of 0.91. In future the resistant genotypes (28-2 or R 9227 or NC Ac 343) may be crossed with any of the susceptible genotypes in the above clusters in order to develop mapping populations for identification of DNA markers linked with *Spodoptera* resistance. But there is a need to screen large number of primers or look for other markers like SSRs or AFLPs in order to explore the QTLs contributing to resistance.

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Appendices

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Appendix I: Mean monthly data at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

	January	February	March	April	May	June	July	August	September	October	November	December	Annual
1998													
Maximum (°C)	30.5	32.1	35.7	38.3	35.8	31.0	27.0	27.7	28.2	28.6	29.6	28.6	31.1
Minimum (°C)	15.3	16.3	19.0	22.1	21.8	21.8	21.2	21.3	20.9	20.1	17.1	14.0	19.2
Relative humidity (%)	73	70	64	58	62	80	86	87	91	93	75	72	75.9
Rainfall (mm)	-	-	-	-	33.2	2224	825	51.4	214.4	98.1	40.2	-	290.5
1999													
Maximum (°C)	29.4	32.8	36.2	36.6	32.2	28.0	26.4	27.1	28.3	28.9	29.6	28.9	30.4
Minimum (°C)	12.4	16.9	20.2	21.1	21.3	21.0	20.8	20.4	20.0	19.8	16.0	13.4	18.6
Relative humidity (%)	75	63	63	65	75	85	89	85	83	79	59	51	73
Rainfall (mm)	0.0	0.0	0.0	14.7	32.8	71.8	113.9	19.7	8.8	161.1	0.0	0.0	422.8
2000													
Maximum (°C)	30.6	32.2	35.2	37.3	33.9	29.0	26.8	27.2	29.0	29.5	30.4	28.3	30.8
Minimum (°C)	15.1	15.7	18.5	21.5	21.1	21.3	20.4	20.2	20.4	20.2	16.9	13.4	18.7
Relative humidity (%)	48.0	52.0	47.0	57.0	67.0	79.0	81.0	81.0	76.0	77.0	63.0	58.0	65.0
Rainfall (mm)	0.0	0.0	0.0	44.1	44.1	50.7	125.2	50.4	32.1	88.3	0.0	3.0	539.2
2001													
Maximum (°C)	29.9	34.0	35.3	35.7	34.8	30.3	26.8	27.2	30.1	30.1	31.0	29.6	31.2
Minimum (°C)	15.0	16.8	18.5	22.0	21.5	21.3	21.1	20.9	20.2	19.9	17.9	13.7	19.1
Relative humidity (%)	55.0	50.0	45.0	55.0	59.0	75.0	81.0	81.0	72.0	66.0	55.0	55.0	62.0
Rainfall (mm)	0.0	0.0	0.0	52.1	23.2	32.5	33.1	58.1	53.6	17.0	0.0	Tr	269.6
2002													
Maximum (°C)	30.1	31.9	36.0	37.2	34.9	29.6	28.4	26.6	29.8	30.7	30.5	30.2	31.3
Minimum (°C)	14.5	17.9	20.0	21.7	23.0	21.9	21.3	20.5	20.0	20.4	17.1	14.4	19.4
Relative humidity (%)	55.0	50.0	49.0	53.0	62.0	80.0	79.0	82.0	73.0	67.0	62.0	47.0	63.0
Rainfall (mm)	0.0	62.7	0.0	67.0	57.9	60.5	15.0	48.1	6.6	103.6	7.0	0.0	428.4

Appendix II : Mean performance of the F₃ progenies of different crosses for pest resistance, disease resistance and productivity parameters in different crosses

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
28-2 X R9227							
28-2	124	22	2	3	14.4	68	54
1-2	170	31	2 *	4 *	19.5 *	66	60 *
1-4	96 *	34	2 *	5 *	17.6 *	62	43
1-7	138	35	3	5 *	18.3 *	63	57 *
1-8	126	34	2 *	3 *	16.6 *	67	57 *
1-12	123	34	4	3 *	20.4 *	72	49
1-14	115	41	4	4 *	15.1	72	60 *
1-16	173	49	2 *	4 *	23.2 *	65	57 *
1-20	137	29 *	2 *	3 *	19.4 *	70	56 *
1-1	120	42	4	2 *	9.7	53	58 *
1-5	131	60	1 *	4 *	24.2 *	61	51
1-6	95 *	46	3	3 *	16.6 *	72	54
1-9	228	42	2 *	4 *	17.6 *	72	42
1-10	74 *	63	1 *	2 *	22.0 *	72	60 *
1-11	152	27 *	1 *	4 *	15.0	71	48
1-13	174	33	3	5 *	19.0 *	61	58 *
1-15	188	23 *	2 *	2 *	11.3	71	66 *
1-17	90 *	37	2 *	3 *	18.2 *	64	59 *
1-18	81 *	52	1 *	3 *	15.6	65	57 *
2-1	89 *	37	2 *	4 *	13.8	70	57 *
2-2	77 *	39	3	4 *	15.8 *	70	51
2-4	117	40	3	4 *	23.0 *	68	54
2-5	164	31	3	4 *	24.8 *	68	60 *
2-6	87 *	23 *	3	4 *	8.8	69	49
2-7	96 *	23 *	2 *	5 *	9.5	69	45
2-8	139	30	2 *	6	14.9	71	53
2-9	140	40	3	7	25.1 *	70	46
2-10	189	38	4	7	19.5 *	69	61 *
2-11	190	43	3	7	20.9 *	70	55 *
2-12	156	43	4	7	12.0	71	52
2-13	155	43	4	5 *	18.2 *	63	50
2-16	111	41	4	6	20.9 *	72	49
2-17	88 *	36	4	7	17.0 *	72	54
2-18	129	21 *	4	7	16.6 *	71	56 *
2-19	219	20 *	3	7	11.7	71	51
2-20	109	40	3	7	17.0 *	72	46
2-21	72 *	29 *	3	7	11.1	60	46
2-22	86 *	35	4	7	13.9	68	58 *
2-23	132	34	3	8	15.2	73 *	59 *
2-24	109	35	3	7	13.5	68	60 *
2-25	189	42	3	6	17.3 *	66	60 *
2-26	175	31	3	7	15.1	73 *	50

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
2-27	128	45	2 *	6	18.1 *	66	62 *
2-28	96 *	36	4	7	16.4 *	74 *	48
2-29	90 *	51	4	6	22.6 *	73 *	48
2-30	65 *	35	3	5 *	15.8 *	74 *	54
2-31	106	41	4	7	18.2 *	68	54
2-32	78 *	52	3	7	16.3 *	73 *	44
2-33	44 *	52	3	6	14.3	70	62
2-34	132	43	3	7	9.7	68	44
2-35	203	32	2 *	7	12.7	68	42
2-36	93 *	39	3	5 *	15.5	71	53
2-37	77 *	47	3	7	14.3	73 *	58 *
2-39	219	56	2 *	7	11.9	72	53
2-40	171	44	3	7	16.9 *	76 *	58 *
2-41	153	30	4	7	19.1 *	73 *	45
2-42	171	29 *	3	8	18.6 *	73 *	55
2-43	144	34	3	8	17.4 *	70	45
2-44	204	57	4	8	15.9 *	65	47
2-45	80 *	48	3	8	13.1	68	51
2-46	139	37	4	8	17.6 *	74 *	55 *
2-46a	77 *	36	4	7	18.5 *	72	50
2-47	74 *	39	4	7	18.5 *	70	44
2-48	65 *	37	2 *	6	13.4	70	47
2-49	66 *	41	4	6	22.2 *	71	50
2-50	38 *	32	4	6	21.4 *	68	57 *
2-51	133	19 *	2 *	5 *	12.9	71	49
2-52	80 *	45	3	8	13.5	71	60 *
2-53	38 *	22 *	3	8	7.5	69	32
2-54	96 *	44	4	5 *	17.1 *	69	67 *
2-55	77 *	37	4	5 *	16.1 *	71	62 *
2-56	45 *	32	4	6	20.0 *	68	51
2-57	63 *	39	4	6	12.9	75	50
2-58	69 *	46	4	7	12.7	69	55 *
2-59	64 *	41	4	5 *	12.4	72	58 *
2-60	28 *	40	4	5 *	16.2 *	75	48
3-2	79 *	41	4	5 *	15.9 *	71	60 *
3-3	43 *	62	4	6	21.2 *	69	48
3-4	93 *	33	5	4 *	14.9	72	48
3-5	63 *	44	4	5 *	17.7 *	72	51
3-6	150	29 *	4	5 *	17.3 *	65	43
3-7	227	33	4	4 *	11.7	66	49
3-8	191	29 *	4	4 *	9.2	63	53
3-9	263	36	4	5 *	15.8 *	62	50
3-10	178	39	4	5 *	11.5	72	41
3-11	202	32	3	5 *	20.9 *	72	51
3-12	190	46	4	5 *	24.5 *	66	45
3-13	122	31	4	4 *	17.3 *	64	53

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
3-14	168	66	2 *	5 *	17.0 *	71	47
3-15	193	37	4	6	23.2 *	68	54
2-3	197	28 *	4	2 *	8.6	61	62 *
2-15	221	22 *	2 *	2 *	15.5	57	47
2-38	200	39	4	3 *	18.8 *	66	57 *
R 9227	117	29	4	7	11.8	64	43
GM	125.87	37.69	2.96	5.25	16.38	68.76	52.62
S.Em ±	38.55	5.5	0.72	0.99	4.51	2.97	4.03
CV(%)	30.62	14.59	24.25 *	18.88	27.57	4.32	7.66
28-2 X NC Ac 343							
28-2	124	22	2	3	14.4	68	54
1-1	247	26 *	2 *	4 *	8.2	66	55 *
1-2	151	33	3	5 *	14.7	73 *	66 *
1-3	262	38	3	5 *	18.7 *	72	67 *
1-4	252	30	3	4 *	17.9 *	63	69 *
1-5	233	42	3	4 *	12.5	76 *	71 *
1-6	183	29 *	3	3 *	21.9 *	62	57 *
1-7	166	34	3	4 *	16.8 *	72	57 *
1-8	203	17 *	2 *	4 *	14.5	57	61 *
1-9	194	38	3	4 *	14.5	72	60 *
1-10	271	46	4	6	26.5 *	68	60 *
1-11	257	47	5	4 *	23.7 *	50	58 *
1-12	211	51	4	5 *	19.7 *	66	54
1-13	231	40	4	6	13.0	46	59 *
1-14	178	41	4	5 *	21.3 *	70	70 *
1-15	210	38	3	4 *	21.7 *	63	54
1-16	150	57	3	6	11.8	56	47
1-17	191	35	4	5 *	18.4 *	73 *	60 *
1-18	91 *	33	3	5 *	25.3 *	68	51
1-19	110	43	4	5 *	21.8 *	67	43
1-21	94 *	51	3	6	26.9 *	69	60 *
1-22	90 *	26 *	3	4 *	15.3	65	55 *
1-24	105	32	3	4 *	22.3 *	70	65 *
1-25	173	38	2 *	6	15.3	73 *	61 *
1-26	136	36	3	3 *	16.4 *	68	70 *
1-27	207	35	3	5 *	21.1 *	71	50 *
1-28	77 *	34	3	5 *	26.4 *	66	63 *
1-29	144	40	4	4 *	27.0 *	69	48
1-30	163	48	2 *	3 *	13.8	67	59 *
1-31	148	45	1 *	4 *	19.3 *	72	49
1-33	204	42	2 *	6	15.8 *	72	68 *
1-34	37 *	54	3	5 *	15.4	67	68 *
1-35	100	52	2 *	2 *	15.3	69	67 *
1-36	44 *	50	2 *	6	14.5	74 *	53
1-38	64 *	45	3	5 *	21.9 *	72	48
1-39	77 *	47	4	4 *	20.4	72	60 *

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
1-40	262	55	5	3 *	21.7*	69	55 *
NC Ac 343	88	18	4	5	6.7	59	36
GM	161.02	38.94	2.82	4.34	18.21	67.18	58.26
S.Em ±	36.3	11.1	0.73	1.24	6.24	4.63	5.83
CV(%)	22.4	28.52	25.92	28.74	34.3	6.89	10.02
28-2 X GPBD 4							
28-2	124	22	2	3	14.4	68	54
1-1	187	65	5	5 *	25.2 *	68	62 *
1-2	72 *	49	5	4 *	26.9 *	66	45
1-3	137	46	4	5 *	25.1 *	73 *	51
1-4	127	54	4	4 *	30.9 *	73 *	65 *
1-5	300	74	4	5 *	21.5 *	63	44
1-6	207	41	4	4 *	12.8	71	50
1-8	178	59	4	5 *	25.3 *	70	64 *
1-9	179	46	4	4 *	27.8 *	72	61 *
1-10	210	41	4	5 *	18.1 *	71	47
1-11	564	60	5	5 *	18.8 *	70	45
1-12	307	47	5	5 *	20.4 *	70	67 *
1-13	201	43	5	6	18.7 *	73 *	51
1-14	202	48	5	5 *	20.4 *	69	57 *
1-15	272	53	5	4 *	18.6 *	68	51
1-16	249	47	4	4 *	26.0 *	72	61 *
1-17	388	56	4	5 *	22.9 *	68	24
1-18	272	55	5	4 *	20.8 *	74 *	47
1-19	117	56	4	3 *	13.8	77 *	50
1-20	129	44	5	4 *	17.5 *	69	44
1-21	114	49	5	4 *	31.1 *	69	45
1-22	84 *	62	5	5 *	18.2 *	68	42
1-23	101	43	4	4 *	22.1 *	69	56 *
1-27	70 *	33	4	3 *	16.1 *	68	49
1-29	173	59	5	5 *	12.4	73 *	46
1-30	97	54	5	5 *	18.6 *	75 *	48
1-31	53 *	50	4	6	23.0 *	75 *	54
1-32	79 *	47	5	4 *	23.6 *	71	55 *
1-33	107	59	5	5 *	22.3 *	71	49
1-34	148	58	4	4 *	21.1 *	71	43
1-37	156	62	5	5 *	19.3 *	74 *	38
1-36	172	48	5	6	38.7 *	70	58 *
1-7	132	41	5	6	28.9 *	66	57 *
GPBD 4	207	44	4	2	15.8	73	37
GM	179.57	50.20	4.26	4.39	21.64	70.51	50.55
S.Em ±	57.24	8.00	0.73	0.63	4.58	347.00	6.57
CV(%)	31.87	15.93	17.34	14.47	21.20	4.92	13.00

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
R 9227 X NC Ac 343							
R 9227	116	29	4	7	11.8	64	42
1-3	112	55	3	7	12.5	75	62 *
1-5	95 *	53	3	7	22.7 *	72	67 *
1-6	149	44	4	7	31.6 *	73 *	68 *
1-7	72 *	47	3	7	18.0 *	69	57 *
1-8	122	52	3	7	17.5 *	69	56 *
1-9	97 *	40	4	7	14.7	77 *	62 *
2-2	97 *	27 *	3	7	14.4	37	65 *
2-3	127	35	3	7	8.7	66	64 *
2-4	78 *	24 *	3	7	17.6 *	71	47
2-5	98 *	35	3	7	22.7 *	73 *	54
2-6	56 *	38	3	8	10.7	70	52
2-8	42 *	42	4	7	18.6 *	66	59 *
2-10	89 *	42	3	8	17.0 *	56	60 *
2-11	111	58	4	7	15.0	74 *	60 *
2-12	120	37	3	7	26.0 *	69	62 *
2-15	58 *	52	3	6	8.5	67	56 *
2-16	83 *	38	3	7	9.7	74 *	61 *
2-17	80 *	45	4	7	21.9 *	66	49
2-19	61 *	29 *	3	7	25.1 *	71	55 *
2-27	63 *	43	3	6	17.1 *	73 *	51
2-30	31 *	38	4	6	14.8	74 *	54
2-31	35 *	33	3	4 *	17.2 *	75 *	47
2-33	67 *	41	4	6	20.1 *	75 *	62 *
2-34	17 *	47	2 *	6	15.7	76 *	58 *
2-35	35 *	48	4	7	22.1 *	71	53
2-36	43 *	40	3	6	13.1	71	53
2-37	32 *	31	3	8	10.4	72	41
2-38	113	33	3	8	24.0 *	75 *	61 *
2-40	80 *	43	4	8	18.9 *	68	57 *
2-41	79 *	45	4	6	17.6 *	70	67 *
2-43	97 *	55	4	6	12.3	69	68 *
1-1	77 *	52	2 *	6	21.0 *	31	50
1-2	52 *	27 *	4	5 *	25.0 *	76 *	60 *
2-1	55 *	42	5	6	17.5 *	54	66 *
2-9	237	47	2 *	5 *	19.0 *	66	69 *
2-13	36 *	42	2 *	6	27.0 *	35	39
2-14	153	38	3	6	27.0 *	54	45
2-20	200	39	3	6	21.4 *	68	54
2-21	177	42	4	6	23.7 *	63	53
2-22	172	19 *	5	6	18.1 *	59	54
2-23	167	34	4	6	16.7 *	56	56 *
2-24	103	29 *	4	6	16.0 *	57	56 *
2-25	57 *	25 *	4	6	19.6 *	48	47
2-26	79 *	49	4	6	21.5 *	68	43

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
2-28	48 *	39	3	6	21.7 *	46	63 *
2-32	98 *	31	5	3 *	16.5 *	78 *	69 *
2-42	116	53	4	3 *	21.5 *	70	67 *
3-2	122	45	4	6	12.4	67	69 *
3-3	42 *	33	4	8	21.5 *	67	43
3-4	73 *	43	4	7	20.0 *	75 *	50
3-5	186	47	4	6	13.9	69	57 *
3-6	212	33	2 *	8	16.0 *	72	63 *
3-8	125	36	4	7	23.0 *	65	58 *
3-10	174	24 *	3	8	19.0 *	72	40
3-11	90 *	33	3	6	20.0 *	67	50
3-12	86 *	51	4	8	11.0	62	52
3-13	46 *	39	4	7	19.9 *	75 *	65 *
3-14	128	56	5	7	24.9 *	70	59 *
3-15	90 *	29 *	2 *	7	10.8	72	43
3-16	55 *	52	4	8	15.6	70	53
3-17	48 *	18 *	4	7	17.4 *	72	45
3-18	73 *	23 *	2 *	8	13.7	69	50
3-19	67 *	30	3	8	16.0 *	69	64 *
3-20	84 *	41	3	7	16.6 *	68	60 *
3-21	72 *	50	4	6	14.6	63	57 *
3-22	140	29 *	5	5 *	7.6	62	64 *
3-23	17 *	40	4	7	19.0 *	71	58 *
3-24	44 *	26 *	2 *	7	15.3	75 *	38
3-25	48 *	33	2 *	6	17.2 *	64	61 *
3-26	44 *	16 *	1 *	7	18.1 *	71	60 *
3-27	21 *	27 *	3	5 *	21.4 *	67	68 *
3-28	81 *	41	4	6	12.7	67	60 *
3-29	25 *	28 *	4	7	12.9	56	59 *
3-30	22 *	50	3	6	19.2 *	66	69 *
3-31	36 *	34	4	7	19.3 *	69	58 *
3-32	41 *	36	4	7	14.6	74 *	58 *
4-1	53 *	41	4	6	22.7 *	70	43
4-2	74 *	44	4	5 *	24.5 *	68	46
4-3	88 *	27 *	4	6	19.0 *	75 *	42
4-4	65 *	18 *	5	7	10.6	64	54
4-5	156	21 *	4	7	12.2	72	47
4-6	179	36	2 *	6	11.2	74 *	43
4-7	175	33	4	7	20.5 *	77 *	57 *
4-8	144	49	3	5 *	10.2	69	54
4-9	108	41	4	7	13.3	71	66 *
4-10	152	36	5	6	19.8 *	74 *	49
4-11	158	26 *	4	4 *	13.1	68	46
4-12	146	40	4	4 *	21.1 *	68	56 *
4-14	238	22 *	5	4 *	18.5 *	67	48
4-15	122	15 *	3	7	9.5	73 *	50

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
4-16	136	30	4	6	15.0	71	53
4-17	200	41	4	5 *	20.2 *	71	58 *
4-18	89 *	41	3	7	7.7	69	48
4-19	170	44	4	6	20.3 *	68	58 *
4-20	117	17 *	4	7	15.0	70	42
4-21	90 *	44	4	5 *	20.3 *	73 *	54
4-23	180	29 *	4	5 *	30.0 *	62	53
4-24	62 *	35	4	6	12.8	72	59 *
4-25	96 *	25 *	3	6	15.8 *	71	49
4-26	196	35	4	6	22.8 *	64	69 *
4-27	150	44	4	6	9.3	66	46
4-28	94 *	34	4	7	19.6 *	74 *	49 *
4-29	59 *	33	2 *	6	8.4	74 *	52
4-30	116	32	3	6	13.3	72	58 *
4-31	195	27 *	4	6	17.3 *	68	34
4-32	261	25 *	4	5 *	7.2	70	57 *
4-33	84 *	39	4	6	29.5 *	72	66 *
4-34	163	35	4	6	15.5	73 *	68 *
4-35	177	33	4	6	16.9 *	69	56 *
4-36	145	39	4	6	20.5 *	71	57 *
4-38	113	43	4	6	31.2 *	70	66 *
4-39	87 *	31	3	6	32.3 *	75 *	68 *
4-40	148	33	4	6	14.8	72	47
4-41	126	38	4	6	21.5 *	74 *	61 *
4-43	165	32	5	7	23.3 *	74 *	44
4-44	91 *	27 *	4	6	6.6	74 *	50
4-45	64 *	36	4	7	8.8	73 *	61 *
5-1	152	49	5	6	29.1 *	78 *	69 *
5-2	141	26 *	3	8	15.6	76 *	53
5-3	127	37	3	7	20.2 *	77 *	69 *
5-5	121	36	4	6	7.3	69	61 *
5-6	105	30	5	7	10.4	71	45
5-7	125	29 *	3	7	14.7	72	54
5-8	51 *	40	4	7	20.0 *	73 *	57 *
6-1	37 *	46	3	5 *	13.3	61	51
6-2	94 *	36	4	7	15.9 *	71	66 *
6-3	119	39	5	7	18.5 *	74 *	50
6-4	79 *	38	4	7	20.6 *	75 *	52
6-5	83 *	41	4	6	16.6 *	67	65 *
6-6	88 *	29 *	4	7	16.7 *	71	60 *
6-7	115	39	3	7	20.3 *	74 *	65 *
6-8	109	25 *	3	7	18.6 *	77 *	66 *
6-10	52 *	32	3	7	12.1	75 *	51
6-11	66 *	33	4	7	19.0 *	72	54
6-12	57 *	30	3	5 *	12.3	71	41
6-13	72 *	36	4	7	17.3 *	68	56 *

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
NC Ac 343	88	18	4	5	6.7	59	36
GM	101.5	36.7	3.3	6.6	17.90	68.9	55.49
S.Em ±	22.7	7.7	0.7	0.8	5.77	4.3	3.31
CV(%)	22.4	20.9	19.7	11.5	32.24	6.2	5.90
R 9227 X GPBD 4							
R 9227	116	29	4	7	11.8	64	42
1-1	241	39	5	5 *	14.6	74 *	39
1-2	90 *	38	5	7	12.8	60	32
1-3	168	34	5	6	18.4 *	75 *	43
1-4	90 *	34	4	6	14.3	75 *	42
1-5	241	29 *	4	6	28.0 *	76 *	42
1-6	102	31	5	6	17.2 *	77 *	43
1-7	76 *	34	4	6	16.1 *	74 *	43
1-9	73 *	37	4	6	13.6	75 *	44
1-11	49 *	29 *	5	7	11.3	74 *	44
1-14	37 *	36	5	6	16.7 *	73 *	41
1-15	21 *	34	4	4 *	13.7	76 *	38
1-16	131	35	4	6	19.5 *	72	41
1-17	78 *	36	4	8	17.1 *	74 *	55 *
1-21	109	27 *	4	8	8.0	73 *	37
1-23	43 *	38	4	6	15.1	73 *	34
1-24	24 *	39	3	6	13.8	73 *	39
1-26	37 *	33	4	6	17.7 *	72	44
1-27	56 *	32	5	7	17.3 *	73 *	38
1-28	139	38	4	7	15.8 *	74 *	41
1-8	50 *	18 *	4	4 *	12.0	66	56 *
1-10	94 *	31	5	7	13.3	72	52
1-13	110	32	4	6	23.6 *	71	43
1-18	99 *	32	4	6	19.8 *	79 *	48
1-19	81 *	28 *	5	5 *	25.6 *	74 *	39
1-20	74 *	31	5	6	18.0 *	74 *	64 *
1-22	84 *	25 *	3	4 *	11.3	63	34
1-25	59 *	26 *	5	6	24.0 *	71	47
2-1	117	31	3	7	12.5	72	44
2-2	172	30	3	6	17.6 *	66	40
2-3	133	28 *	5	6	9.2	75 *	35
2-4	156	30	4	7	21.1 *	76 *	43
2-5	134	39	4	6	9.3	77 *	42
2-6	102	32	4	7	12.0	80 *	40

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
2-7	24 *	31	3	9	15.3	76 *	45
2-8	137	36	4	8	19.4 *	73 *	43
2-9	105	30	5	6	16.4 *	78 *	50
2-12	90 *	28 *	4	7	13.4	76 *	42
2-13	111	31	5	4 *	15.1	72	38
2-14	150	35	4	6	19.4 *	69	46
2-15	53 *	35	4	7	13.8	73 *	40
2-16	164	32	5	6	19.4 *	67	52
2-17	129	31	4	7	16.9 *	71	39
2-18	130	29 *	4	7	22.7 *	72	42
2-19	104	34	4	7	27.2 *	77 *	46
2-20	25 *	30	4	7	12.0	66	36
2-21	60 *	30 *	4	7	18.7 *	74 *	44
2-22	55 *	26 *	5	7	16.3 *	73 *	30
2-23	98 *	27 *	4	6	16.6 *	72	46
2-24	214	30	4	8	14.7	69	37
2-25	157	25 *	4	6	21.0 *	76 *	45
2-26	185	30	5	6	16.2 *	75 *	34
2-27	84 *	27 *	4	6	16.0 *	79 *	36
2-30	172	25 *	4	8	18.7 *	73 *	42
2-28	284	34	4	6	16.5 *	74 *	39
GPBD 4	207	44	4	2	15.7	72	37
GM	110.72	31.93	4.02	6.38	17.16	73.28	42.75
S.Em $\pm$	27.28	4.39	0.51	0.81	3.80	3.73	5.13
CV(%)	24.64	13.74	12.71	12.72	22.24	5.10	12.00
TAG 24 X NC Ac 343							
TAG 24	300	34	4	8	12.9	66	49
1-2	153	19 *	2 *	5 *	21.6 *	65	47
1-3	194	22 *	3	6	10.7	74	56 *
1-5	68 *	32	5	6	21.0 *	61	54
1-6	169	21 *	4	8	10.8	67	40
1-7	198	24 *	3	7	16.3 *	67	53
1-8	114	24 *	3	6	20.5 *	55	61 *
1-9	203	23 *	3	7	14.3	56	39
1-10	101	26 *	3	7	21.3 *	65	58 *
1-11	170	23 *	4	6	12.5	71	53
1-15	100	21 *	4	7	14.0	61	59 *
1-16	89 *	28 *	3	8	11.0	65	47

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
1-17	167	23 *	4	8	20.8 *	60	54
1-18	133	23 *	4	9	10.8	73 *	54
1-21	148	24 *	4	7	9.0	71	47
1-22	202	23 *	4	7	08.1	71	64 *
2-1	180	30	3	6	15.7	64	47
2-6	174	29 *	3	8	24.4 *	72	56 *
3-2	209	30	4	8	17.5 *	66	52
3-3	139	61	4	8	20.4 *	68	51
3-4	152	44	4	7	32.9 *	64	66 *
3-5	156	16 *	4	6	6.7	62	34
4-1	151	22 *	4	7	19.3 *	72	63 *
4-2	128	26 *	4	7	20.3 *	64	47
4-4	155	14 *	4	6	16.0 *	72	51
4-5	173	24 *	3	7	15.6	73 *	43
4-7	162	31	3	6	9.1	74 *	43
4-8	183	39	4	6	18.1 *	68	49
5-1	141	38	3	7	17.3 *	71	43
5-4	203	33	3	7	14.4	64	45
1-1	137	11 *	2 *	6	10.7	58	52
1-4	187	34	3	7	15.2	79 *	57 *
1-12	168	42	3	7	16.6 *	61	55 *
1-13	111	29 *	4	7	21.6 *	75 *	51
1-14	175	22 *	3	7	27.0 *	67	53
1-20	79 *	17 *	5	7	17.8 *	69	69 *
1-23	85 *	18 *	5	6	10.5	73 *	58 *
1-24	100	27 *	3	8	9.4	75 *	45
2-2	67 *	24 *	4	6	11.4	73 *	63 *
2-3	54 *	31	3	5 *	15.2	69	65 *
2-4	81 *	23 *	2 *	6	12.7	64	49
2-5	139	27 *	3	6	34.4 *	66	48
3-1	77 *	24 *	3	5 *	16.5 *	71	42
4-3	90 *	30	3	7	21.7 *	67	48
4-6	150	25 *	4	6	13.5	67	25
5-2	96 *	21 *	4	7	7.6	61	52
5-5	50 *	42	4	6	18.7 *	67	48
5-6	75 *	20 *	3	6	12.4	69	65 *
5-7	131	21 *	4	6	20.4 *	56	56 *
6-1	95 *	19 *	4	4 *	25.0 *	43	25
6-2	66 *	22 *	5	6	12.4	63	49
6-4	30 *	23 *	5	6	11.5	58	47

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
6-5	73 *	32	4	7	22.2 *	69	68 *
7-1	104	32	4	8	20.0 *	77 *	58 *
7-2	89 *	35	4	7	14.6	67	52
7-3	57 *	39	4	8	17.3 *	67	50
7-4	125	30	4	7	18.0 *	66	58 *
7-5	68 *	29 *	4	7	17.3 *	69	53
7-6	88 *	28 *	3	7	22.8 *	74 *	53
7-7	32 *	29 *	4	6	8.5	77 *	54
7-8	95 *	29 *	3	7	24.8 *	66	59 *
7-9	105	29 *	3	7	20.0 *	73 *	60 *
7-11	71 *	50	4	6	14.0	67	50
7-12	25 *	20 *	4	7	16.0 *	70	57 *
NC Ac 343	88	18	4	5	6.7	59	36
GM	124.36	27.44	3.31	6.46	16.59	67.09	52.14
S.Em ±	24.89	3.67	0.51	0.93	4.12	4.98	5.79
CV(%)	20.01	13.39	15.53	14.34	24.86	7.43	11.12
TAG 24 X R 9227							
TAG 24	300	34	4	8	12.9	66	49
1-1	95 *	19 *	3	9	15.0	67	31
1-4	63 *	24 *	3	9	9.4	69	45
1-5	121	25 *	4	9	18.1 *	71	43
1-6	40 *	29 *	4	9	19.5 *	74 *	43
1-7	33 *	29 *	3	8	20.0 *	73 *	45
1-8	69 *	23 *	4	8	14.6	74 *	54
1-9	125	27 *	4	9	21.9 *	73 *	42
1-10	79 *	26 *	3	9	14.3	77 *	38
1-12	62 *	31	3	9	21.7 *	69	47
1-13	98 *	27 *	4	8	14.2	76 *	44
1-14	107	31	4	9	19.7 *	71	42
1-15	54 *	26 *	4	8	16.4 *	72	43
1-16	37 *	25 *	4	9	11.8	75 *	42
1-17	50 *	35	4	8	16.5 *	73 *	48
1-18	156	26 *	4	8	10.8	73 *	61 *
1-20	62 *	27 *	4	8	17.6 *	76 *	47
1-21	151	31	4	9	16.9 *	73 *	49
1-22	16 *	24 *	4	8	15.6	70	54
1-23	66 *	27 *	4	9	18.0 *	75 *	43
1-24	42 *	28 *	4	9	9.1	72	52
1-25	68 *	23 *	3	9	16.0 *	69	40

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
1-26	80 *	26 *	3	9	15.3	73 *	40
1-27	57 *	30	4	9	22.0 *	75 *	36
1-28	70 *	31	4	9	17.1 *	75 *	46
1-29	131	29 *	4	9	12.7	74 *	48
1-30	122	34	3	9	16.0 *	74 *	42
1-31	104	32	4	8	18.6 *	69	42
1-32	90 *	25 *	4	9	21.6 *	70	41
1-33	49 *	30	4	9	16.5 *	71	50
1-34	32 *	26 *	4	9	15.4	72	40
1-35	157	25 *	4	9	20.6 *	73 *	47
1-37	41 *	24 *	4	9	16.1 *	75 *	46
1-38	51 *	28 *	4	9	15.8 *	73 *	51
2-1	39 *	28 *	4	7	27.3 *	73 *	50
2-2	27 *	41	5	8	12.8	76 *	42
2-3	34 *	31	4	7	16.6 *	71	44
2-4	26 *	27 *	4	8	18.5 *	72	48
2-5	37 *	27 *	4	8	17.3 *	74 *	45
2-6	27 *	35	4	8	17.7 *	70	41
2-7	23 *	47	4	8	14.2	76 *	42
2-8	168	27 *	4	7	21.1 *	73 *	45
2-9	68 *	25 *	4	7	17.1 *	32	18
1-2	40 *	25 *	4	8	6.7	72	45
1-3	34 *	19 *	3	8	14.2	74 *	58 *
1-11	29 *	21 *	4	7	13.0	59	44
1-19	18 *	20 *	3	7	18.0 *	71	53
1-36	22 *	28 *	4	9	9.8	72	35
R 9227	116	29	4	7	11.8	64	42
GM	73.28	28.02	3.59	8.19	16.20	71.63	45.50
S.Em ±	11.31	6.11	0.51	0.5	4.5	2.24	5.54
CV(%)	15.44	21.8	14.39	6.10	27.78	3.13	12.18
GPBD 4 X NC Ac 343							
GPBD 4	207	44	4	2	15.7	73	37
1-1	79 *	46	4	5 *	25.6 *	63	54
1-2	57 *	29 *	4	5 *	16.4 *	62	51
1-3	100	31	5	5 *	16.2 *	71	47
1-5	94 *	31	3	5 *	15.1	71	38
1-7	70 *	36	4	4 *	21.4 *	73 *	53
1-8	58 *	41	3	5 *	23.5 *	74 *	47

Contd..

Lines	GIW	DS	THRIPS	LLS	PY	SP	HSM
1-9	135	45	4	5 *	16.7 *	77 *	56 *
1-4	127	19 *	4	5 *	17.0 *	70	26
1-6	102	41	4	6	21.0 *	73 *	51
1-10	135	16 *	4	6	11.7	64	53
2-1	145	29 *	4	5 *	11.2	79 *	48
2-2	107	46	4	4 *	23.2 *	75 *	62 *
2-3	34 *	36	4	5 *	19.5 *	77 *	43
2-4	78 *	37	3	5 *	17.0 *	75 *	65 *
2-5	70 *	27 *	4	6	16.0 *	74 *	47
2-7	105	30	3	5 *	15.9 *	73 *	63 *
2-8	70 *	32	5	5 *	11.2	82 *	38
2-9	103	32	3	5 *	13.2	73 *	43
2-10	54 *	39	3	5 *	17.5 *	80 *	41
2-11	46 *	37	3	4 *	25.5 *	71	41
NC Ac 343	88	18	4	5	6.73	59	36
GM	94.06	33.98	3.59	4.39	17.44	72.27	47.53
S.Em ±	24.00	6.01	0.57	0.58	1.59	01.18	0.911
CV(%)	25.51	17.72	15.97	11.95	09.31	1.63	1.89

* Superior progeny

Appendix III: Performance of *Spodoptera* resistant segregants for productivity over environments

Appendix III: Performance of <i>Sporophylla</i> resistant segregants for <i>Sporophylla</i> resistance															
Lines	Pod yield (g)				Shelling outturn (g)				Hundred seed mass (g)						
	R 01	PR 01	R 02	PR 01	Mean	R 01	PR 01	R 02	PR 01	Mean	R 01	PR 01	R 02	PR 01	Mean
28-2 X R 9227 (MR)															
MR 2-6	8.8	23.4	8.2	19.6	15.0	69	68	65	66	67	49	43	39	50	45
MR 2-7	9.5	22.5	13.6	14.5	15.0	69	65	66	67	67	46	51	51	43	48
MR 2-21	11.0	16.2	6.6	14.0	11.9	61	63	52	64	60	46	31	42	46	41
MR 2-53	12.7	12.9	12.3	17.3	13.8	69	71	69	65	68	55	29	39	31	38
28-2 X NC Ac 343 (MN)															
MN 1-22	15.3	8.1	4.4	3.7	7.9	46	64	40	47	49	66	53	38	53	52
R 9227 X NC Ac 343 (RN)															
RN 2-4	17.5	5.9	11.2	12.0	11.6	72	61	60	68	65	47	37	40	67	48
RN 2-19	19.0	11.1	14.6	13.9	14.4	71	63	56	61	63	56	39	40	48	46
RN 2-25	19.6	10.0	13.1	14.0	14.2	47	65	68	55	58	46	37	52	53	47
RN 3-17	17.4	8.9	10.5	6.5	10.8	72	63	52	54	60	46	32	35	44	39
RN 3-24	15.4	11.0	9.8	11.2	11.8	76	72	62	64	68	39	39	33	42	38
RN 3-26	17.5	9.6	10.2	12.0	12.3	73	68	51	64	64	60	48	65	52	56
RN 3-29	13.0	15.8	9.5	7.6	11.4	56	69	66	57	62	59	33	43	49	46
RN 4-4	10.9	17.0	7.1	11.1	11.5	65	74	73	63	69	40	49	46	37	43
R 9227 X GPBD 4 (RG)															
RG 1-11	11.4	2.7	10.5	24.5	12.3	74	68	67	72	71	44	14	47	45	37
RG 1-8	12.0	16.7	13.2	15.2	14.3	66	61	66	65	64	56	37	64	51	52
RG 2-21	18.8	18.2	10.0	16.5	15.9	75	65	68	63	67	45	39	38	37	40
RG 2-22	16.3	20.8	8.3	16.0	15.3	74	56	59	66	63	31	29	41	36	34
TAG 24 X NC Ac 343 (TN)															
TN 1-16	11.1	6.2	6.8	18.0	10.5	65	62	58	66	63	47	33	42	63	46
TN 1-20	17.8	10.9	7.5	8.0	11.0	69	55	62	72	65	68	60	56	57	60
TN 1-23	10.5	15.2	11.0	16.2	13.2	73	66	57	75	68	58	52	47	51	52
TN 2-2	11.8	15.5	11.7	19.1	14.5	73	61	60	71	66	63	58	62	71	63
TN 2-4	12.7	13.9	5.7	14.5	11.7	64	45	44	67	55	49	22	41	63	44
TN 3-1	16.5	8.2	10.0	19.8	13.6	42	46	66	66	55	71	39	44	49	51
TN 6-2	12.5	9.1	5.5	17.0	11.0	65	51	48	59	55	49	47	45	51	48
TN 6-4	11.5	14.4	8.5	15.4	12.5	53	60	42	61	54	47	41	37	51	44

Appendix IV: Performance of single plant selections for productivity across seasons

Appendix IV: Performance of single plant selections for productivity across seasons															
Line No.	Pod yield (g)					Shelling outturn (g)					Hundred Seed Mass (g)				
	R 01	PR 01	R 02	PR 01	Mean	R 01	PR 01	R 02	PR 01	Mean	R 01	PR 01	R 02	PR 01	Mean
MR 1-7-1	9.5	21.5	14.5	21.6	16.8	69	60	60	70	64	45	36	48	46	44
MG 1-2a	26.9	9.0	14.8	14.8	16.4	66	57	66	65	63	45	31	51	41	42
RN 1-13-1	20.0	6.7	18.4	22.4	16.9	75	57	56	75	66	65	38	54	73	58
RD 1-8a	12.0	8.8	13.2	15.7	12.4	66	42	68	74	62	57	46	62	59	56
MR 1-18-1	16.6	35.6	18.4	23.6	23.5	71	50	66	76	66	56	43	49	60	52
MR 1-10-1	22.0	10.9	7.2	19.8	15.0	72	63	48	66	62	60	58	27	51	49
MR 1-51-1a	12.9	20.2	18.9	10.7	15.7	71	54	74	74	68	49	44	40	58	48
MR 2-37-1	14.3	16.5	15.0	20.3	16.5	73	51	61	72	64	58	40	52	53	51
MR 1-51-1b	12.9	21.4	7.0	27.2	17.1	71	35	53	69	57	49	54	36	62	50
MN 1-31-1	19.3	16.5	12.2	22.5	17.6	72	53	74	68	67	50	40	45	45	45
RG 2-25-1	21.0	9.4	13.7	27.5	17.9	76	50	62	70	65	46	45	41	57	47
TN 2-3-1	15.7	26.3	8.7	27.3	19.5	69	39	46	65	55	65	53	38	51	52
TN 5-5-1	17.3	11.2	26.6	20.4	18.9	71	63	69	74	69	44	37	54	50	46
TR 1-7-1a	20.9	17.3	29.2	23.6	22.8	73	62	66	71	68	46	37	56	64	51
MR 1-49-1	22.2	21.9	20.7	14.6	19.9	71	58	62	70	65	50	48	42	60	50
MR 1-51-1c	12.9	20.2	9.9	32.0	18.7	71	54	39	75	60	49	44	36	66	49
MG 1-31-1	23.0	30.8	19.2	17.2	22.5	75	36	72	73	64	55	35	47	52	47
MN 1-28-1	26.4	11.7	6.1	26.0	17.6	66	51	46	70	58	63	45	27	57	48
TR 1-7-1b	20.9	17.3	19.7	22.3	20.0	46	62	63	70	60	73	37	44	55	52
TR 1-19-1	18.0	23.6	12.0	29.6	20.8	75	57	58	61	63	53	34	26	53	41
TR 1-33-1	16.5	32.6	14.3	19.0	20.6	71	49	62	70	63	50	55	43	54	50
TR 1-6-1	19.5	28.4	14.9	20.9	20.9	74	64	58	71	67	44	47	39	46	44
TR 2-1-2	27.3	26.1	8.7	19.1	20.3	73	60	62	73	67	50	44	45	47	47
TR 2-4-1	18.5	20.0	11.1	22.6	18.0	73	73	61	71	69	48	47	45	47	47
TR 2-7-1	14.2	17.3	11.4	24.8	16.9	72	64	68	68	68	43	37	51	46	44

Appendix IV. Contd..

Line No.	Pod yield (g)					Shelling outturn (g)					Hundred Seed Mass (g)				
	R 01	PR 01	R 02	PR 01	Mean	R 01	PR 01	R 02	PR 01	Mean	R 01	PR 01	R 02	PR 01	Mean
Parents															
28-2	14.4	13.9	16.0	15.2	14.9	68	48	57	55	57	55	44	51	46	49
R 9227	11.8	18.7	11.6	22.4	16.1	65	57	62	69	63	43	38	42	42	41
NC Ac 343	6.7	8.3	5.7	10.8	7.9	59	61	58	68	61	36	29	32	30	32
GPBD 4	15.8	20.4	15.9	15.4	16.9	73	73	66	67	70	37	36	29	35	34
TAG 24	12.9	17.1	9.1	19.3	14.6	71	69	70	72	70	67	49	43	43	50
Mean	16.9	16.4	13.5	19.4	16.6	69.6	56.6	60.5	68.5	63.8	51.4	41.5	44.3	51.0	47.2
CV	26.1	18.6	22.8	22.4	33.8	11.9	11.3	9.9	6.0	11.4	9.0	17.4	12.4	16.7	16.7
CD(5%)	8.6	6.0	6.0	8.5	7.8	16.2	12.5	11.7	8.0	10.1	9.1	14.1	10.8	16.7	11.0
Environmental Index	0.29	-0.16	-3.02	2.89	-	5.81	-7.24	-3.35	4.79	-	4.44	-5.48	-2.68	3.72	-

R: Rainy season RP: Post rainy season 01: 2001 02: 2002

GENETIC ENHANCEMENT OF HOST PLANT RESISTANCE TO *Spodoptera litura* (Fab.) IN GROUNDNUT

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ABSTRACT

In groundnut, *Spodoptera litura* a defoliating insect is a major pest causing substantial yield loss. Screening germplasm against *Spodoptera* and further evaluation under high infestation in the field revealed significantly low damage in Mutant (28-2), NC Ac 343, ICGV 86031, R 9227 and TAG 24. In laboratory, the genotypes 28-2, NC Ac 343, R 9227 and ICGV 86031 affected various components of growth and development of the insect indicating antibiosis as the principal mechanism of resistance. Gain in weight of larvae was most efficient in differentiating the genotypes and was later proved to be a good criterion for screening segregating material in the laboratory. Though the genotype, TAG 24 suffered low damage in the field, the larval and pupal development was normal in the laboratory. Because of determinate habit of TAG 24, growth ceases at 55 days and lack of much preferred young leaves at peak pest activity lead to reduced damage in the field due to escapism/seasonal avoidance.

To combine high level of resistance with desirable agronomic features, 8 crosses involving resistant and susceptible genotypes were generated. Replicated evaluation of F₃ Progenies revealed continuous and unimodal distribution of damage indicating quantitative nature of resistance. Among the crosses TAG 24 x NC Ac 343 and TAG 24 x R 9227 were found most potential for resistance. The crosses involving resistant parent 28-2 produced very low frequency of resistant segregates revealing its poor combining ability. Five progenies RN 3-17, RN 3-26, RN 4-4, RD 1-8 and TN 1-20 recorded enhanced level of resistance compared to the best parent NC Ac 343. The progeny RD 1-8 also combined resistance to late leaf spot with high yield. The segregate MR 1-7 combined resistance to *Spodoptera*, thrips and late leaf spot along with yield indicating multiple pest and disease resistant nature. These lines could be profitably exploited in breeding programme or registered as resistant germplasm after detailed evaluation.

Biochemical analysis of resistant genotypes for the known insect deterrent compounds revealed lack of any role of trypsin inhibitor and lectin activity in imparting resistance. RAPD assay of genotypes with varied level of resistance to *Spodoptera* indicated limited variability. But the resistant genotypes (28-2, NC Ac 343 & R 9227) distinctly clustered away from the susceptible genotypes thus revealing scope for developing mapping populations by crossing with susceptible genotypes to identify markers associated with resistance to *Spodoptera*.