IDENTIFICATION OF RESISTANT SOURCES TO GROUNDNUT BUD NECROSIS DISEASE (GBND) IN GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES

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B.Sc. (Ag.)

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(PLANT PATHOLOGY)

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BY

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B.Sc. (Ag.)

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2014

DECLARATION

I, **AFSHA TABASSUM,** hereby declare that the thesis entitled **" IDENTIFICATION OF RESISTANT SOURCES TO GROUNDNUT BUD NECROSIS DISEASE (GBND) IN GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES "** submitted to the **Acharya N. G Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Hyderabad **(AFSHA TABASSUM)**

Date: **ID. No: RAM/12-67**

CERTIFICATE

Ms. AFSHA TABASSUM has satisfactorily prosecuted the course of research and that thesis entitled **"IDENTIFICATION OF RESISTANT SOURCES TO GROUNDNUT BUD NECROSIS DISEASE (GBND) IN GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date: **(Dr. BHARATI N. BHAT)**

Place: Hyderabad Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"IDENTIFICATION OF RESISTANT SOURCES TO GROUNDNUT BUD NECROSIS DISEASE (GBND) IN GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES "** submitted in partial fulfilment of the requirements for the degree of **'Master of Science in Agriculture'** of the Acharya N.G. Ranga Agricultural University, Hyderabad is a record of the bonafied original research work carried out by **Ms. AFSHA TABASSUM** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

g	:	gram(s)
ha	:	hectare
ha^{-1}	:	per hectare
i.e.,	:	which is to say, in other words
kg ha^{-1}	:	kilogram per hectare
m	:	metre
mm	:	millimetre
m^2	:	metre square
t ha^{-1}	:	tonnes per hectare
viz.,	:	namely
ICRISAT	:	International Crops Research Institute for the Semi-Arid Tropics
DAC-ELISA	:	Direct antigen coating- Enzyme linked Immunosorbent Assay
GBND	:	Groundnut Bud Necrosis Disease
GBNV	:	Groundnut Bud Necrosis Virus
mm	:	Milligram
kg	:	Kilogram
t	:	Tonne
ml	:	Milliliter
µl	:	Microliter
TSWV	:	Tomato Spotted Wilt Virus
APS	:	American Phytopathological Society
DGR	:	Directorate of Groundnut Research
DAS	:	Days After Sowing
DAI	:	Days After Inoculation
M	:	Million

No.	:	Number
etc.	:	and so on; and other people/things
%	:	per cent
@	:	at the rate of
cm	:	centimetre
cm^2	:	square centimetre
et al.	:	and others people
pH	:	negative logarithm of hydrogen ion
SAS	:	statistical application software
m	:	metre
mm	:	millimetre
^oC	:	degree Celsius
Fig.	:	Figure
min	:	Minute
h	:	Hour
ANOVA	:	Analysis of Variance
PDI	:	Per cent Disease Incidence
nm	:	Nanometer

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ABSTRACT

Groundnut Bud Necrosis Disease (GBND) caused by Groundnut Bud Necrosis Virus (GBNV) has been considered as one of the major virus diseases in Andhra Pradesh. Survey conducted in groundnut growing areas of Anantapur district during *kharif* and *rabi* 2013-14 and in Karimnagar and Warangal districts during *rabi* 2013-14 season revealed the natural occurrence of GBND in different mandals with an overall average incidence of 3.47 per cent. Higher incidence of GBND was observed in Anantapur (8.50 per cent) followed by Karimnagar (0.97 per cent) and Warangal (0.94 per cent) districts. Groundnut cultivar K-6 was grown extensively in all the districts surveyed. The infected groundnut leaf samples collected from Anantapur district tested positive when subjected to DAC-ELISA.

Of the 15 common weed species found in and around the surveyed groundnut fields, *Parthenium hysterophorus*, *Celosia argentea*, *Tridax procumbens*, *Achyranthus aspera* and *Cynodon dactylon* were more predominant and found in all the surveyed fields during *rabi* 2013-14 which may serve as reservoir weed hosts for GBND.

Evaluation of 40 groundnut genotypes for vector resistant sources under natural field conditions during late *kharif* 2013 revealed GBND incidence ranging from 2.57 to 22.71 per cent compared to 4.04 per cent in ICGV 86031 (resistant check) and 25.45 per cent in JL 24 (susceptible check). Of the 40 genotypes tested, 8 genotypes were resistant with disease incidence of 2.57 - 4.99 per cent, 24 genotypes were moderately resistant (5.13 - 9.93 per cent) and remaining 8 genotypes were moderately susceptible (10.21 - 22.71 per cent). The mean GBND severity in these genotypes under field conditions ranged from 1.99 to 4.32 compared to 2.33 in ICGV 86031 and 4.67 in JL 24. Further, resistance or susceptibility of genotypes was confirmed through DAC-ELISA.

Screening of groundnut genotypes inoculated with GBNV inoculum at 1:10 dilution under greenhouse conditions revealed mean disease incidence ranging from 64.71 to 100 per

cent compared to 72.22 per cent in resistant check and 94.44 per cent in susceptible check. All the genotypes were highly susceptible to GBNV at higher virus concentration (1:10 dilution). The mean disease incidence at lower virus concentration (1:100 dilution) ranged from 5.56 to 100 per cent compared to 26.67 in resistant check and 77.78 per cent in susceptible check, at 21 days after inoculation.

At low virus concentration, two genotypes (ICGV 00213, 06146) were moderately resistant (disease incidence of 5.56 and 7.14 per cent), four genotypes were moderately susceptible (11.11 - 25 per cent), 10 genotypes were susceptible (26.67 - 50 per cent) and remaining 24 genotypes were highly susceptible (52.94 - 100 per cent). None of the genotypes recorded highly resistant or resistant reaction.

The mean GBND severity in genotypes under greenhouse conditions, at 1:10 virus concentration, ranged from 2 to 5 compared to 4 in ICGV 86031 (resistant check) and 5 in JL 24 (susceptible check). At 1:100 virus concentration, disease severity was slightly less, which ranged from 2 to 4 compared to 2 in ICGV 86031 (resistant check) and 4 in JL 24 (susceptible check).

The genotype, ICGV 06146 showed resistance in field and moderate resistance in greenhouse screening by artificial inoculation. ICGV 00213 showed moderate resistance in both field and greenhouse screening. The genotypes, ICGV 07222, 03057 and ICGS 76 that showed moderate resistance in field, exhibited moderate susceptibility in greenhouse. Genotypes *viz.*, ICGV 00187, 00191, 00202, 00203, 06100, 93260, 05155 and ICR 48 which showed moderate resistance in field were grouped under susceptible group in greenhouse. ICGV 07220 showed resistance in field and moderate susceptibility in greenhouse. These genotypes had Spanish bunch growth habit except ICGS 76 and ICR 48 which had Virginia bunch growth habit.

The present study revealed that the genotypes which were resistant or moderately resistant to the vector/disease under field conditions showed relative degree of susceptibility under high disease pressure in greenhouse conditions. The genotypes ICGV 06146, 00213, 07222, 03057 and ICGS 76 which were found promising with combined resistance to the vector and GBNV can be further evaluated over 2 - 3 seasons and genotypes with stable performance can be used in resistance breeding programme.

Chapter I

INTRODUCTION

Chapter I

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a popular legume crop, cultivated over 100 countries across six continents as a rich source of edible oil (48 - 50 %), protein (26 - 28 %), dietary fiber, minerals, and vitamins (Ntare *et al.,* 2008). With an annual world production of 41.19 Mt from 24.71 Mha (Food and Agriculture Organization of the United States, 2014), groundnut is a major oilseed crop that is grown commercially throughout the tropical, sub-tropical and warm temperate regions of the world (Nwokolo, 1996). It is largely a small-holder crop, grown under rainfed conditions in semi-arid areas characterized by unpredictable rainfall, and these areas contribute over 90 % of world groundnut production.

In India, groundnut is grown in an area of 47.66 lakh ha with estimated production of 47.49 lakh tonne and average productivity of 996 kg ha^{-1} (DGR Annual Report, 2013). Groundnut in India is mostly grown in five states *viz.*, Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka and Maharashtra. Out of them, Andhra Pradesh and Gujarat account for more than half of the groundnut area in the country. In Andhra Pradesh, groundnut is grown in an area of 13.45 lakh ha with estimated production of 11.09 lakh tonne and average productivity of 825 kg ha^{-1} (DGR Annual Report, 2013). In Andhra Pradesh, groundnut is majorly grown in Anantapur, Chittoor, Kurnool, Kadapa, Mahabubnagar, Warangal, Nalgonda, Srikakulam and Visakhapatnam districts.

Low inputs, rainfed cultivation of the crop in marginal lands, non-availability of seed of suitable high-yielding varieties, and the occurrence of many insect pests, fungal diseases, and numerous viral diseases at different stages of crop growth are primary factors responsible for low yields in groundnut (Reddy *et al.,* 1992). Natural infection of about 30 viruses representing 14 virus groups has been recorded on groundnut from different countries (Sreenivasulu, 2005). Among these Tospoviruses are emerging as serious pathogens (Varma *et al*., 2002).

The name *Tospovirus* was given after the discovery of *Tomato Spotted Wilt Virus* (TSWV) in Australia in 1915. In Indian sub-continent, Five *Tospovirus* species *viz., Groundnut bud necrosis virus* (GBNV), *Groundnut yellow spot virus*, *Watermelon bud necrosis virus*, *Iris yellow spot virus* and *Capsicum chlorosis virus* have been identified from India, however, only GBNV has been reported to infect leguminous hosts (Jain *et al*., 2007)

GBNV belongs to family *Bunyaviridae* and responsible for causing Groundnut Bud Necrosis Disease (GBND) in groundnut (Reddy, 1991). GBNV is an economically important *Tospovirus* and its distribution is confined to South and Southeast Asian countries namely India, China, Pakistan, Nepal, Sri Lanka and Thailand (Dwivedi *et al.,* 1995). The disease was first recorded in India at Indian Agricultural Research Institute in 1949 (Reddy *et al.,* 1995). GBND in India until 1990 was reported to be caused by TSWV. Serological comparisons and sequencing of nucleic acids revealed the existence of several distinct Tospoviruses and GBNV was found to be serologically distinct from other Tospoviruses such as TSWV and *Impatiens necrotic spot virus* (INSV) (Reddy *et al*., 1995). This virus is mechanically transmissible, but in nature, it is transmitted by the vector *Thrips palmi* in persistent manner (Vijayalakshmi, 1995).

GBND has been considered as one of the major virus diseases of groundnut apart from Groundnut Stem Necrosis Disease (GSND) in Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, Gujarat and Maharashtra on *kharif* groundnut crop. The hot spot locations for GBND are Jagtial and Hyderabad (ICRISAT) in Andhra Pradesh; Latur in Maharashtra; Tikamgarh in Madhya Pradesh; Raichur in Karnataka; Mainpuri in Uttar Pradesh and Saurashtra in Gujarat (Basu, 1995).

Symptoms initially appear on young quadrifoliates as mild chlorotic mottle or spots, which develop into necrotic or chlorotic rings and streaks. This is followed by death of terminal bud. Secondary symptoms are stunting, auxiliary shoot proliferation, and malformation of leaflets (Reddy *et al.,* 1995). However, the symptomatology varies depending on the strain, host species and genotype, and is also influenced by environmental factors such as temperature.

Thrips-transmitted Tospoviruses cause significant losses in yield and quality of produce from vegetable, legume and ornamental crops in many parts of the world (Mumford *et al.,* 1996; Pappu, 1997; Pearce, 2005 and Persley *et al.,* 2006). GBND became economically important during the late 1960's when incidences up to 100 % were recorded in many groundnut growing regions of the country. Incidence of GBND ranging from 5 to 80 %, and yield losses of up to 50 %, worth more than $89 million in India alone, have been reported (American Phytopathological Society, 2013). Substantial decrease in plant stand occurs, during infection at early stages of crop growth leading to considerable yield losses, but infection at later stages may still cause significant losses in the yield and quality of produce (Culbreath *et al.,* 2003).

In India, 80 % of groundnut sowing is taken up in *kharif* season and sometimes with late onset of monsoon, July-August sowings are taken up. Maximum thrips populations were observed from 2nd week of July to end of August resulting in complete crop loss (Vijayalakshmi, 1995). There is no practical control measure currently available for GBNV in groundnut. However, by using certain cultural practices such as adjustment of planting date coinciding with low levels of thrips activity, intercropping with fast growing cereals (Reddy *et al.,* 2000) and close planting (Basu, 1995; Buiel and Parlevliet, 1996 and Wongkaew, 1995), the disease incidence can be reduced. Control of this virus through crop rotation and removal of alternate weed hosts have met with limited success (Rao *et al.,* 2013). Efforts to control vector with insecticides have been mostly unsuccessful. Indiscriminate use of insecticides is leading to the development of resistance in vector. In this context, genetic resistance in host plants is the most economical method for the resource poor farmers. So far, the released varieties are found to be susceptible to GBND. Identification of GBND resistant sources in advanced breeding lines would help in direct release of resistant genotypes for hot spot locations.

Keeping in view the economic importance of the disease in most of the groundnut growing areas and lack of resistance sources to GBND, the present investigation is proposed with the following objectives

Objectives of investigation:

1. Survey on incidence and severity of Groundnut Bud Necrosis Disease in major groundnut growing areas of Andhra Pradesh.
2. To identify field resistant sources to Groundnut Bud Necrosis Disease in selected groundnut genotypes.
3. To differentiate Groundnut Bud Necrosis Virus resistant and Vector resistant sources in identified resistant sources.

Chapter II

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

The literature pertaining to the various aspects of groundnut bud necrosis disease (GBND) and other related literature is reviewed briefly in this chapter under the following headings.

2.1 Occurrence of the disease

Delfosse *et al.* (1995) conducted a survey in major groundnut growing areas of Pakistan (Attock, Chakwal and Rawalpindi districts) during July 1995 for the incidence of groundnut viral diseases and 5 to 15 per cent incidence of GBND was recorded. Symptoms were often unclear in fields observed. ELISA test results confirmed the presence of *Groundnut Bud Necrosis Virus* (GBNV) in Pakistan.

Field survey carried out in *kharif,* 1999 at three crop growth stages (seedling, flowering and pod formation) revealed the occurrence of GBNV on groundnut in all the farmers' field surveyed in the states of Andhra Pradesh, Tamil Nadu and Karnataka in India. Low incidence of up to 5 per cent and maximum of up to 19 per cent was observed at seedling and maturity stages, respectively. Disease incidence of 25 per cent was recorded in Chittoor and Kadapa districts of Andhra Pradesh and 20 per cent in Kolar districts of Karnataka (Pande and Rao, 2000).

Kendre *et al.* (2000) carried out a study in 1992-93 to determine the occurrence of *Thrips palmi* Karny in the Marathwada region of Maharashtra state on 85 different groundnut plants. On the basis of *Thrips palmi* population, the level of *Thrips palmi* was graded as high, moderate and absent. About 30 plant species were found to be heavily infested by the thrips.

Sunkad *et al*. (2012) conducted survey during *kharif* 2007 and *rabi/summer* 2007-08 which revealed GBND prevalence in Upper Krishna Project and Tungabhadra Project area. During *kharif* season, disease incidence varied from 1 to 44 per cent while, in *rabi/summer* disease incidence of 1 to 84 per cent was recorded.

Bhat *et al.* (2001) conducted survey during August to October, 1999 in New Delhi, India to know the occurrence of tospovirus infections on black gram, cowpea, green gram and soybean. Maximum disease incidence of 2-20 per cent was recorded in different cultivars of

green gram, 2-12 per cent in soybean cultivars, 0.4-6 in cowpea and 0.2-2 per cent in black gram.

Sreekanth *et al.* (2002a) conducted survey in different districts of Andhra Pradesh to know the occurrence of thrips and incidence of GBNV on green gram during *kharif* (2000, 2001 seasons), *rabi* (2000, 2001 seasons) and *summer* (2001 season). *Thrips palmi* was the most dominant thrips species (51 per cent of the total population), followed by *Scirtothrips dorsalis, Frankliniella schultzei* and *Megalurothrips usitatus* (24.9, 14.9 and 9.3 per cent, respectively). Thrips infestation and GBNV incidence was higher in 2001 than in 2000. Thrips infestation and GBNV incidence was also much higher in *kharif* than in *rabi* and *summer*.

Survey conducted by Rao *et al.* (2003a) in Nalgonda, Khammam, Medak, Warangal and Karimnagar districts of Telangana region during *kharif* 2000-01 and 2001-02 seasons revealed leaf curl disease incidence on mung bean ranging from 0.24 to 18.94 per cent and 14.12 to 33.96 per cent in two seasons respectively. Whereas, the leaf curl incidence on urd bean (*Vigna mungo*) ranged from 10.04 to 11.98 per cent in the 2001- 02 *rabi* season in Guntur, Krishna and Prakasam districts. Disease incidence of 2.92-5.73 per cent was recorded in Guntur and Krishna district on urd bean grown in rice fallow. Of 372 leaf curl samples of mung bean and urd bean obtained from different districts of Andhra Pradesh, 337 samples were positive to GBNV when subjected to ELISA test.

Jagadeeshwar *et al.* (2005) conducted extensive survey in the predominant chilli growing areas of Northern Telangana zone in Andhra Pradesh from *kharif*, 2000 to *kharif*, 2002 and reported mosaic virus incidence ranging from 1.0 to 48.5 per cent with overall average incidence of 18.5 per cent.

Manjunatha *et al.* (2010a) carried out the survey in parts of Belgaum, Dharwad, Haveri and Kolar district during *summer* season in irrigated tomato fields to know the incidence of the bud blight on tomato. The disease incidence ranged from 12.5 to 94.4 per cent. Maximum disease incidence was noticed in Kyalanur (94.4 per cent) of Kolar district and minimum was in Guledkoppa (12.5 per cent) in Dharwad taluk. Cultivar Abhinav recorded highest incidence and cultivar Utsav had relatively less incidence among the varieties/hybrids surveyed.

Gopal *et al.* (2011) carried out survey to know the occurrence of GBND in Karnataka and Andhra Pradesh, the major groundnut growing states in South India. Apart from

groundnut, GBND incidence was observed on other different crops, *viz.*, green gram, black gram, tomato, watermelon, cowpea, chilli, cucumber and sesame. In Andhra Pradesh, disease incidence of 27.6 to 47 per cent in green gram; 4.33 to 18.6 per cent in chilli; 8.33 to 40.4 per cent in cowpea was observed. In Karnataka, Raichur recorded highest mean GBND incidence of 5.3-37.8 per cent in post rainy season and 3.5-45.5 per cent in rainy season, while Tumkur recorded lowest GBND incidence of 0.9-6.6 per cent in post rainy season on groundnut. *Achyranthus aspera*, *Ageratum conyzoides*, *Alysicarpus rugosus*, *Commelina bengalensis* and *Vigna trilobata* weed species were found abundant in Andhra Pradesh and Karnataka. When compared to other weeds more infection was observed on *Ageratum conyzoides* (17.56 per cent)

2.2 Etiology

2.2.1 Association of virus with groundnut bud necrosis disease

HoXuan *et al.* (2003) noticed tospovirus infection on mung bean (up to 70 per cent) in different varietal trials, at Indian Agricultural Research Institute experiment farm, New Delhi. Positive reaction with GBNV and watermelon silver mottle virus (WSMV) antisera in DAC-ELISA was shown by symptomatic mung bean plants.

Biswas *et al.* (2009) studied occurrence and incidence of the single and multiple viral diseases in urd bean (*Vigna mungo* L.) caused by *Mung bean yellow mosaic India virus* (MYMIV), *Groundnut Bud Necrosis Virus* (GBNV), Urd bean leaf crinkle virus complex (ULCD). During *pre-kharif and kharif* season of 2006 and 2007, nine cultivars and 40 Initial Varietal Trial (IVT) lines of urd bean were sown in experimental farm at Indian Agriculture Research Institute, New Delhi. The study showed maximum disease incidence of 65.5-72.0 per cent in urd bean cv. P 2056 by MYMIV, 66.1 per cent in cv. T9 by ULCD, 14.5 per cent in Pant U35 by GBNV, 21.5 per cent in Barabanki Local by MYMIV+ULCD, 6.5 per cent in T9 by MYMIV+GBNV, 4.8 per cent in Pant U35 by ULCD+GBNV and 3.0 per cent in P 2056 by MYMIV+ULCD+GBNV.

Kunkalikar *et al.* (2011) conducted a survey from 2002 to 2009 in the major vegetable growing areas of India for GBNV and other viruses. GBNV was reported widely in tomato and chilli peppers in 14 states representing southern, north-western, north-eastern, and central regions of India. Expansion of the host range of GBNV to watermelons and other cucurbits and WBNV to tomato and chili peppers led to natural mixed infection of the two viruses.

2.2.2 Association of GBNV and TSV with other hosts and weeds

Jain *et al.* (2000) encountered unusual disease in potato in parts of northwestern /central plains of India (Madhya Pradesh and Rajasthan) since 1982, which was characterized by extensive stem and petiole necrosis, leaf spotting/deformation and stunting. Electron microscopic analysis of the diseased tissue revealed the presence of spherical virus particles measuring 70-90 nm in diameter and the virus was designated as potato stem necrosis virus (PSNV). In order to identify and classify PSNV isolates from Madhya Pradesh and Rajasthan, nucleocapsid protein (NP) serology and NP-gene amplification using reverse transcription polymerase chain reaction (RT-PCR) were attempted. Though primers derived from NP-gene and 3'-terminal non-coding regions of GBNV and watermelon silver mottle virus were unable to amplify target sequences in a specific and reproducible manner from potato tissue extracts, yet the PSNV reacted only with polyclonal NP antiserum to GBNV and watermelon bud necrosis virus recorded from India.

During 2002-03, soybean cv. JS-335 grown in Rajendranagar, Hyderabad showed chlorotic/necrotic spots on young trifoliate leaves and terminal bud blight symptoms. Groundnut cultivars which were surrounded around the soybean field showed GBNV incidence ranging from 5 to 23 per cent. A total of 20 samples of soybean showing early chlorotic spot symptoms on young leaves and 5 samples of groundnut showing bud necrosis symptoms tested by DAC-ELISA, reacted strongly with GBNV antiserum. Based on serology and infectivity assay, the pathogen was identified as GBNV (Kumari *et al*., 2003).

Rao *et al.* (2003c) reported *Parthenium hysterophorus* as major weed in epidemic areas of Groundnut Stem Necrosis Disease (GSND) caused by *Tobacco Streak Virus*. *M. usitatus, F. schultzei* and *S. dorsalis* were vectors in the presence of infected pollen. *Helianthus annuus* (sunflower) and *Tagetus patula* acted as source of inoculum among crop species. These also reported that virus was not seed transmitted in the peanut cultivar JL-24 or in the sunflower hybrids KBSH-41, -42, -44, and -50, MSFH-17 and ZSH- 976.

Nagaraja *et al.* (2005a) randomly collected suspected weed species belonging to several families showing the associated virus symptoms of GBNV from the fields (Karnataka) and subjected them to DAC-ELISA. Out of 39 weed flora tested, 16 were found to be infected with GBNV. *Acanthospermum hispidum*, *Commelina benghalensis*, *Ageratum conyzoides*, *Achyranthes aspera*, *Borreria hispida*, *Euphorbia geniculata* and *Datura stramonium* showed strong reaction to GBNV antisera in DAC-ELISA.

Pranav *et al.* (2008) collected virus infected sunflower samples from Raichur and Dharwad districts of Karnataka. Total RNA was isolated and using GBNV partial Cp gene

specific primers, RT-PCR was done. The amplicon was cloned and confirmed by sequencing. The sequencing result revealed the amplicon to be part of GBNV Cp gene. This study shows sunflower as new host of GBNV, which could be devastating for sunflower cultivation in the future.

Reddy *et al.* (2008) studied tomato necrosis, a devastating disease in tomato, which was caused by a *Tospovirus* belonging to the family *Bunyaviridae*. The bioassay of necrosis affected tomato samples produced chlorotic lesions on cowpea cv. C-152 and chlorotic cum necrotic lesions on *Chenopodium amaranticolar*. Symptomatic parts of the plant used for assay showed positive reaction with the GBNV polyclonal antiserum both in DAC-ELISA and dot immunobinding assay indicating the association of tospovirus serologically related to GBNV. Alcobasa-V and PKM-1 cultivars were resistant to GBNV/tomato *Tospovirus* during the field screening.

Hemalatha *et al.* (2008) propagated tospovirus infecting tomato in the fields of Karnataka on greenhouse grown *Nicotiana benthamiana* by mechanical inoculation. The N gene of tomato tospovirus showed 98 per cent homology with GBNV and only 82 per cent identity with N gene of GBNV-To isolate from Taiwan. The results indicated that tospovirus infecting tomato in Karnataka is strain of GBNV and designated as GBNV – To (K).

Survey conducted in 2008 by Damayanti and Naidu (2009) in farmers' fields of Warung Kondang, Cianjur, West Java, Indonesia showed stunting and leaf symptoms of either bronzing or general chlorosis with vein-banding on tomato. Chlorosis and vein-banding symptoms was observed in Salabintana, Sukabumi, West Java on chilli pepper whereas groundnut plants showed chlorotic rings and necrosis spots on leaves in Darmaga Bogor. An analysis of the nucleotide sequence obtained from the groundnut sample (FJ177300) showed 94 per cent sequence identity with the corresponding L RNA sequence of a GBNV isolate from India (AF025538).

Akram and Naimuddin (2010a) observed 20 per cent disease incidence in *Vigna mungo* var. *silvestris* grown in the experimental field of the Indian Institute of Pulses Research, Kanpur, India, during Autumn 2008. On the basis of the symptoms on the diagnostic host, and the reverse transcription polymerase chain reaction (RT-PCR) using specific primers of the NSm and NP genes the virus was identified. According to them, this is the first report of GBNV on *V. mungo* var. *silvestris*.

Akram and Naimuddin (2010b) observed disease incidence in the range of 1-10 per cent in different pea fields grown during the winter season in Uttar Pradesh, India. They failed to isolate the fungal or bacterial pathogens from the leaves and pods showing symptoms. Sap inoculation on cowpea cv. Pusa Komal, from leaves and pods showing symptoms, resulted in the development of characteristic necrotic local lesions on the inoculated primary leaves, and subsequent systemic infection developed on newer leaves. These indicated tospovirus disease etiology. This is thought to be the first report of GBNV affecting pea (*Pisum sativum*) in India.

Sivaprasad *et al.* (2011a) suspected the natural occurrence of GBNV on Taro (*Colocasia esculenta*) in Nellore district, Andhra Pradesh during one of the survey in 2010. Detection was done by ELISA using an antiserum raised against GBNV and RT- PCR using coat protein specific primers. 93-99 per cent and 95-99 per cent identity at nucleotide and amino acid levels respectively with other reported GBNV isolates was revealed during sequence analysis. According to them, this is the first report of GBNV infecting taro.

Sivaprasad *et al.* (2011b) observed 15-20 per cent of plants showing viral symptoms in two commercial jute fields in the Chittoor district of Andhra Pradesh in August 2010. By using polyclonal antibodies for GBNV and TSV the leaves with symptoms were tested for viruses by DAC - ELISA. Only GBNV was detected and this was confirmed by RT-PCR with total RNA extracted from GBNV-positive jute with ELISA using GBNV coat protein gene-specific primers. This is the first report of the natural occurrence of GBNV infecting jute in Andhra Pradesh, India.

Sujitha *et al.* (2012) collected onion samples (showing straw coloured, mosaic and necrotic lesions) from commercial onion fields in the Kadapa district of Andhra Pradesh, during November, 2011. Natural occurrence of GBNV on onion was identified by ELISA using an antiserum raised against GBNV and RT- PCR using coat protein gene specific primers. 93-100 per cent and 95-100 per cent identity at nucleotide and amino acid levels respectively with other reported GBNV isolates was revealed in sequence analysis. According to them, this is the first report of GBNV infecting onion.

Survey conducted from 2010 to 2011 in Godagari Upzila, Rajshahi district in Bangladesh (Akhter *et al* ., 2012) revealed the presence of unusual disease of tomato characterized by leaf mottling and necrotic streaks on veins, shortened internodes, necrosis of terminal buds, and concentric rings on fruits. Disease incidence in popularly grown F_1 hybrid cultivars, which included Sobal, Abhiruchi, Salamat, Bangobir, BARI hybrid tomato-5 and

BARI hybrid tomato-6, in approximately 40 commercial fields ranged from 40 to 90 per cent. Extracts from the field samples reacted with polyclonal antiserum to GBNV in DAC-ELISA, suggesting the association of a *Tospovirus* antigenically related to serogroup IV tospovirus.

Meng *et al.* (2013) conducted a field survey from 2010 to 2011 in Guangxi, China. The incidence of virus-like diseases of mulberry ranged from 40 to 80 per cent and deep sequencing of small RNAs was conducted to identify the viruses infecting mulberry. Among the contigs assembled, a 445-bp contig (GenBank Accession No. JX268597) was found to share 76.6 % nucleotide identity and 83.0 % amino acid identity to GBNV (*Tospovirus* : Accession Nos. U42555 and AAC55521). This is thought to be the first report of a *Tospovirus* infecting *M. alba*.

Akram and Naimuddin (2013) during post-rainy season (*rabi*) of 2009–10, 2010–11 and 2011–12 observed a disease in rajmash at Kanpur, India with disease incidence between 4-5 per cent. The causal virus from field infected plants was successfully sap transmitted to healthy plants of rajmash. Using primer pair (HRP26/HRP28) specific to the coat protein (CP) gene of the GBNv, the identity of the virus was confirmed as GBNV and has been designated as GBNV-[Frb-KNP].

2.3 Effect of bud necrosis disease on yield and yield parameters of groundnut

In, two seasons field trials at ICRISAT, ICGV 86699 showed field tolerance to GBND with average incidence of 17.9 per cent compared to 37.9 per cent incidence in JL 24. Field trials in hot spot location in Northern India for one season showed 7.9 per cent GBND compared to 47.1 per cent in Kadiri 3. In, 20 replicated trials, conducted in different locations in India during 1987-90, ICGV 86699 produced an average pod yield of 1.25 t ha^{-1}, 47 per cent greater than Kadiri 3. (Reddy *et al.,* 1996)

Singh *et al.* (1997) evaluated incidence and losses due to GBND and peanut mottle disease (PMD) in groundnut cv. Kaushal during *kharif* 1992 at Kanpur. The incidences of GBND and PMD were 7.5 per cent and 11.2 per cent, respectively. At 35 DAS, GBND began to appear in the field whereas PMD appeared at 50 DAS. Compared with healthy plants, both the diseases caused significant loss in plant height, number and weight of pods/plant.

Bhargava *et al.* (1999) conducted field trails in June 1995 in Durgapura, Jaipur, Rajasthan, India to assess the effects of GBNV infection on performance of groundnut cv.

Chitra. They reported that increasing disease severity was related to fall in number and dry weight of pods, plant height and biomass. Infected plants were more branched than uninfected plants, although branching was greatest with low levels of infection.

Lokesh *et al.* (2008) conducted experiment during *kharif* 2006 to evaluate new groundnut genotypes against GBND. Of the 3 groundnut varieties evaluated *viz.*, R-2001-3, R-8808 and local check (TMV-2), the genotypes R-2001-3 and R-8808 showed moderate resistance reaction to necrosis disease (7-14 per cent) with highest pod yield of 1,325 and 1,740 kg ha^{-1} respectively, when compared to local check (TMV-2) that recorded highest necrosis disease incidence with low pod yield of 478 kg ha^{-1} exhibiting susceptible disease reaction and higher disease pressure.

Farmer participatory field trials were conducted in selected locations of Tamil Nadu State in India to evaluate the performance of selected tomato cultivars and hybrids against natural infection of GBNV. Although none of the cultivars showed resistance, the data indicated that some cultivars and hybrids exhibited field tolerance with higher fruit yield compared to susceptible ones. Decrease in lycopene, β- carotene, vitamin A, zinc, total sugars and carbohydrates in tomatoes harvested from GBNV infected plants indicating that nutritive quality of the fruit was affected by virus infection. Virus infected seedlings from commercial nurseries helped in GBNV spread in new seedlings. During and soon after transplanting, roguing of virus – infected tomato seedlings significantly reduced disease incidence (Gandhi *et al.*, 2011).

2.4 Identification of the causal virus

2.4.1 Serology

Wongkaew and Chuapong (1995) studied the major groundnut producing areas of Thailand during December 1992 to April 1993 for viral diseases of groundnut. To determine the incidence of diseases, disease symptoms in the field were subjected to ELISA test. It was concluded that bud necrosis caused by GBNV was most prevalent especially in northeastern and eastern areas of Thailand.

Thakur *et al.* (1996) evaluated 19 soybean samples showing bud blight symptoms using DAC-ELISA with GBNV antiserum. Of the 19 samples, 14 were positive for BNV alone whereas, one was positive for BNV, cowpea mild mottle carlavirus and groundnut stripe virus.

Golnaraghi *et al.* (2002) reported the first occurrence of GBNV in groundnut fields in the Golestan province of Iran. Mechanical inoculation, triple-antibody sandwich enzyme-linked immunosorbent assay (ELISA) and polyclonal (As) combined with monoclonal antibodies (MAbs) tests were performed to confirm the occurrence of GBNV.

In New Delhi and Kerala, India, infected mung bean, cowpea, and tomato plants were characterized by severe necrosis of leaves, stem, meristems, buds, pods, and fruits. Jain *et al.* (2002) identified tospovirus isolates based on serological and nucleocapsid protein gene sequence analyses. Symptomatic leaf samples reacted positively to a polyclonal antiserum against a nucleocapsid protein of GBNV in DAC-ELISA.

To detect the natural infection of viral diseases in standing crops of urd and mung bean in experimental trials at Allahabad Agricultural Institute – Deemed University (AAI-DU), Allahabad and farmers' fields in the vicinity of AAI-DU, survey was conducted during *kharif* 2004 and 2005. Subsequently, the young tissue samples collected from suspected plants was subjected to DAC-ELISA. The bioassayed samples tested positive in DAC-ELISA. Using serology, symptomatology and physical properties of buffered sap, the virus inciting leaf curl in mung and urd bean was confirmed as GBNV (Manoj *et al.* 2007).

Srinivasaraghavan *et al.* (2011) collected groundnut samples with bud necrosis symptoms from different parts of north-eastern Karnataka and subjected them to DAC-ELISA technique using polyclonal antiserum of GBNV and TSWV. All samples showed positive reaction with GBNV and negative reaction with TSWV antisera. Negative reaction to GBNV antisera was also shown by the weed samples prevailing in groundnut ecosystem.

2.5 Screening of genotypes for vector resistance sources

Dwivedi *et al.* (1993) developed Spanish type peanut (*Arachis hypogaea* L. subsp. *fastigiata* Waldron var. *vulgaris* Hartz) at ICRISAT, Patancheru, India. In 1991, it was released by Plant Materials Identification Committee of ICRISAT because of its resistance to Thrips (*Thrips palmi* Karny), Jassid (*Empoasca kerri* Pruthi), Spodoptera (*Spodoptera litura* Deventer) and BNV which causes GBND.

Thakur *et al.* (1998) screened 60 soybean germplasm cultivars under field conditions during *kharif*, 1993 and spring, 1994. In this experiment KHJB 1, JS 84-1 and JS 71-05 germplasm cultivars were not infected, whereas JS 81-227, ES 5, JS 2, JS 79-81 and JS 340 were highly resistant to bud blight. The number of plants infected with bud blight was high at

flowering or pod initiation when compared to pre-flowering or podding. Infected plants were significantly higher in JS 75-46 than in JS 335.

Desai (1998) during *kharif* 1993 and *kharif* 1994 season recorded reaction of 137 groundnut genotypes to GBNV under conditions of natural infection in Northern Dry Zone – 3 (Region II) of Karnataka. The disease incidence ranged from 2.38 to 21.75 per cent with lowest (2.38 per cent) on ICG 2866 and highest (21.75 per cent) on ICG 2330. More than 20 per cent disease incidence was recorded on ICGS 4937, 2330 and 9320. Less than 5 per cent disease incidence was recorded in ICG 5323, ICG 2866, NRCG 1015, R13 and NRCG 4400 genotypes.

Thakur *et al.* (1999) reported average incidence of bud blight of soybean (caused by GBNV) from 5.39 to 15.65 per cent in different varieties of soybean grown in Raipur, India in *kharif*, 1996. The variety Bragg showed lowest incidence of disease (5.39 per cent) whereas, variety PK 472 showed highest disease incidence (15.65 per cent). JS 75-46 was found to be most susceptible to bud blight.

Sunkad *et al*. (2000) screened 172 groundnut collections during *kharif* and post rainy/summer seasons from 1996 to 1999 at Regional Research Station, Raichur. Screening was done under natural disease incidence conditions and observations were recorded one week before harvest of the crop based on standard disease rating scale (0-5). Seven highly resistant, 33 resistant, 52 moderately resistant, 53 moderately susceptible, 25 susceptible and 2 highly susceptible genotypes were recorded.

Forty-eight genotypes of soybean were evaluated during *kharif*, 2001 to identify field resistant sources to bud blight known to be caused by a strain of GBNV. All the genotypes showed visible disease symptoms. The lines MACS-754, NRC-55, VLS-55, JS-SH-96-04, TS-128-5, DSb-228 and SL-528 were found to be highly resistant (0.1-1 per cent infected plants), while the lines HIMSO-1597, PK-1308, DSb-3, MACS-756, RKS-7 and MACS-798 were moderately resistant against GBNV. Line JS-95-60 was highly susceptible (100 per cent mortality) to GBNV (Lal *et al.*, 2002).

Sreekanth *et al.* (2002b) screened 38 green gram genotypes under field conditions in Hyderabad, Andhra Pradesh, India, during 2000 *rabi* and 2001 *kharif* seasons for resistance to *Thrips palmi* and GBNV. LGG 460, 480, 491, and 582 genotypes consistently showed resistance to *T. palmi* in both 2000 (2.0-2.3 thrips per 5 terminals) and 2001 (4.7-5.0 thrips

per 5 terminals). The same genotypes also recorded resistance and moderate resistance to GBNV, in 2000 (7.1-10.0 per cent) and 2001 (12.5-15.0 per cent), respectively.

Thakare *et al*. (2002) screened 44 germplasm lines of groundnut at Oilseeds Research Station, Jalgaon during *kharif* 2000. Each line was of 5 m length and a spacing of 30 x 10 cm was used. Observations were recorded at pod filling stage and just before harvest. Six highly resistant, 8 resistant and 14 moderately resistant genotypes were identified during screening of groundnut germplasm for field resistance to GBND.

Nagaraja *et al.* (2005b) evaluated 12 groundnut genotypes during *kharif* 2003 at Bangalore, Karnataka under field conditions with three replications to assess their reaction to both GBNV and its vector thrips (*Thrips palmi*). The per cent incidence of the disease ranged from 2.84 to 24.75. Among the genotypes evaluated, GPBD-4, JSSP-9 and DH-53 were recorded lowest disease incidence. Genotypes, JL 24 and TMV-2 recorded the highest incidence ranging from 0.94 to 24.75 per cent and 0.43 to 22.05 per cent, respectively. Among the evaluated genotypes, numbers of thrips per plant ranged from 7.13 to 13.37 and 10.25 to 18.80 at 30 and 45 DAS, respectively, and were at par in all the genotypes.

Singh and Ali (2005) screened about 86 groundnut genotypes in Uttar Pradesh, India over four *kharif* seasons (1999-2002) against GBNV. Per cent disease incidence was recorded at 90 DAS in test entries and susceptible control (JL 24). The genotypes were classified as highly resistant (0-5 per cent), resistant (5.1-10.0 per cent), moderately susceptible (10.1-20 per cent), or highly susceptible (at least 20.1 per cent) based on disease reaction. Based on the overall performance of the genotypes over the years, 8 lines showed highly resistant reaction, 15 lines were resistant, 40 lines were moderately susceptible, and 23 lines were highly susceptible. The overall per cent disease incidence in the different genotypes varied from 1.5 to 40.6 per cent, while in JL 24, it was 42.4 per cent.

Kesmala *et al.* (2006) evaluated ten groundnut genotypes (KK 60-3, KKU 72-1, KKU 72-2, Luhera 11, Tainan 9, JL 24, IC 10, IC 34, ICGV 86031 and ICGV 86388) for their reaction to GBNV under field conditions in Thailand in 2001. The genotypes IC 10, IC 34, ICGV 86031 and ICGV 86388 were identified as good sources of GBNV resistance.

Gopal *et al.* (2010) tested 242 groundnut genotypes both in epiphytotic field and laboratory conditions. The genotypes *viz.,* ICGV 90009, 86699, 86329, 91177, 91234, 94252 and TG 26 were found promising both with low incidence of GBND and longer incubation periods.

Manjunatha *et al.* (2010b) screened 22 tomato varieties against bud blight disease caused by GBNV under field conditions in *summer* 2008. The disease incidence ranged from 10-100 %. Marikrit and NS- 2535 cultivars were moderately resistant and moderately susceptible, respectively. 18 cultivars were highly susceptible.

Krishnaiah *et al.* (2012) using 32 groundnut genotypes, screened for thrips to obtain resistant/tolerant genotypes at dry land farm of Sri Venkateshwara Agricultural College, Tirupati in 2010-2011 *rabi* seasons. The maximum leaf damage of 30-31 per cent leaf damage was noticed in K-6, ICG (FDRS-79), GPBD-4 and TCGS-1014 genotypes at 50 DAS.

Sain and Chadha (2012) conducted field experiments in 2007 and 2008 at AVRDC - The World Vegetable Center's-Regional Center for South Asia in Hyderabad to evaluate 30 improved lines of tomato for yield performance and field tolerance/resistance against *Tomato leaf curl virus* and GBNV. Yields in 2007 and 2008 ranged from 27.92 to 83.74 t ha^{-1} and 62 to 80 t ha^{-1}, respectively. In lines, DR2-1 (BL1173) (0.0 per cent) and NC 3220x57-27-3 (0.0 per cent) low GBND was recorded.

Srinivasraghavan *et al.* (2013) evaluated, a total of 419 interspecific derivatives of groundnut collected from Junagadh, Gujarat, India, during the *kharif* season of 2010 and *rabi* season of 2010-11 at MARS, Raichur , for yield and resistance to GBNV under natural infestation. The genotypes were classified as highly resistant (42 genotypes), resistant (77 genotypes), moderately resistant (135 genotypes), susceptible (148 genotypes) and highly susceptible (18 genotypes) based on their performance over two seasons. Seven highly resistant genotypes (CS-51, CS-55, CS-82, CS-86, CS-246, CS-262 and CS-268) and 13 resistant genotypes (CS-43, CS-45, CS-54, CS-73, CS-77, CS-83, CS-92, CS-94, CS-104, CS-137, CS-156, CS-202 and CS-212) were superior in terms of pod characteristics, shelling percentage (73.0-78.0 per cent), sound mature kernels (89.0-94.0 per cent) and 100-seed weight (29.0-36.0 g).

Ruth *et al.* (2013) screened 98 genotypes and cultivars of tomato under field conditions. Among the 50 NBPGR lines, 20 AICRIP genotypes, 5 IIHR genotypes and 23 cultivars and hybrids, EC-514117, EC-514190, LE-23, LE-30, Arka Vikas, Akra Abhaya and Akra Saurabh were found resistant to GBNV-Tomato isolate.

2.6 Screening of genotypes for vector and virus resistance sources

Dwivedi *et al*. (1995) reported that field resistance was the result of resistance to the vector, the virus, or a combination of both. 141 varieties and inter-specific derivatives of groundnut were evaluated in the field for resistance to the vector, on the basis of thrips injury on a 1-9 scale. Disease incidence was in the range of 4.8 per cent to 20 per cent with 54.4 per cent in JL 24 (susceptible control). Under controlled greenhouse conditions the vector-resistant genotypes were then screened for GBNV resistance by mechanical inoculation (using a 10^{-1} and 10^{-2} dilution of infected plant extract). Screening of about 42 genotypes for resistance to GBNV revealed all of them susceptible to GBNV at higher virus concentration (10^{-1}). ICGV 86031 and ICGV 86388 in addition to field resistance showed resistance to GBNV when mechanically sap was inoculated with low virus concentration (10^{-1}).

Pensuk *et al.* (2002) evaluated 6 groundnut genotypes (ICGV 86388, IC 34, IC 10, JL 24, Khon Kaen 60-1 and Khon Kaen 4) for their reaction to GBNV in the field (natural infestation) and greenhouse in Thailand in 2000 and 2001. The results indicated that differences among genotypes could be better observed at 40 DAS but not at 30 DAS. Lower field disease incidence was observed in ICGV 86388, IC 34 and IC 10 genotypes than in JL 24, Khon Kaen 60-1 and Khon Kaen 4 genotypes. Similar results were observed with greenhouse test. For breeding for resistance to GBNV, genotypes ICGV 86388, IC 34 and IC 10 were identified as potential resistant sources.

Reddy *et al.* (2000) evaluated 83 wild *Arachis* germplasm accessions, belonging to 24 species of five sections and one natural hybrid derivative of a cross between the cultivated and a wild *Arachis* species, along with a susceptible groundnut cultivar for resistance to GBNV in a replicated field trial at ICRISAT, Patancheru, India. One accession each of *A. benensis* and *A. cardenasii*, and two accessions of *A. villosa*, in the section *Arachis*, two accessions of *A. appressipila* in the section *Procumbentes*, and one accession of *A. triseminata* under section *Triseminatae* were not infected by GBNV. These 7 field-resistant accessions were tested under glasshouse conditions for virus resistance by mechanical sap inoculations. One accession of *A. cardenasii* and two accessions of *A. villosa* did not show systemic infection. In another glasshouse test, 13 *A. cardenasii* accessions of section *Arachis* were evaluated, two accessions did not show systemic infection. In all these resistant accessions, the inoculated leaves showed infection, but the systemic leaves did not show the presence of virus in spite of repeated mechanical sap inoculations. So, the resistance in these accessions appears to be due to a block in systemic movement of the virus. According to

them, this is the first report on the identification of resistance to GBNV in wild *Arachis* species.

Kalyani *et al.* (2005) screened eleven field resistant sources of GBND to TSV under glasshouse conditions at two virus concentrations (1:10 and 1:100) and at two plant ages (14 and 21 DAI). The results indicated that five genotypes ICGV 99029 (29.7 %), ICGV 01276 (34.2 %), ICGV 92267 (35.0 %) and ICGV 00068 (37.4 %) showed less TSV infection than JL 24 (68.6 %). These genotypes have also showed tolerance to GBND, rust and late leaf spot in addition to TSV making them good parents in multiple disease resistance breeding programs.

Ramana *et al.* (2006) selected 63 tomato entries (which included 20 cultivars, 36 genotypes and 7 wild species) for screening resistance to GBNV under field conditions during *kharif*, 2003. EC 5888 showed a highly resistant reaction, while EC 8630 and EC 26512 were resistant. Pusa Uphar, EC 251709, EC 35446, EC 165700, LE 23, IIHR 2187, IIHR 2272, IIHR 2273 and IIHR 2274 were moderately resistant. For confirmation of resistance, these field promising genotypes were further tested by mechanical sap inoculation in the greenhouse. Genotypes *viz.*, EC 8630 and EC 5888 were highly resistant; LE 23 and EC 26512 were resistant and EC 165700 displayed a moderately resistant reaction.

Rao *et al.* (2006) developed about 48 groundnut transgenic plants by using viral coat protein (CP) / nucleotide transgene (np) of GBNV through *Agrobacterium tumefacians* and micro-projectile mediated genetic transformation. Using two levels of concentrations in greenhouse conditions the progeny of transgenic plants were subjected to mechanical inoculation. At 1:100 concentration of disease leaf sap inoculum 24 to 36 transgenic plants did not develop any disease whereas at 1:50 concentration 24 plants exhibited no symptoms. On station field trials with these 24 transgenic plants showed similar results.

Kalyani *et al.* (2007) screened 56 germplasm accessions from 20 wild *Arachis* spp. in four sections (*Arachis, Erectoides, Procumbente*, and *Rhizomatosae*), along with susceptible peanut cultivars (JL 24 and K 1375) for resistance to TSV under greenhouse conditions using mechanical sap inoculation. Systemic virus infection, ranged between 0 and 100 % in the test accessions determined by ELISA. Twenty-four accessions in section *Arachis* that had 0 to 35 % systemically infected plants were retested, and systemic infection was not detected in eight of these accessions in repeated trials in the greenhouse. These were ICRISAT groundnut (ICG) accession nos. 8139, 8195, 8200, 8203, 8205, and 11550 belonging to *A. duranensis*; ICG 8144 belonging to *A. villosa*; and ICG 13210 belonging to *A. stenosperma*. Even though

the resistant accessions had 0 to 100 % TSV infection in inoculated leaves, TSV was not detected in the subsequently emerged leaves. According to them, this is the first report of TSV resistance in *Arachis* spp. The eight TSV resistant accessions were cross compatible with *A. hypogaea* for utilization in breeding for stem necrosis disease resistance.

Rao *et al.* (2013) developed over 200 transgenic lines of JL 24 using the gene encoding for the nucleocapsid protein (N gene) of GBNV. Using PCR, Southern hybridization, RT-PCR and western blot analysis, integration and expression of the transgenes was confirmed. By using mechanical sap inoculation at 1:100 and 1:50 dilutions of GBNV in the greenhouse T_1 and T_2 generation transgenic plants were assayed. Three transgenic plants from T2 generation showed considerable reduction in disease incidence in greenhouse and field. Out of them, only one transgenic plant showed over 75 per cent reduction in disease incidence when compared to untransformed control. This shows the partial and non-durable resistance to GBND using the viral N-gene.

Chapter III

MATERIALS AND METHODS

Chapter III

MATERIALS AND METHODS

Present investigation on "Identification of resistant sources to Groundnut Bud Necrosis Disease (GBND) in groundnut (*Arachis hypogaea* L.) genotypes" was carried out at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Andhra Pradesh, India.

3.1 Survey on incidence and severity of Groundnut Bud Necrosis Disease

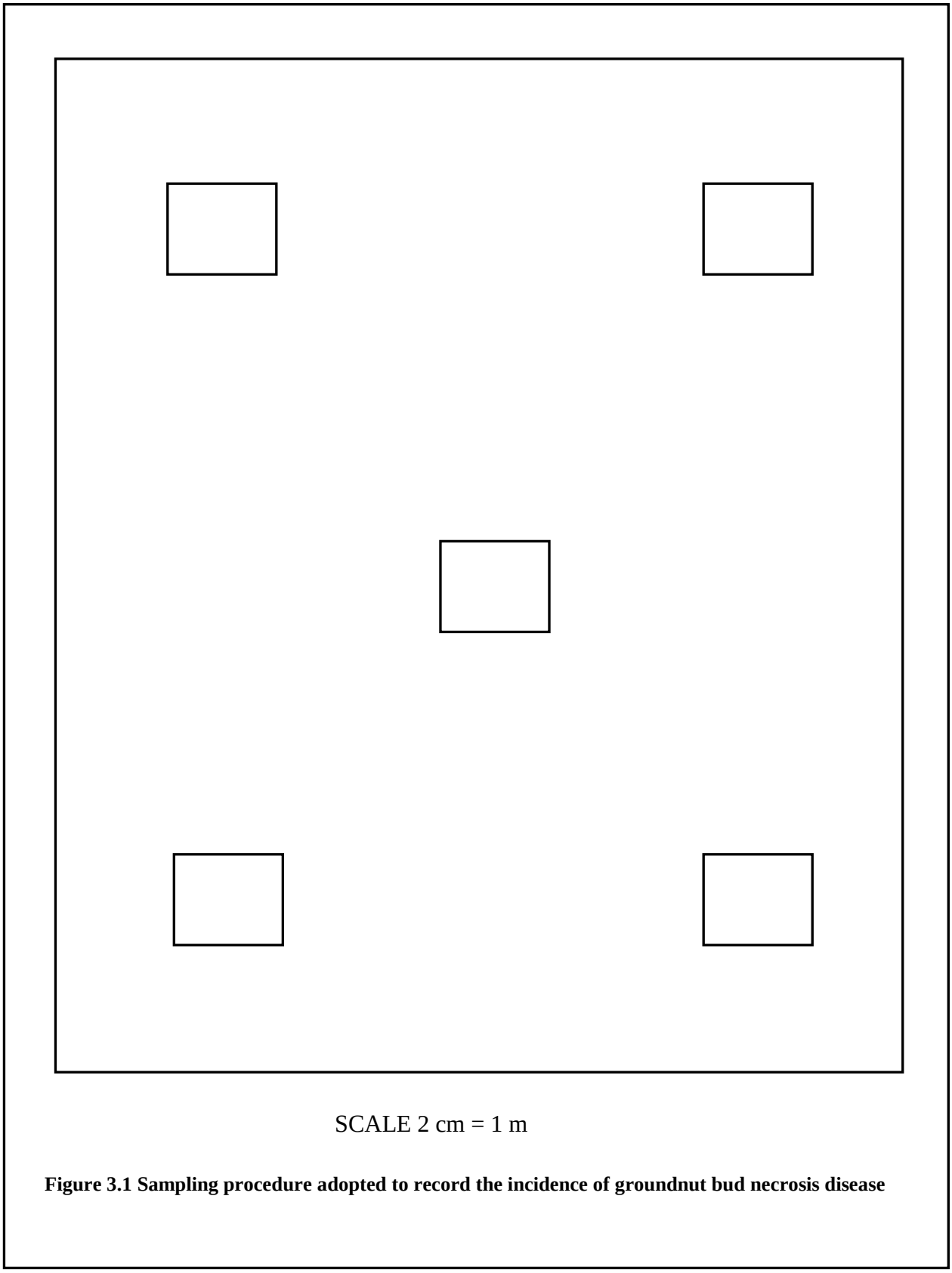
A survey was undertaken in farmers' fields for the incidence and severity of GBND in Anantapur (*kharif* 2013 and *rabi* 2013-14), Warangal and Karimnagar (*rabi* 2013-14) districts of Andhra Pradesh, India.

Anantapur and Warangal are among the major groundnut growing districts of Andhra Pradesh. Karimnagar district was selected, since Jagtial region is a hot spot for GBND. To assess GBND incidence and severity a minimum of two major groundnut growing mandals in each district and a minimum of two villages in each mandal were surveyed.

In the selected field, 1 m^2 area in each of the four corners leaving border rows and another 1 m^2 area at the center was observed (Fig. 3.1) to record the incidence of GBND (Arunkumar *et al.,* 2006 and Upendhar, 2004).

The number of infected plants and the total number of plants in that area were recorded. Per cent disease incidence was calculated by using the following formula (Sunkad *et al.,* 2000)

$$PDI = \frac{\text{Number of GBND infected plants}}{\text{Total number of plants}} \times 100$$



SCALE 2 cm = 1 m

Figure 3.1 Sampling procedure adopted to record the incidence of groundnut bud necrosis disease

Disease severity (DS) scoring was given following five point (1-5) scale in the fields surveyed (Pensuk *et al.*, 2002).

Rating Scale	Description of symptoms
1	No disease symptoms
2	No systemic symptoms but with spots on some leaves
3	Systemic symptoms with top chlorosis but no stunting
4	Systemic symptoms with strong leaf distortion and stunting
5	Severe necrosis and stunting

Samples showing typical GBND symptoms such as leaves with chlorotic spots, chlorotic top leaves, stunted plants with auxiliary shoot proliferation and distorted leaves were collected from the fields visited in air sealed polythene bags and kept in vasculum containing ice. The samples were subjected to Direct Antigen Coating - Enzyme Linked Immuno Sorbent Assay (DAC-ELISA) in the laboratory using polyclonal antiserum of *Groundnut Bud Necrosis Virus* (GBNV) (Source: ICRISAT, Patancheru, India) to confirm the presence of virus as described in 3.4 of this section.

During the survey, information on source of seed material, name of the cultivar/variety, soil type, cropped area, stage of the crop, type of symptoms, and weeds found in and around the field were also recorded.

3.2 Screening for field resistance to groundnut bud necrosis disease

3.2.1 Planting material/Source of seeds

Seeds of 42 groundnut advanced breeding lines including resistant check (ICGV 86031) and susceptible check (JL 24) were obtained from groundnut breeding program, ICRISAT, Patancheru, Hyderabad.

3.2.2 Seed Treatment

Seed treatment with thiram (3g kg^{-1} seed) was done prior to sowing in the field. Sufficient quantity of synthetic adhesive was added and shaken well to form uniform coating over the seed. Then required quantity of seed dressing chemical was added to the gum coated seeds and shaking was continued till uniform coverage of seeds with the fungicide was obtained. The treated seeds were dried in shade and used for sowing in the field.

3.2.3 Screening of groundnut advanced breeding lines

To determine the incidence and severity of GBND in groundnut advanced breeding lines, a field trial was conducted at ICRISAT, Patancheru, Hyderabad in Alpha Lattice Design using 42 treatments including resistant and susceptible check in three replications during *kharif* 2013. The crop was sown deliberately late in the season (In the month of August) anticipating more disease pressure. The layout of experimental field is presented in Fig. 3.2 and Plate 3.1.

Each genotype (treatment) was sown in three rows of 4 m each with 60 cm distance between rows and 25 cm distance between plants. All the recommended package of practices was followed and field was irrigated on need basis. Weeding operations was done manually twice at 30 and 60 days after sowing (DAS).

Table 3.1 List of groundnut advanced breeding lines screened for GBND resistance

Treatment	Genotype	Growth Habit	Duration	Traits for selection
1	ICGV 99058	SB	MEDIUM	FDR
2	ICGV 99072	SB	MEDIUM	FDR
3	ICGV 00162	SB	MEDIUM	FDR
4	ICGV 00187	SB	MEDIUM	FDR
5	ICGV 00189	SB	MEDIUM	FDR
6	ICGV 00191	SB	MEDIUM	FDR
7	ICGV 00201	SB	MEDIUM	FDR
8	ICGV 00202	SB	MEDIUM	FDR
9	ICGV 00203	SB	MEDIUM	FDR
10	ICGV 00206	SB	MEDIUM	FDR

11	ICGV 00211	SB	MEDIUM	FDR
12	ICGV 00213	SB	MEDIUM	FDR
13	ICGV 00241	VB	MEDIUM	FDR
14	ICGV 00246	VB	MEDIUM	FDR
15	ICGV 00247	VB	MEDIUM	FDR
16	ICGV 86590	SB	MEDIUM	FDR
17	ICGV 86699	VB	MEDIUM	FDR
18	ICGV 91114	SB	SHORT	SD
19	ICGV 00308	SB	SHORT	SD
20	ICGV 03042	SB	MEDIUM	MD
21	ICGV 03057	SB	MEDIUM	DR
22	ICGV 06100	SB	MEDIUM	MD
23	ICGV 07222	SB	MEDIUM	DR
24	ICGV 07220	SB	MEDIUM	DR
25	ICGV 05155	SB	MEDIUM	DR
26	ICGV 06146	SB	MEDIUM	FDR
27	ICGV 02266	SB	MEDIUM	DR
28	ICGV 87846	VB	MEDIUM	DR
29	ICGV 93468	SB	SHORT	SD
30	ICGV 00348	SB	MEDIUM	DR
31	ICGV 00350	SB	MEDIUM	DR
32	ICGV 00351	SB	MEDIUM	DR
33	ICGV 93260	SB	MEDIUM	FDR
34	ICGV 93261	SB	MEDIUM	FDR
35	ICGV 89280	SB	MEDIUM	MD
36	ICGV 92195	SB	SHORT	SD

37	ICGV 92035	SB	SHORT	SD
38	ICGS 44	SB	MEDIUM	MD
39	ICGS 76	VB	MEDIUM	MD
40	ICR 48	VB	MEDIUM	DR
41	ICGV 86031 (Resistant check)	SB	SHORT	SD
42	JL 24 (Susceptible check)	SB	SHORT	SD

Source: ICRISAT, Patancheru, India.

SB – Spanish bunch VB – Virginia bunch

SD – Short duration MD – Medium duration FDR – Foliar Disease Resistance

DR – Drought resistant

3.2.4 Observations

In each treatment, data on plant stand, disease incidence and severity were recorded. The data collected in different observations were statistically analyzed as per the design.

3.2.4.1 Plant stand in each treatment

In each treatment, number of germinated plants was recorded at 12 DAS

3.2.4.2 Incidence of the disease

The incidence of GBND was recorded against total plant stand at 30, 45, 60, 75 and 90 DAS in each treatment by counting the diseased plants and per cent disease incidence was calculated as described in 3.2 of this section.

REPLICATION-I					
Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**
9	6	22	41	33	40
21	30	20	42	15	25
1	24	17	8	10	2
4	5	23	37	19	39
7	34	18	27	36	14
29	26	12	31	13	35
32	16	38	11	28	3
REPLICATION-II					
Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**
26	37	25	35	16	15
41	30	20	42	13	36
9	18	28	33	14	11
10	19	8	17	7	39
38	5	6	34	3	12
40	32	27	21	22	4
23	2	29	1	31	24
REPLICATION-III					
Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**
35	10	5	28	8	27
11	30	33	26	41	24
9	16	12	7	22	21
15	25	2	14	39	40
18	23	29	17	19	20
3	4	31	37	1	13
6	42	38	36	34	32

Plot size: 4 x 1.8 m

Spacing: 60 x 25 cm

1 - 40: Advanced breeding lines

41: Resistant check; 42: Susceptible check

Figure 3.2 Layout of the field trial for screening of genotypes against groundnut bud necrosis disease in alpha lattice design

Disease reaction: Based on per cent disease incidence, the test lines were categorized into six distinct group using 0-5 scale (Sunkad *et al.,* 2000).

Scale	Disease Incidence (%)	Grade
0	0-1.0	Highly resistant
1	1.1-5.0	Resistant
2	5.1-10.0	Moderately resistant
3	10.1-25.0	Moderately susceptible
4	25.1-50.0	Susceptible
5	50.1 and above	Highly susceptible

3.2.4.3 Severity of the disease

The disease severity of GBND was recorded at 30, 45, 60, 75 and 90 DAS in each treatment based on the symptom intensity of the infected plants following five point scale (1-5) as described in 3.2 of this section by randomly tagging five plants treatment^{-1}.

3.2.4.4 Confirmation through DAC-ELISA

The groundnut advanced breeding lines, resistant and susceptible checks screened under natural infestation of the vector *Thrips palmi* were further tested serologically using polyclonal antiserum of GBNV by DAC-ELISA as described in the section 3.4.

3.3 Screening to groundnut genotypes to differentiate vector and virus resistant sources

3.3.1 Isolation and maintenance of the virus

Young leaves showing typical symptoms of GBND were collected from naturally infected plants of cv. JL 24. The standard extract of the inoculum was prepared, by grinding collected samples in a chilled mortar and pestle using phosphate buffer (0.05M, pH 7.0) @ 1:10 (w/v). After homogenizing, the pulp was squeezed through muslin cloth and filtrate was used as inoculum. The virus inoculum was multiplied on cv. JL 24 at 3-4 leaf stage by mechanical inoculation using standard extract of the virus (Plate 3.2). In order to maintain purity of the virus, young infected tissues showing primary symptoms with distinct chlorotic lesions were transmitted to JL 24 and maintained for further use.

Plate 3.1 Screening of groundnut advanced breeding lines against GBNV during *kharif* 2013

Plate 3.2 Preparation of standard extract of the virus

3.3.2 Sap Transmission

Buffer used for sap inoculation

Phosphate buffer (0.05 M; pH 7.0)

Potassium di- hydrogen phosphate (KH_2PO_4) : 2.4 g

Di- potassium hydrogen phosphate (K_2HPO_4) : 5.4 g

Thioglycerol : 0.75 ml

Distilled water : 1000 ml

A small quantity of fine carborundum powder was dusted on the leaves of test plants before inoculation. The inoculum was rubbed on the upper surface of young leaves with the help of pestle previously dipped in the inoculum. During inoculation, the leaves were supported from below with left hand palm to avoid any injury and to assure uniform pressure and spread of inoculum. The inoculated leaves were washed immediately with a fine jet of distilled water using wash bottle to remove excess inoculum and carborundum powder. The plants were maintained in an insect proof greenhouse for about six weeks to observe development of symptoms.

3.3.3 Test Plants

For greenhouse screening, 40 groundnut advanced breeding lines together with resistant and susceptible checks were raised in plastic pots (5" diameter) @ 3 seeds pot^{-1} in three replications under insect proof conditions (Plate 3.3). Ten days old (3-4 leaf stage) seedlings were inoculated with freshly prepared standard extract of virus inoculum @ 1:10 and 1:100 concentrations. Suitable uninoculated controls were maintained. The plants were observed for the development of symptoms up to six weeks under greenhouse conditions.

3.3.4 Observations

In each replication, disease incidence and severity of the disease was recorded.

3.3.4.1 Incidence of the disease

The incidence of GBND was recorded on total plant stand at weekly intervals after sap inoculation in each replication by counting the diseased plants and per cent disease incidence was calculated as described in 3.2 of this section.

3.3.4.3 Severity of the disease

The disease severity of GBND was recorded at weekly intervals in each replication based on the symptom intensity of the infected plants following five point scale (1-5) as described in 3.2 of this section.

3.4.4.4 Confirmation through DAC-ELISA

The groundnut advanced breeding lines, resistant and susceptible check screened under insect proof greenhouse conditions were further tested serologically by DAC-ELISA using polyclonal antiserum of GBNV as described in 3.4 section.

3.4 Serology

The serological relationship of the virus causing GBND was studied using polyclonal antiserum of GBNV by DAC-ELISA (Hobbs *et al.,* 1987).

Leaf samples showing typical bud necrosis symptoms collected from farmers' field during survey, test samples of genotypes from field and greenhouse screening were used for serological studies.

3.4.1 Direct Antigen – Coated Enzyme Linked Immunosorbent Assay (DAC - ELISA)

3.4.1.1 Materials

- **ELISA plates:** 'Greiner labortechnik' 96 well polystyrene microtitre plates
- **Micropipettes:** 1- 40 µl, 40-200 µl and 200-1000 µl single channel pipettes. 40-200 µl multichannel pipette of Finpipette.
- **ELISA plate reader:** Bio-RAD iMark Microplate reader provided with 405 nm filter.
- GBNV polyclonal antibodies
- ALP-labelled anti-rabbit (goat antibodies)
- Penicillinase enzyme
- Mortars and pestles
- Muslin cloth
- pH meter
- p- nitro phenyl phosphate (PNPP)
- Light box
- Incubator
- Refrigerator

3.4.1.2 Solutions

3.4.1.2.1 Carbonate buffer or coating buffer, pH 9.6

Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g
Distilled water	1.0 l

1.71 g of sodium diethyl dithiocarbamate (DIECA) was added after dissolving the above two compounds.

3.4.1.2.2 Phosphate buffer saline (PBS), pH 7.4

Na_2HPO_4	1.19 g
KH_2PO_4	0.2 g
KCl	0.2 g
NaCl	8.0 g
Distilled water	1.0 l

3.4.1.2.3 Phosphate buffered saline Tween (PBS-T)

PBS	1.0 l
Tween- 20	0.5 ml

3.4.1.2.4 Antibody buffer (PBS-TPO)

PBS –T	100 ml
Polyvinyl pyrrolidine	2.0 g
Ovalbumin	0.2 g

3.4.1.2.5 Healthy leaf extract

Healthy groundnut leaf tissue	1 g
Antibody buffer	20 ml
DIECA	0.1 g

3.4.1.2.6 Substrate buffer (diethanolamine buffer) for ALP system

10 % diethanolamine was prepared in distilled water and stored at 4 ^{0}C. pH was adjusted to 9.8 with conc HCl. 0.5 mg ml^{-1} PNPP in 10 % diethanolamine, pH 9.8 (for each 5 mg tablet 10 ml substrate buffer was used) solution was freshly prepared.

3.4.1.3 Procedure

GBNV infected samples macerated with carbonate coating buffer @ 100 mg ml^{-1} (1:10 w/v) were dispensed into new ELISA plate @ 150 µl / well and incubated in humid chamber at 37 ^{0}C for 1h. The plate was washed three times with PBS-T, allowing 3 min between each wash. GBNV polyclonal antiserum which was diluted in PBS-TPO to 1:20,000 was dispensed into each well @ 150 µl and the plate was covered and incubated in humid chamber at 37 ^{0}C for 1h. Subsequently, the plate was washed with PBS-T for three times allowing 3 min between each wash. Anti-rabbit ALP-conjugate was diluted to 1:5000 in PBS-TPO and dispensed into each well @ 150 µl. The plate was covered and incubated in humid chamber at 37 ^{0}C for 1h. The plate was then washed with PBS-T three times allowing 3 min for each wash. 150 µl of PNPP substrate was dispensed into each well of the plate and was incubated in dark at room temperature for 15-20 min. The reaction was stopped by adding 50 µl of 3 M NaOH per well. Absorbance values were measured in an ELISA plate reader (Bio

RAD iMark Microplate reader) (Plate 3.4.) at 405 nm. The reaction was considered positive, if there was change in substrate color to yellow and the absorbance value was five times higher than healthy sample (-ve control).

3.5 Weather Parameters

The data pertaining to the different weather parameters during growth period of field experiment and during survey period in Warangal, Karimnagar and Anantapur districts were obtained from Resiliant Dryland Systems (ICRISAT), Regional Agricultural Research Station (Warangal and Jagtial, Karimnagar) and Agricultural Research Station (Kadiri, Anantapur) respectively.

3.6 Statistical Analysis

ANOVA was performed using PROC MIX SAS 9.3 to determine the difference in disease incidence and severity data collected in field experiment.

Plate 3.3 Screening of groundnut genotypes against GBNV by sap inoculation under greenhouse conditions

Plate 3.4 ELISA Reader (BioRAD iMark Microplate reader)

Chapter IV

RESULTS AND DISCUSSION

Chapter - IV

RESULTS AND DISCUSSION

The results and discussion pertaining to the present investigation are presented in the following sections.

4.1 Survey for the occurrence of groundnut bud necrosis disease

A survey for assessing the incidence of groundnut bud necrosis disease (GBND) was undertaken in major groundnut growing areas of Anantapur district of Andhra Pradesh during *kharif* 2013 and *rabi* 2013-14. Similar, survey was undertaken in Karimnagar and Warangal districts of Andhra Pradesh, India during *rabi* of 2013-14. Groundnut cultivar kadiri-6 (K-6) was seen prominently in all the districts surveyed. In surveyed areas, the crop was sown between last week of June to first week of August (*kharif*) and first fortnight of September to first fortnight of November (*rabi*).

A total of 42 fields, spread over 23 villages of three districts were surveyed during *rabi,* 2013-14 which revealed widespread occurrence of GBND in Anantapur district. Different types of symptoms such as chlorotic spots, chlorosis of top leaves, severe leaf distortion and severe necrosis with stunting was observed in the fields surveyed. Disease incidence ranged from 0 to 20 per cent, with mean maximum incidence of 8.50 per cent in Anantapur district and minimum of 0.94 per cent in Warangal district.

The details of GBND incidence, disease severity observed in the surveyed areas are furnished in Tables 4.1 to 4.4, Figs. 4.1 to 4.4 and Plates 4.1 to 4.4).

4.1.1 Anantapur district

Survey carried out in Gorantla, Puttaparthi, Amadagur and Obuladevaracheruvu mandals during *kharif,* 2013 revealed zero per cent disease incidence and disease severity of 1 on 1-5 scale.

Mean GBND incidence in the district during *rabi* 2013-14 was 8.50 per cent with disease severity of 2 and 3 on 1-5 scale. Mandal-wise mean GBND ranged from 5.63 per cent in Obuladevaracheruvu mandal to 12.75 per cent in Nallamada mandal. The highest mean disease incidence of 13 per cent was recorded in Mulappagaripalli village of Nallamada mandals. The lowest disease incidence of 2.5 per cent was recorded in Gachiguntapalli

village of Obuladevaracheruvu mandal.

4.1.2 Warangal district

Mean GBND in the district during *rabi* 2013-14 was 0.94 per cent with disease severity of 1 and 2 on 1-5 scale. Mandal-wise mean GBND ranged from 0.63 per cent in Mahabubabad mandal to 1.25 per cent in Kuravi mandal. Highest disease incidence of 3.75 per cent was recorded in Mogilicherla village of Kuravi. Bethole village of Mahabubabad recorded disease incidence of 2.5 per cent, whereas no disease incidence was recorded in Rajole, Narayanapur villages of Kuravi and in Laxmipur, Reddial, Ammangal villages of Mahabubabad mandal.

4.1.3 Karimnagar district

Mean GBND in the district during *rabi* 2013-14 was 0.97 per cent with disease severity of 1 and 2 on 1-5 scale. Mandal wise mean GBND in the district ranged from 0 per cent in Korutla mandal to 1.67 per cent in Mallapur mandal. Highest disease incidence of 5 per cent was recorded in Raghavapeta village of Mallapur mandal. Vempet village of Metpalle mandal recorded disease incidence of 2.5 per cent. Muthampet, Mallapur villages of Mallapur mandal, Regunta of Metpalle mandal and in Joganpalle, Venkatapur villages of Korutla mandal were free from the disease.

In the present study, the disease incidence varied within the mandals of a district and among the districts surveyed. The maximum mean incidence of GBND under field conditions was only 13 per cent across the locations surveyed in major groundnut growing areas. This may be attributed to the amount of inoculum, presence of thrips, agronomic practices followed and weather conditions that prevailed during the susceptible stage of the crop.

Survey carried out in *kharif* 2013 in Anantapur district revealed no disease incidence and samples tested for GBNV by DAC-ELISA showed negative results. Similar findings were reported (Vemana, 2014) during survey carried out in Kadiri, Mudigubba, Nallacheruvu and Nallamada mandals of Anantapur district. The weed, *Parthenium hysterophorus*, which is a reservoir host to thrips has come to flowering late in the season

Table 4.1 Incidence of GBND in different districts of Andhra Pradesh during *rabi*, 2013-14

Districts	No. of mandals	No. of villages	No. of fields surveyed	*Per cent disease incidence		Cultivars
				Range	Average	
Anantapur	4	9	15	0-20	8.50	K-6
Warangal	2	7	14	0-7.5	0.94	K-6
Karimnagar	3	7	13	0-10	0.97	K-6
Total	**9**	**23**	**42**	**0-20**	**3.47** (average)	

*Based on apparent disease symptoms in the field.

Table 4.2 Incidence of GBND in different mandals of Anantapur during *rabi*, 2013-14

Name of the mandal / village	No. of fields surveyed	Per cent disease incidence based on symptoms		Severity (1-5 scale)
		Range	Average	
Mudigubba				
Malakavemula	2	10-15	12.5	2
Yerravankapalli Tanda	2	2.5-5	3.75	2
	4	**2.5-15**	**8.13**	
Obuladevaracheruvu				
Mittapalli	2	5-12.5	8.75	2
Gachiguntapalli	1	0-5	2.50	2
	3	**0-12.5**	**5.63**	
Kadiri				
Kadiri	1	0-10	5.00	2
Patanam	2	5-20	12.50	3
Kalasamudram	1	0-10	5.00	2
	4	**0-20**	**7.50**	
Nallamada				
Nallamada	2	10-15	12.50	3
Mulappagari palli	2	12-14	13.00	3
	4	**10-15**	**12.75**	
Total	**15**	**0-20**	**8.50**	

Table 4.3 Incidence of GBND in different mandals of Warangal during *rabi*, 2013-14

Name of the village / mandal	No. of fields surveyed	Per cent disease incidence based on symptoms		Severity (1-5 scale)
		Range	Average	
Kuravi				
Mogilicherla	2	0-7.5	3.75	2
Rajole	2	0	0	1
Narayanapur	2	0	0	1
	06	**0-7.5**	**1.25**	
Mahabubabad				
Bethole	2	0-5	2.50	2
Laxmipur	2	0	0	1
Reddial	2	0	0	1
Ammangal	2	0	0	1
	08	**0-5**	**0.63**	
Total	**14**	**0-7.5**	**0.94**	

Table 4.4 Incidence of GBND in different mandals of Karimnagar during *rabi*, 2013-14

Name of the village / mandal	No. of fields surveyed	Per cent disease incidence based on symptoms		Severity (1-5 scale)
		Range	Average	
Mallapur				
Muthampet	2	0	0	1
Raghavapeta	1	0-10	5	2
Mallapur	2	0	0	1
	5	**0-10**	**1.67**	
Metpalle				
Vempet	2	0 -5	2.5	2
Regunta	2	0	0	1
	4	**0-5**	**1.25**	
Korutla				
Joganpalle	2	0	0	1
Venkatapur	2	0	0	1
	4	**0**	**0**	
Total	**13**	**0-10**	**0.97**	

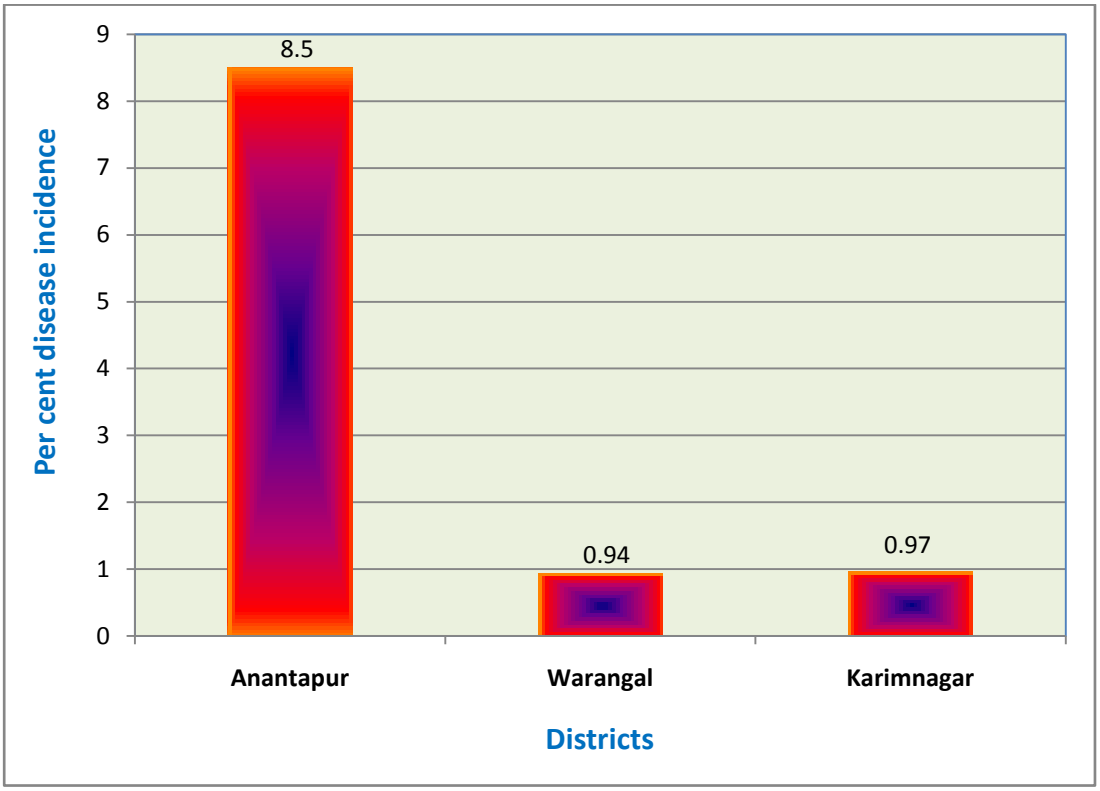


Figure 4.1 Incidence of GBND in different districts of Andhra Pradesh during *rabi*, 2013-14

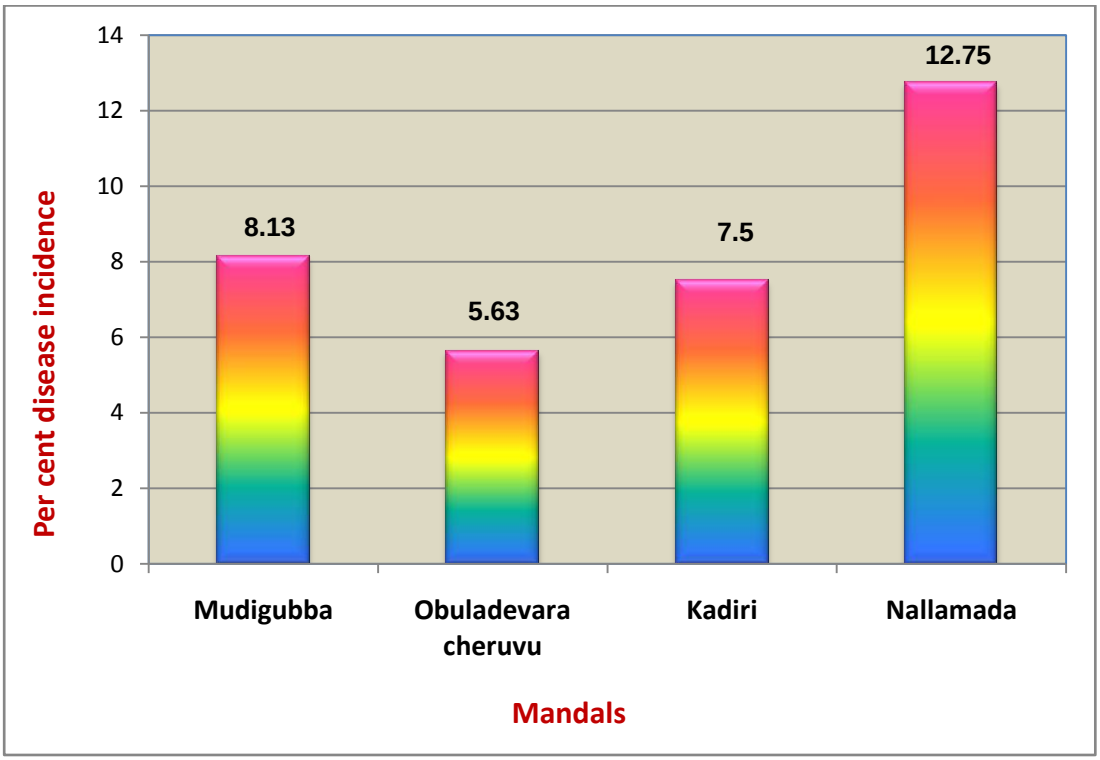


Figure 4.2 Incidence of GBND in different mandals of Anantapur during *rabi*, 2013-14

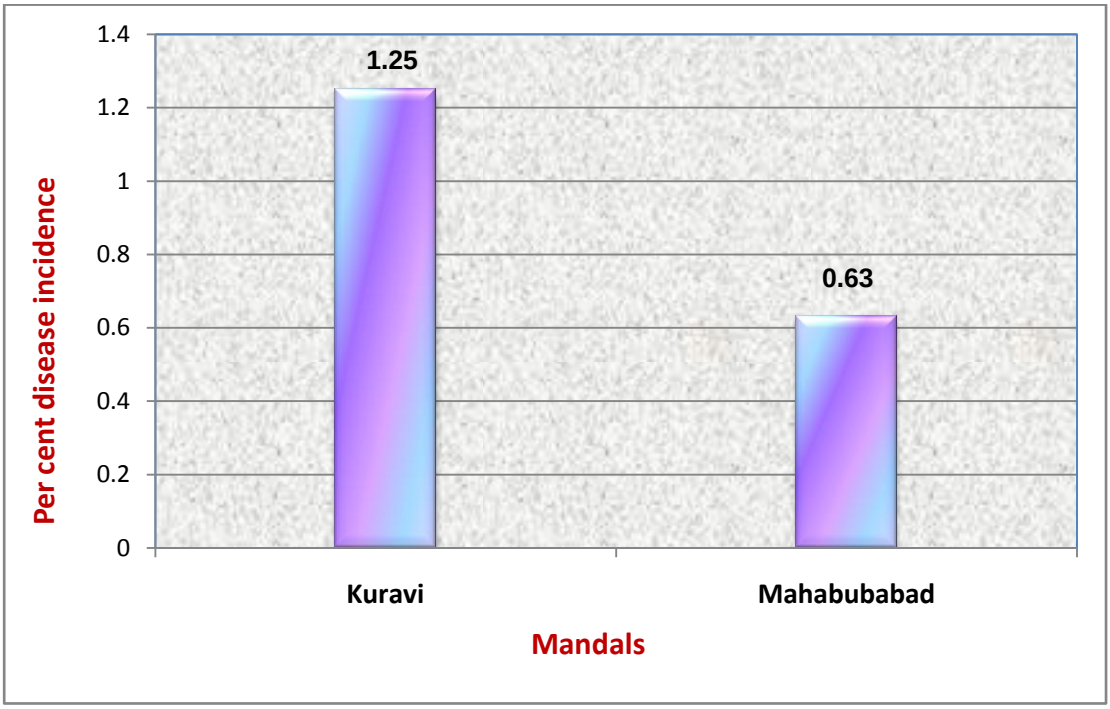


Figure 4.3 Incidence of GBND in different mandals of Warangal during *rabi*, 2013-14

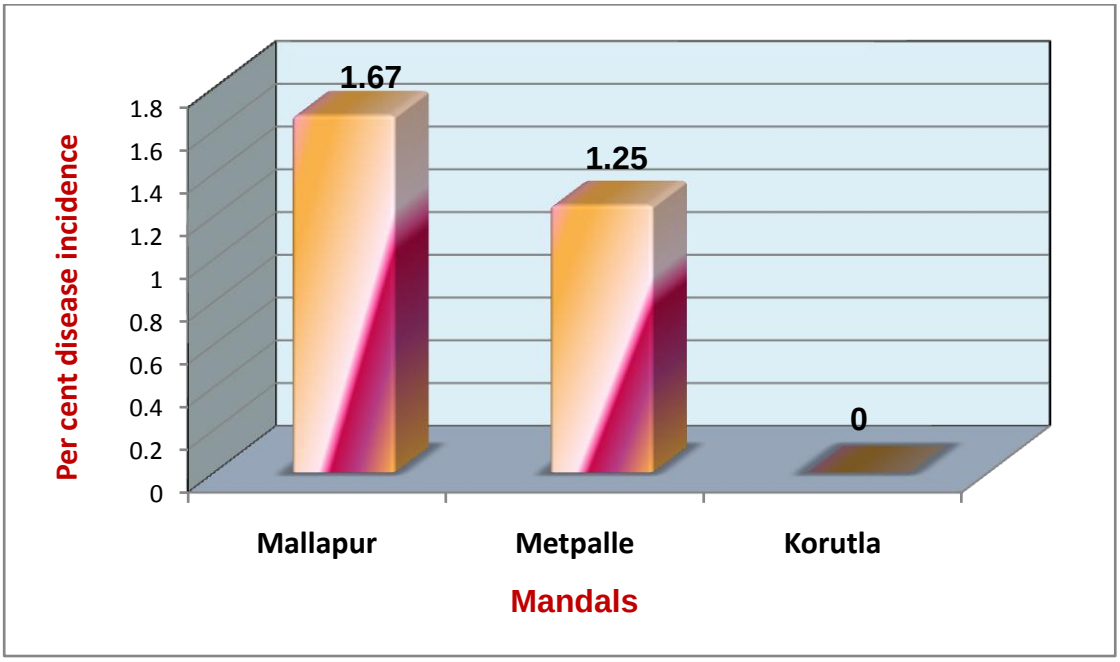


Figure 4.4 Incidence of GBND in different mandals of Karimnagar during *rabi*, 2013-14

Plate 4.1 Groundnut field in Anantapur district during *kharif* 2013

Plate 4.2 Groundnut field in Anantapur district during *rabi* 2013-14

due to delayed rains during *kharif* 2013 in Anantapur district. So, the thrips population might have declined due to the absence of reservoir host. The flowering of *Parthenium* did not coincided with the susceptible stage of groundnut, thereby resulting in less disease incidence in *kharif*.

Since the survey was conducted only one time during the growth period, incidence might have occurred in later stages. The other probable reason might be the incidence of groundnut stem necrosis disease (GSND) caused by *Groundnut stem necrosis virus* (GBNV) in *kharif* season whose symptoms are similar to GBNV at later stages.

Rao *et al.* (2003b) reported the epidemic of GSNV in the rainy season of 2000 in Anantapur district. The disease caused the estimated losses of more than Rs. 3 billion (US $ 65 million). Survey conducted by Rao *et al.* (2003c) during *kharif* 2001 to *kharif* 2004 in Anantapur district indicated the presence of both GBNV and GSNV through ELISA test.

It is an established fact that GBND incidence was found to be more in *kharif* and less in *rabi* seasons. This may be attributed to the decreased activity of the thrips during *rabi* due to low temperatures. Several researchers reported significantly lower incidence of thrips population (Reddy *et al.,* 1983, Sreenivasulu, 1994 and Gopal *et al.,* 2011) and GBNV incidence in groundnut and other crops during *rabi*. Wider spacing, improper agronomic practices and late sowing of crop in Anantapur district during *rabi,* 2013-14 could have contributed to the increase in disease incidence.

In Karimnagar and Warangal districts, groundnut is predominantly grown during *rabi* season under irrigated conditions and hence survey was carried out during *rabi*, 2013-14. Dense cropping, intercropping with fast growing cereals and good agronomic practices that eliminated reservoir host could have contributed to low disease incidence in these districts during *rabi*, 2013-14 (Plates 4.3 and 4.4)

The field survey also indicated the difference in incidence of the disease with the crop age as the incidence of GBND was more at vegetative stage than at later developmental stages of the crop. The difference in GBND incidence may also be attributed to the presence of thrips population at the susceptible stage of the crop. Further, thrips population is also influenced by weather conditions prevailing in an area.

Plate 4.3 Dense cropping in Karimnagar district during *rabi* 2013-14

Plate 4.4 Border crop with fast growing cereals in Warangal district during *rabi* 2013-14

Occurrence of GBNV in groundnut and other crops of Andhra Pradesh, India were reported by several workers. Pande and Rao (2000) reported 4 - 25 per cent incidence of GBND in Chittoor, 10 - 25 per cent disease incidence in Kadapa, 3 - 18 per cent disease incidence in Anantapur, 6 -15 per cent disease incidence in Kurnool, 4-16 per cent disease incidence in Mahabubnagar districts of Andhra Pradesh during *kharif* 1999.

Gopal *et al.* (2011) reported higher incidence of GBND in greengram, blackgram, cowpea and soybean than in groundnut. The highest mean incidence of 17.81 $\pm$ 4.23 per cent (range of 10.3 – 24.7 per cent) was recorded in Karimnagar during rainy season and 25.59 $\pm$ 4.11 per cent (range of 19.8–29.1 per cent) in post rainy season in Rangareddy district. The lowest incidence of 8.94 $\pm$ 3.58 per cent (range of 4.3–13.3 %) was recorded in Kurnool district of Andhra Pradesh.

Jagadeeshwar *et al.* (2005) reported the occurrence of 18.5 per cent GBNV in major chilli growing areas of Northern Telangana zone in Andhra Pradesh during survey conducted in *kharif* 2000, 2001 and 2002.

Rao *et al.* (2003a) reported the occurrence of leaf curl incidence on mung bean ranging from 0.24 to 18.94 and 14.12 to 33.96 per cent in Nalgonda, Khammam, Medak, Warangal and Karimnagar districts of Telangana region during *kharif* 2000 – 01 and 2001-02 seasons respectively. In Guntur, Krishna and Prakasam districts, the leaf curl incidence on urd bean (*Vigna mungo*) ranged from 10.04 to 11.98 per cent in the 2001- 02 *rabi* season and 2.92 - 5.73 per cent in Guntur and Krishna district on urd bean grown in rice fallow.

4.1.2 Detection of GBNV through DAC- ELISA

Visual diagnosis of GBNV can be done easily, however *Tomato spotted wilt virus* (TSWV) and *Tobacco streak virus* (TSV) produce symptoms similar to GBNV (Srinivasaraghavan *et al.,* 2011). So, identification of disease based on symptoms alone is often unreliable. To confirm the virus causing GBND, DAC- ELISA was used to test the samples collected during survey in Anantapur, Karimnagar and Warangal districts.

Details of DAC-ELISA results pertaining to Anantapur district are furnished hereunder (Table 4.5 and Plate 4.5).

The samples collected from Karimnagar and Warangal district did not test positive for GBNV. In Anantapur district, results were in correlation with observed symptoms with

Table 4.5 Detection of virus causing GBND in groundnut samples collected from Anantapur district during *rabi* 2013-14 through DAC ELISA

S. No.	Mandal	Village	No. of samples	Absorbance Value (405nm) range	Per cent infection based on ELISA
1	**Mudigubba**	Malakavemula	6	0.342 - 3.703	83.33
		Yerravankapalli Tanda	3	0.554 - 2.648	33.33
2	**Obuladevaracheruvu**	Mittapalli	8	0.534 - 2.222	62.50
		Gachiguntapalli	3	0.661 - 2.590	33.33
3	**Kadiri**	Kadiri	3	0.252 - 2.275	66.67
		Patanam	5	0.386 - 2.786	80.00
		Kalasamudram	3	0.132 - 0.642	33.33
4	**Nallamada**	Nallamada	6	1.951 - 2.731	83.33
		Mulappagari palli	4	0.144 - 1.951	75
	GBNV (+ ve control)		2	2.470 - 2.472	100
	Healthy (–ve control)		2	0.135 - 0.145	0
	Buffer control		2	0.454 - 0.798	0

Note: The samples were considered positive when the absorbance values were five times higher than healthy samples (-ve control).

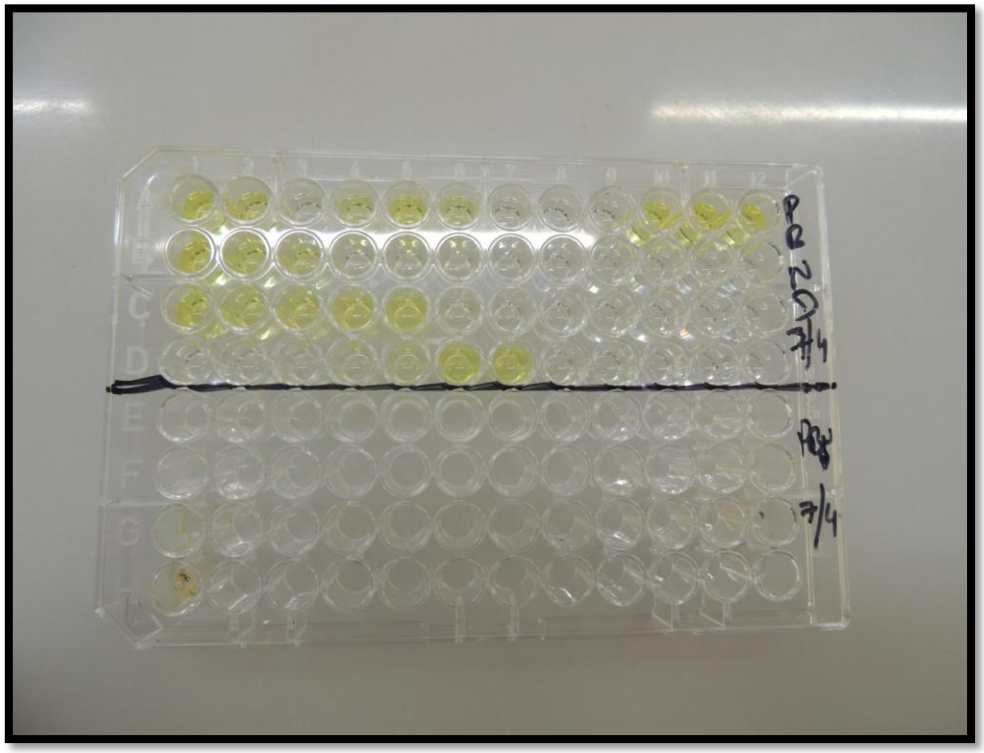


Plate 4.5 Detection of GBNV in groundnut samples collected from Anantapur district during *rabi* 2013 -14 by DAC-ELISA

0.554 - 2.648), Gachiguntapalli (0.661 - 2.590) and Kalasamudram (0.132 - 0.642) villages of Mudigubba, Obuladevaracheruvu and Kadiri mandal respectively. Highest per cent infection of 83.33 per cent was found in Malakavemula (0.342 - 3.703) and Nallamada (1.951 - 2.731) of Mudigubba and Nallamada mandal respectively. This discrepancy in results might be due to similar kind of symptoms such as necrosis, yellowing and wilting, necrosis of petiole, top growing bud and stem, auxiliary shoot proliferation with small leaflets, stunting, reduction in pod size and seed discoloration found due to infection of GBNV and TSV (Vemana and Jain, 2012).

Rao *et al*. (2003a) subjected 372 leaf curl samples of mung bean and urd bean collected from different districts of Andhra Pradesh to DAC-ELISA. Only 337 samples were tested positive to GBNV.

Srinivasaraghavan *et al*. (2011) confirmed the occurrence of GBND in different parts of north eastern Karnataka by subjecting the samples collected during survey to polyclonal antiserum of GBNV and TSWV. All the samples showed positive reaction with GBNV and negative reaction with TSWV confirming the disease incidence.

4.1.3 Survey for weed flora

A total of fifteen weed species were observed in three districts of Andhra Pradesh (Plates 4.6 to 4.8) in all the surveyed fields across locations and seasons.

In the present study, the weed species *viz., P. hysterophorus*, *Celosia argentea, Tridax procumbens, Achyranthus aspera* and *Cynodon dactylon* were found in all groundnut growing areas, and where incidence of GBND was recorded.

Occurrence and distribution of weed flora in and around groundnut fields surveyed during *rabi* 2013-14 season is presented in Table 4.6. In Anantapur district, frequency of occurrence of *P. hysterophorus* was maximum (82 %) followed by *C. argentea* (42 %) and *T. procumbence* (18 %). In Warangal, *P. hysterophorus* was maximum (22 %) followed by *Cyperus rotundus* (17 %) and *C. dactylon* (11 %) whereas in Karimnagar *P. hysterophorus* was maximum (15 %), followed by *C. dactylon* (13 %) and *C. argentea* (12 %).

Since, *P. hysterophorus* was very predominant weed both in cultivated and barren fields in the three districts of Andhra Pradesh surveyed, it is quite possible that *P. hysterophorus* being perennial can serve as reservoir of GBNV and act as primary source

Table 4.6 Occurrence and distribution of weed species in groundnut fields in different districts of Andhra Pradesh during *rabi*, 2013-14

District	Name of the weed	Frequency (%)
Anantapur	*Parthenium hysterophorus* L.	82
	Celosia argentea L.	42
	Argemone mexicana L.	11
	Tridax procumbens L.	18
	Dactyloctenium aegyptium (L.) Willd	01
	Blainvillea acmella Cass.	01
	Cymbopogan refractus Spreng.	02
	Leucas aspera L.	01
	Achyranthus aspera L.	09
	Cyperus rotundus L.	03
	Cynodon dactylon (L.) Pers.	05
Karimnagar	*Amaranthus viridis* L.	06
	Parthenium hysterophorus L.	15
	Celosia argentea L.	12
	Achyranthus aspera L.	08
	Tridax procumbens L.	10
	Cynodon dactylon (L.) Pers.	13
Warangal	*Cleome viscose* L.	02
	Phyllanthus niruri Auct.	07
	Cyperus rotundus L.	17
	Cynodon dactylon (L.) Pers.	11
	Commelina bengalensis L.	05
	Celosia argentea L.	03
	Parthenium hysterophorus L.	22
	Tridax procumbens L.	04
	Achyranthus aspera L.	01

Parthenium hysterophorus

Commelina bengalensis

Celosia argentea

Achyranthus aspera

Tridax procumbens

Plate 4.6 Weed species found in and around groundnut fields during survey in major groundnut growing areas of Andhra Pradesh

Amaranthus viridis

Argemone Mexicana

Cleome viscose

Phyllanthus niruri

Dactyloctenium aegyptium

Plate 4.7 Weed species found in and around groundnut fields during survey in major groundnut growing areas of Andhra Pradesh

Blainvillea acmella

Cymbopogan refractus

Leucas aspera

Cyperus rotundus

Cynodon dactylon

Plate 4.8 Weed species found in and around groundnut fields during survey in major groundnut growing areas of Andhra Pradesh.

of inoculum to groundnut throughout the season. Therefore, measures to eliminate *Parthenium* from field bunds, waste lands and from within the crop was expected to be beneficial in reducing the incidence of GBND. The incidence of disease in groundnut may be correlated with the presence of infected *Parthenium* plants in and around groundnut crop.

Asymptomatic weeds (eg. *Parthenium*) that harbour the virus as well as thrips and produce copious pollen throughout season act as a primary source of inoculum initiating and sustaining the TSV infection in groundnut during a crop season. Thrips colonizing flowers of *Parthenium* can become externally contaminated with pollen and their further movement to new hosts results in introduction of the virus into fields (Rao *et al.*, 2003 b & c).

Host range studies of *Thrips palmi* carried out by Vijayalakshmi (1995) revealed presence of more than 10 thrips per 25 terminals on *P. hysterophorus* and *C. bengalensis*, 1 to 10 thrips 25 per terminals on *C. argentea, Cleome viscosa* and *Amaranthus viridis* and no thrips on *T. procumbens, Dactyloctenium aegyptium and Phyllanthus niruri*. This study clearly indicated *P. hysterophorus, C. bengalensis, C. viscosa, A. viridis* and *C. argentia* as reservoir hosts to thrips.

Gopal *et al.* (2011) reported *A. aspera* and *C. benghalensis* as alternate hosts for GBND.

Since the earlier workers clearly revealed the possible role of some of the weeds as alternate host to GBND, the role of other weed species *viz*., *Argemone mexicana, C. dactylon, Cyperus rotundus, Leucas aspera, Blainvillea acmella, Cymbopogan refractus,* in harboring the virus causing GBND during different seasons of the year, needs to be further investigated. This would demonstrate the extent to which these weed hosts play a role in survival and spread of the virus causing GBND occurring in major groundnut growing areas of the state surveyed.

4.2 SYMPTOMATOLOGY

The symptoms of GBND were studied in detail both under natural and artificial inoculated conditions.

4.2.1 Field symptoms

Under field conditions, the first recognizable symptoms of the disease was noticed when the crop was 25 - 30 days old. Primary symptoms appeared as mild chlorotic spots on young, quadrifoliate leaves (Plate 4.9). The disease extended to the petiole, leading to chlorosis of top leaves (Plate 4.10), necrosis of the terminal bud (Plate 4.11) and ultimately death of plants in early infected ones (Plate 4.12). Secondary symptoms included stunting, (Plate 4.13) auxiliary shoot proliferation and malformation of leaflets (Plate 4.14).

4.2.2 Greenhouse symptoms

The symptoms on groundnut genotypes under artificially inoculated conditions were studied. Mechanically sap inoculated plants at four leaf stage showed chlorotic spots (Plate 4.15) after 7 days of inoculation. These chlorotic spots later turned necrotic (Plate 4.16). Newly produced leaves showed severe chlorosis symptoms (Plate 4.17) in 15 - 20 days after inoculation. Prominent brown streaks were observed on petiole leading to bending of plant (Plate 4.18). It ultimately led to necrosis of terminal bud and death of plants (Plate 4.19).

In the present study, GBND produced similar symptoms in natural and artificial conditions with variations in severity. Variations in symptoms and severity of GBND was influenced by age of the plant during infection, disease pressure applied, presence of vector and the prevailing environmental conditions.

Symptoms of GBND were described by several workers (Delfosse *et al.*, 1995; Thakur *et al.*, 1996; Srinivasaraghavan *et al.*, 2011 and American Phytopathological Society, 2013) both under natural and artificial inoculated conditions, which are in conformity with present findings.

4.3 SCREENING OF GROUNDNUT GENOTYPES FOR VECTOR RESISTANCE UNDER NATURAL CONDITIONS

4.3.1 Incidence of GBND in groundnut genotypes during *kharif* 2013

The data pertaining to incidence of GBND in groundnut genotypes is presented in Table 4.7 and Figs. 4.5 to 4.9.

SYMPTOMS OF GBND OBSERVED UNDER FIELD CONDITIONS

Plate 4.9 Chlorotic spots on leaves

Plate 4.10 Severe leaf chlorosis

Plate 4.11 Necrosis of terminal bud

Plate 4.12 Death of plants

Plate 4.13 Stunting of plants

Plate 4.14 Auxiliary shoot proliferation and malformation of leaflets

SYMPTOMS OF GBND OBSERVED UNDER ARTIFICAL INOCULATED CONDITIONS

Plate 4.15 Chlorotic spots on leaves

Plate 4.16 Severe necrotic spots on leaves and malformation of leaflets

Plate 4.17 Severe chlorosis of top leaves

Plate 4.18 Brown streaks on petiole

Plate 4.19 Severe necrosis and death of plants

Table 4.7 Incidence of GBND in groundnut genotypes during *kharif* 2013

S. No.	Genotype	Per cent Disease Incidence* at					Grade
		30DAS	45DAS	60DAS	75DAS	90DAS	
1	ICGV 99058	4.22	9.32	11.49	11.49	11.49	MS
2	ICGV 99072	3.95	5.59	10.65	10.65	10.65	MS
3	ICGV 00162	4.22	6.93	9.01	10.75	11.44	MS
4	ICGV 00187	0.86	4.36	6.99	6.99	6.99	MR
5	ICGV 00189	2.42	2.42	6.36	7.84	8.58	MR
6	ICGV 00191	0.66	4.30	5.89	6.72	6.72	MR
7	ICGV 00201	1.45	3.57	4.99	4.99	4.99	R
8	ICGV 00202	1.60	5.22	5.91	5.91	6.61	MR
9	ICGV 00203	0.84	3.42	5.13	5.13	5.13	MR
10	ICGV 00206	0.03	2.60	3.65	5.52	6.56	MR
11	ICGV 00211	0.81	1.58	4.02	4.02	4.02	R
12	ICGV 00213	1.47	4.38	5.93	5.93	5.93	MR
13	ICGV 00241	1.79	4.21	6.34	7.35	7.35	MR
14	ICGV 00246	4.04	6.17	7.07	7.07	7.07	MR
15	ICGV 00247	2.40	5.53	7.07	7.07	7.07	MR
16	ICGV 86590	6.38	9.58	9.58	10.23	10.23	MS
17	ICGV 86699	0.63	2.48	3.10	4.33	4.33	R
18	ICGV 91114	7.98	19.09	22.71	22.71	22.71	MS
19	ICGV 00308	3.82	10.72	10.72	10.72	10.72	MS
20	ICGV 03042	2.08	4.20	4.20	4.92	4.92	R
21	ICGV 03057	3.34	5.03	5.71	5.71	5.71	MR
22	ICGV 06100	2.59	4.14	4.92	5.79	6.67	MR
23	ICGV 07222	0.71	3.07	6.04	6.04	6.04	MR
24	ICGV 07220	0.63	1.25	1.89	2.57	2.57	R
25	ICGV 05155	2.09	4.40	5.04	5.87	6.71	MR
26	ICGV 06146	1.40	2.18	3.63	4.31	4.31	R

Table 4.7 contd..							
27	ICGV 02266	3.80	6.94	6.94	7.57	8.20	MR
28	ICGV 87846	1.22	4.34	6.21	6.21	6.21	MR
29	ICGV 93468	4.09	11.75	13.08	13.08	13.08	MS
30	ICGV 00348	2.17	2.92	5.90	7.45	7.45	MR
31	ICGV 00350	2.02	2.64	2.64	2.64	2.64	R
32	ICGV 00351	2.74	2.74	3.36	3.36	3.36	R
33	ICGV 93260	1.99	3.40	4.73	5.38	5.38	MR
34	ICGV 93261	2.47	6.83	8.08	8.70	8.70	MR
35	ICGV 89280	3.18	7.03	7.73	7.73	7.73	MR
36	ICGV 92195	2.92	6.51	7.93	8.67	8.67	MR
37	ICGV 92035	3.74	7.58	8.30	9.12	9.93	MR
38	ICGS 44	3.40	8.17	8.89	9.57	10.21	MS
39	ICGS 76	3.00	4.42	4.42	5.12	5.12	MR
40	ICR 48	0.03	1.20	2.51	5.16	6.47	MR
41	ICGV 86031 (Resistant check)	1.34	4.04	4.04	4.04	4.04	R
42	JL 24 (Susceptible check)	4.88	10.88	18.78	20.96	25.45	S
	Mean of all genotypes	2.51	5.41	6.94	7.51	7.81	

Per cent disease incidence				
Effect	Num DF	Den DF	F Value	Pr > F
GEN	41	77.5	2.63	0.0001
TIME	4	338	94.74	<.0001
GEN*TIME	164	324	1.24	0.0513

*Mean of three replications

SAS analysis was performed and the values mentioned are angular transformed values

R- Resistant; MR- Moderately Resistant; MS- Moderately Susceptible

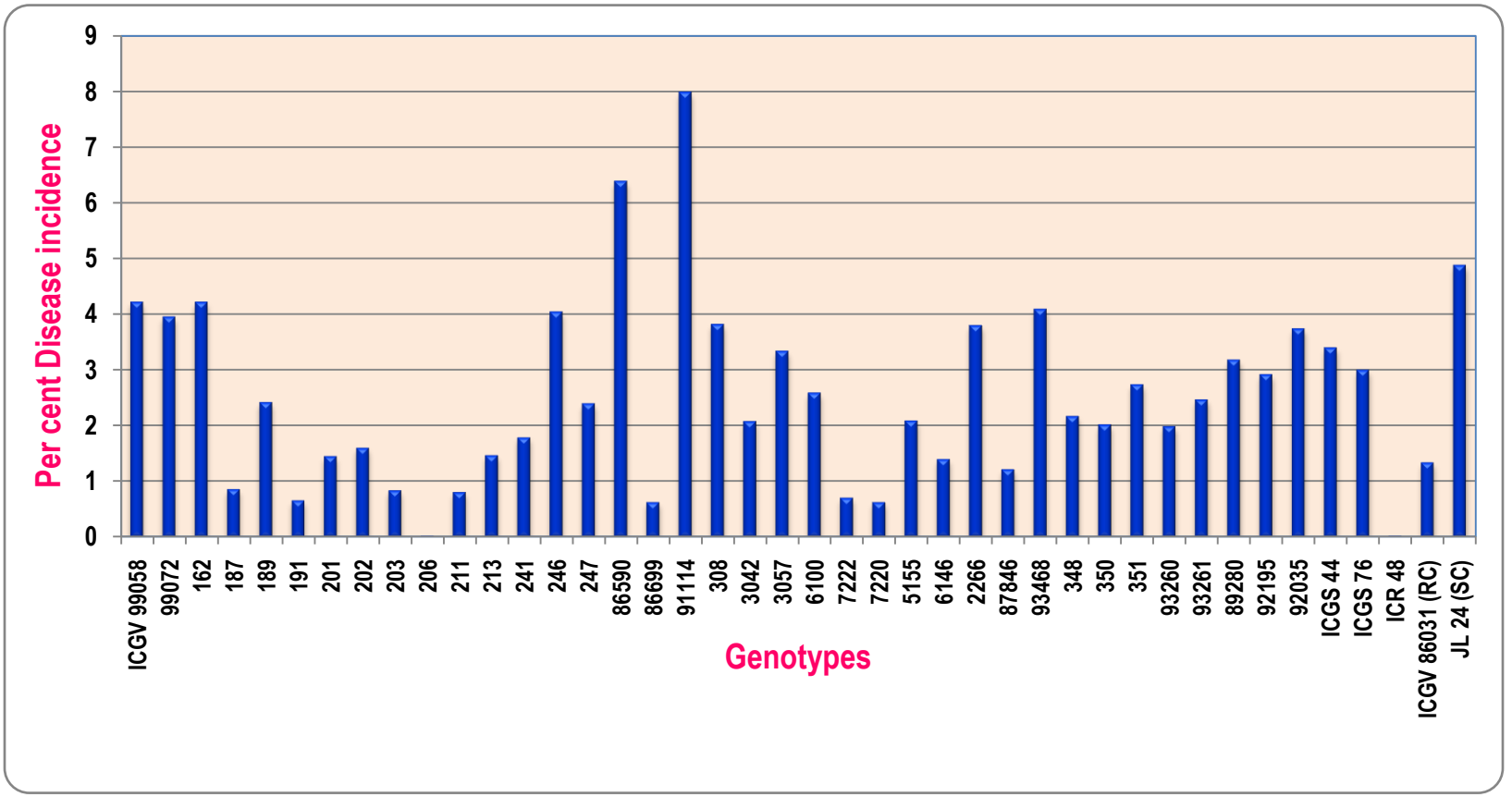


Figure 4.5 Incidence of GBND in groundnut genotypes during *kharif* 2013 at 30 DAS

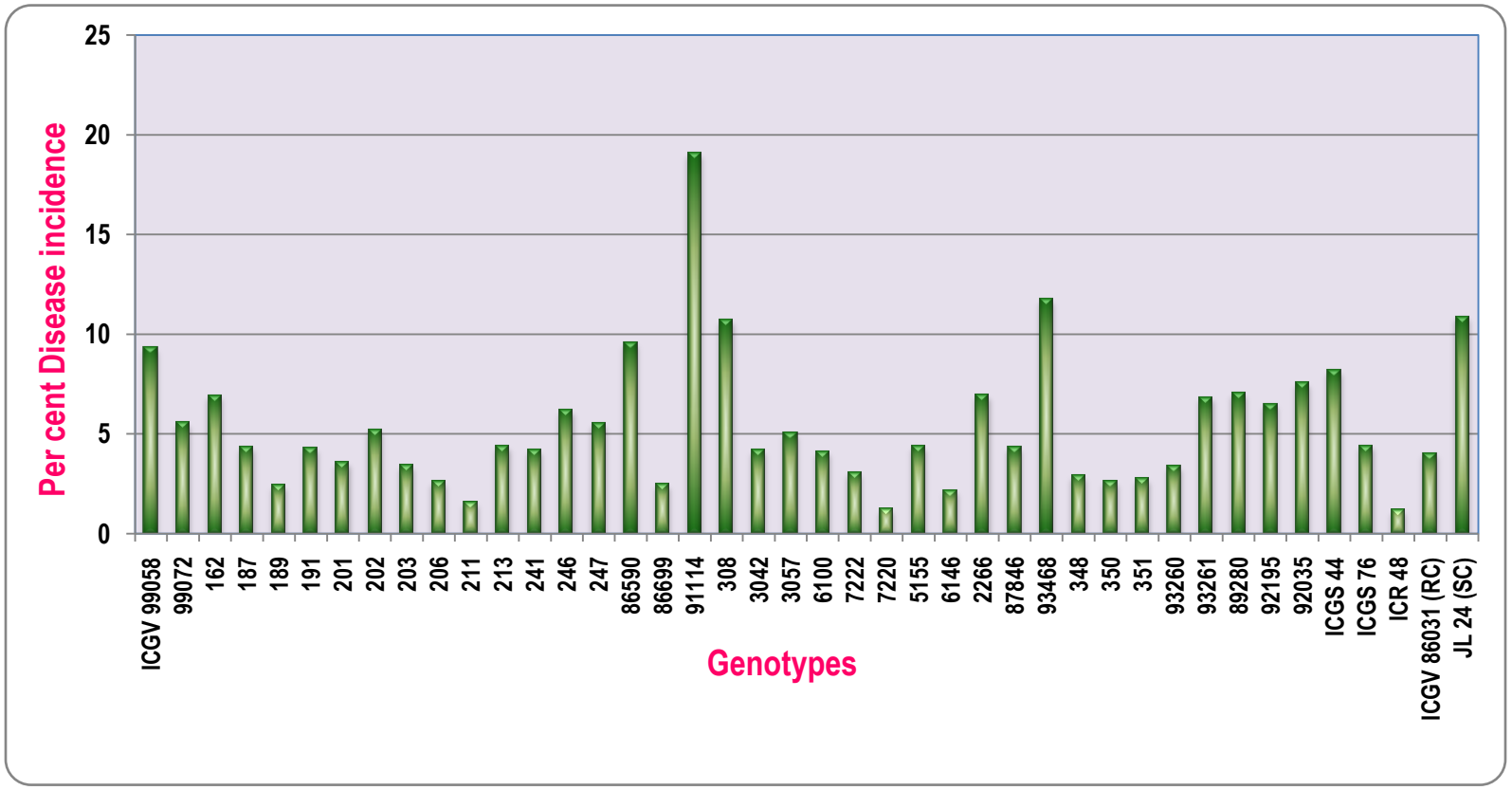


Figure 4.6 Incidence of GBND in groundnut genotypes during *kharif* 2013 at 45 DAS

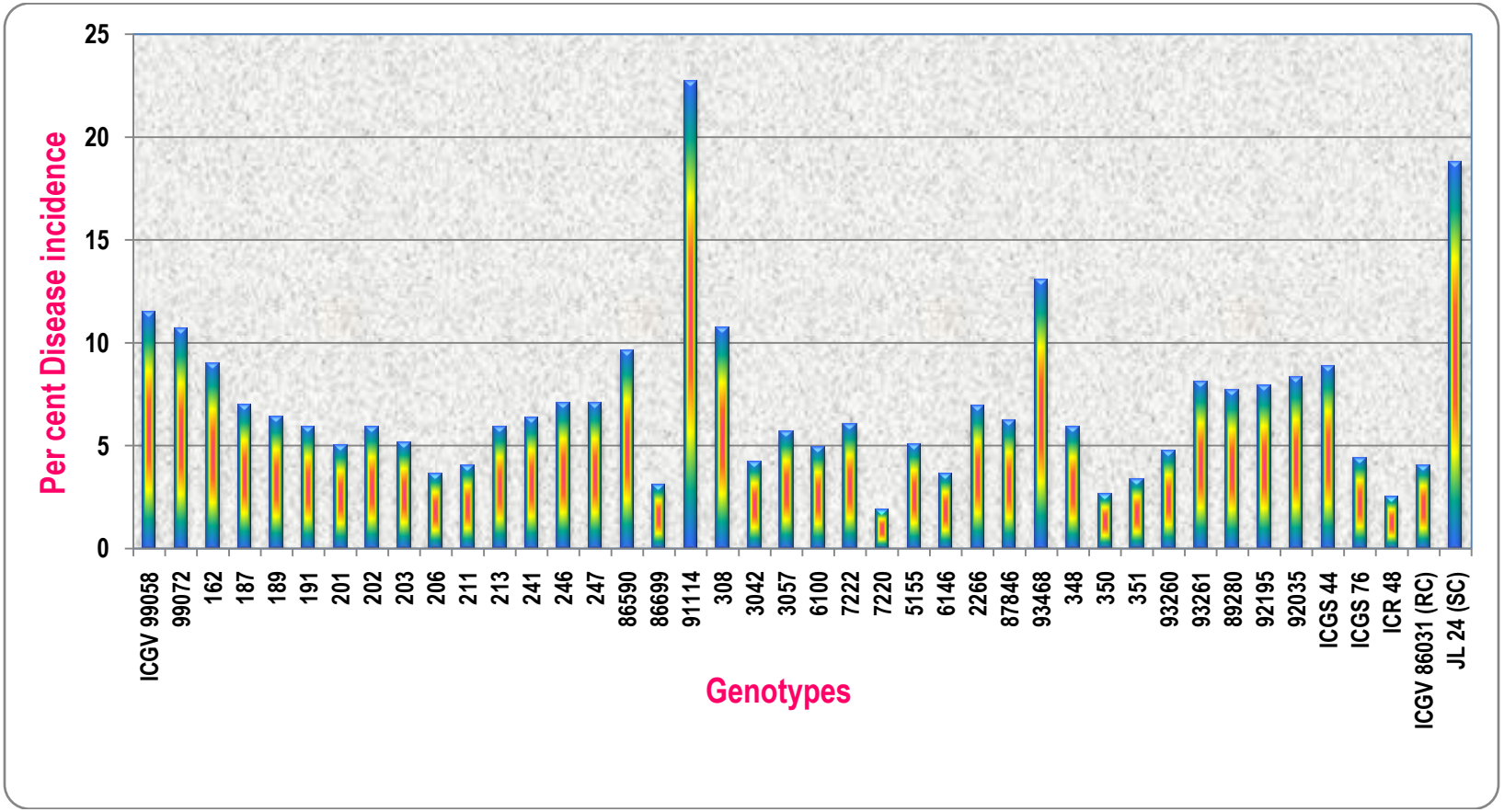


Figure 4.7 Incidence of GBND in groundnut genotypes during *kharif* 2013 at 60 DAS

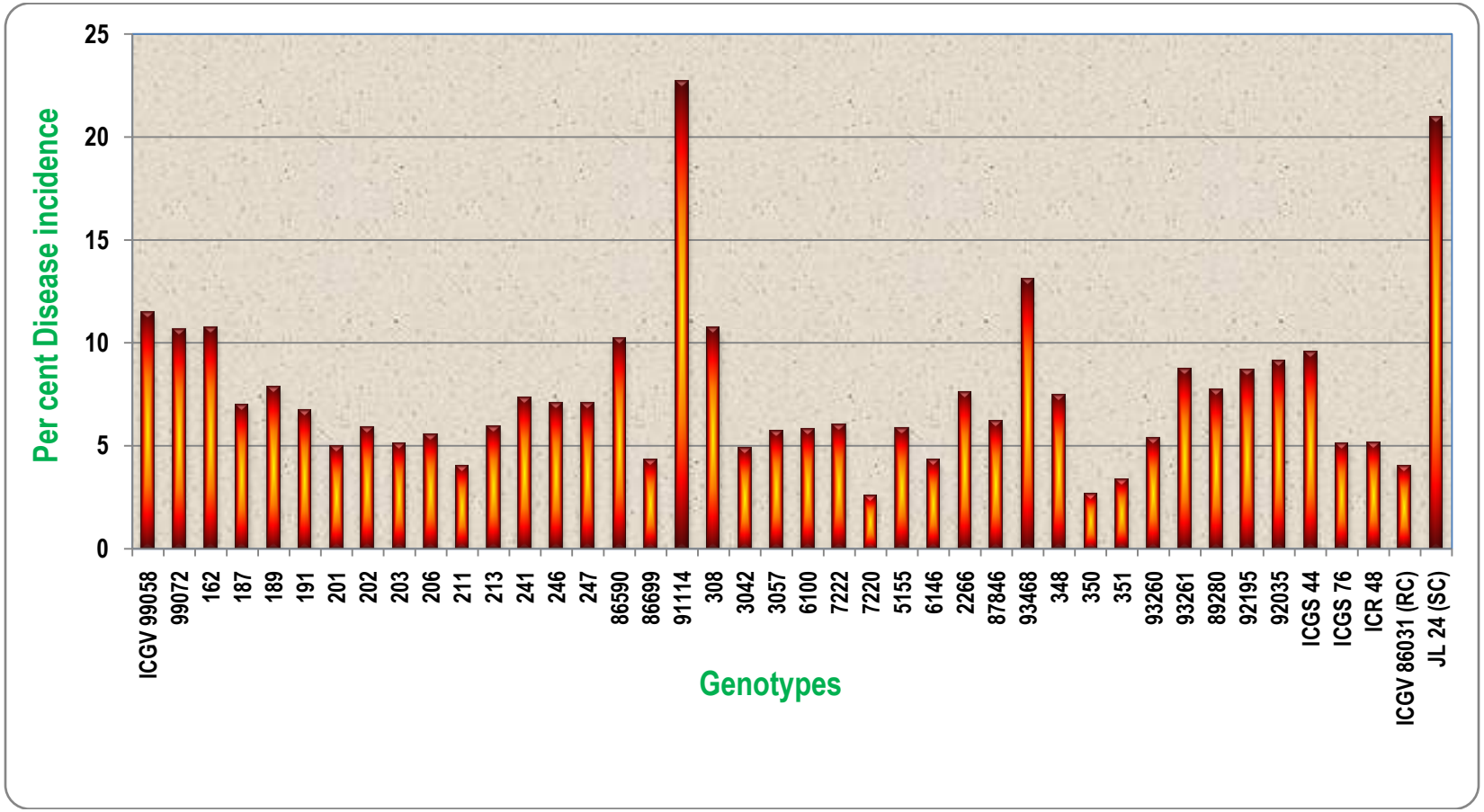


Figure 4.8 Incidence of GBND in groundnut genotypes during *kharif* 2013 at 75 DAS

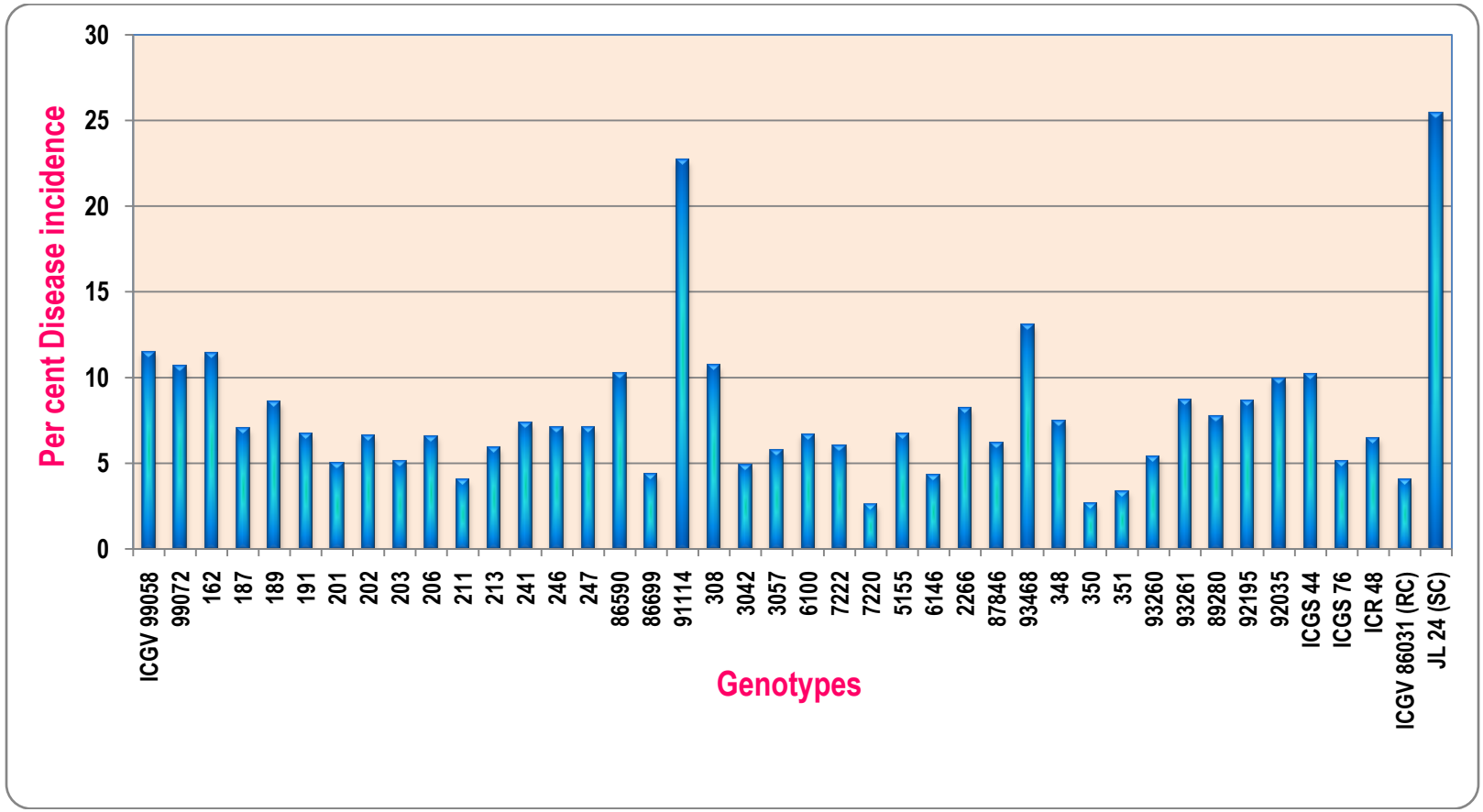


Figure 4.9 Incidence of GBND in groundnut genotypes during *kharif* 2013 at 90 DAS

The average GBND incidence in the tested genotypes ranged from 2.57 to 22.71 per cent compared to 4.04 per cent in ICGV 86031(Plate 4.20) (resistant check) and 25.45 per cent in JL 24 (Plate 4.21) (susceptible check).

The data revealed that the per cent disease incidence ranged from 0.03 to 7.98 at 30 DAS, 1.20 to 19.09 at 45 DAS, 1.89 to 22.71 at 60 DAS, 2.57 to 22.71 at 75 DAS and 2.57 to 25.45 at 90 DAS among the genotypes. The data also revealed that there was a progressive increase in mean disease incidence from 2.51 (30 DAS) to 7.81 (90 DAS) per cent.

The typical symptoms of GBNV such as chlorotic or necrotic spots on leaves, thrips injury on leaves (Plate 4.22), severe chlorosis of top leaves, bushy and stunted growth, severe necrosis and death of bud subsequently death of plants along with vector *Thrips palmi* (Plate 4.23) was observed during 30 - 60 DAS.

Significant difference in disease incidence was observed at different stages of the crop. Although, there were significant differences in disease incidence among genotypes at 30 DAS, some of the resistant lines could not be differentiated from susceptible lines. The mean disease incidence was low at 30 DAS and reached peak levels at 60 DAS when the crop was at flowering. The young plants are more succulent and attract the thrips for feeding. Thereafter, constant or gradual increase in disease incidence was observed at senescence stage. In natural conditions, the decrease in susceptibility of the plant with the age of the crop may be due to increase in resistance of plants to the virus infection.

Sreekanth *et al.* (2002c) observed significant differences in *T. palmi* populations at different stages of green gram crop. Low population (15.6) was observed at 15 DAS and thereafter increased progressively up to 45 DAS to reach higher levels (72.1). At 60 DAS, population dwindled to lower levels (17.1) almost similar to the levels at 15 DAS.

Since assessment at 45 and 60 DAS for disease incidence clearly differentiated groundnut genotypes for resistance to GBND, the appropriate time for assessment could be considered by the magnitude of genotypic variations in disease incidence.

Significant difference in disease incidence was found between genotypes ICGV 91114 and ICGV 99058, ICGV 99072, ICGV 00162, ICGV 86590, ICGV 00308, ICGV 93468, ICGS 44. This might be due to difference in genetic makeup and leaf characters

Plate 4.22 Thrips injury on leaves

Plate 4.23 Photomicrograph of *Thrips palmi* found in groundnut field

such as hairiness, glossy, smooth etc. that resist the vector feeding on them and subsequent block in movement of virus once it enters the plant. The genotypes with thick leaves, glossiness and hairiness showed less disease incidence compared to genotypes having thin, smooth and non glossy leaves.

With regard to per cent GBND incidence in the field, four genotypes *viz.,* ICGV 07220 (2.57 %), ICGV 00350 (2.64 %), ICGV 00351(3.36 %), ICGV 00211 (4.02 %) were found to be resistant and significantly superior to the resistant check ICGV 86031 (4.04 %).

The data pertaining to grouping of groundnut genotypes for reaction to GBND during *kharif 2013* is presented in Table 4.8.

The data revealed that out of the 40 genotypes tested, eight genotypes *viz.,* ICGV 00201(Plate 4.24), ICGV 00211, ICGV 86699, ICGV 03042, ICGV 07220, ICGV 06146, ICGV 00350 and ICGV 00351 were resistant (disease incidence of 2.57 - 4.99 per cent). Twenty four genotypes *viz.,* ICGV 00187, ICGV 00189, ICGV 00191, ICGV 00202, ICGV 00203, ICGV 00206, ICGV 00213, ICGV 00241, ICGV 00246, ICGV 00247, ICGV 03057, ICGV 06100, ICGV 07222, ICGV 05155, ICGV 02266, ICGV 87846, ICGV 00348, ICGV 93260, ICGV 93261, ICGV 89280, ICGV 92195, ICGV 92035, ICGS 76 and ICR 48 were moderately resistant (5.13 – 9.93 per cent). Eight genotypes *viz.,* ICGV 99058, ICGV 99072, ICGV 00162, ICGV 86590, ICGV 91114, ICGV 00308, ICGV 93468 and ICGS 44, were moderately susceptible (10.21 – 22.71 per cent). There were no genotypes pertaining to highly resistant, susceptible and highly susceptible disease reaction grade.

Similarly, grouping of genotypes was done by Sunkad *et al.* (2000) based on disease incidence. Out of 172 lines tested, seven were highly resistant (incidence 0-1 per cent), 33 resistant (1.1-5 per cent), 52 moderately resistant (5.1-10 per cent), 53 moderately susceptible (10.1-25 per cent), 25 susceptible (25.1-50 per cent) and two highly susceptible genotypes (50.1 and above).

Ramana *et al.* (2006) grouped 63 test entries into six distinct categories based on final GBND incidence under field conditions. One entry was highly resistant (0 per cent incidence), two resistant (1-10 per cent), nine moderately resistant (11-20 per cent), one

Table 4.8 Grouping of groundnut genotypes based on reaction to GBND under field conditions during *kharif* 2013

Scale	Disease Incidence (%)	Grade	No. of entries	Genotypes Name
0	0-1.0	Highly Resistant	0	Nil
1	1.1-5.0	Resistant	8	ICGV 00201, 00211, 86699, 03042, 07220, 06146, 00350, 00351
2	5.1-10.0	Moderately Resistant	24	ICGV 00187, 00189, 00191, 00202, 00203, 00206, 00213, 00241, 00246, 00247, 03057, 06100, 07222, 05155, 02266, 87846, 00348, 93260, 93261, 89280, 92195, 92035, ICGS 76, ICR 48
3	10.1–25.0	Moderately susceptible	8	ICGV 99058, 99072, 00162, 86590, 91114, 00308, 93468, ICGS 44,
4	25.1-50.0	Susceptible	0	Nil
5	50.1 and above	Highly susceptible	0	Nil

Plate 4.20 Performance of resistant check ICGV 86031 against GBNV in the field during *kharif* 2013

Plate 4.21 Susceptible check JL 24 showing susceptible reaction to GBNV in the field during *kharif* 2013

Plate 4.24 Performance of resistant genotype ICGV 00201against GBNV under field conditions during kharif 2013

moderately susceptible (21-30 per cent), twenty one susceptible (31-50 per cent) and twenty nine highly susceptible (51 per cent and above).

Thiara *et al.* (2004) reported that thrips population was maximum during 30 June to 30 August after which it reduced to zero level on 30 September. This observation coincided with incidence of GBND.

Results of Sreekanth *et al.* (2002c) indicated that thrips infestation was highest in July sowing (75.2 thrips per 25 terminal), followed by August (64.6), June (57.1), September (48.8), October (41.3), November (31.7), December (28.8), January (26.0), May (18.5), February (17.9), March (16.0) and April (15.3) sowings. Correspondingly, GBNV incidence was maximum in July (50.2) followed by August (46.2), June (41.4), September (34.1), October (30.5), November (25.2), December (22.8), January (21.8), May (11.2), February (9.0), March (6.1) and April (4.7) sowings.

In our study, late sowing of the genotypes fairly coincided with the reasonably high vector populations. Yet, our findings indicate that low disease incidence in these genotypes is due to their superiority in curtailing the thrips feeding and subsequently disease incidence.

Field resistant varieties reported here are not immune to the disease but have reduced disease incidence under field conditions. Resistance in these genotypes might be due to non preference by the thrips vector and/or resistance to GBNV infection or multiplication and spread.

Amin (1985) opined that resistance in case of groundnut cv Robut 33-1 is due to resistance to the vector, perhaps combined with resistance or tolerance to GBNV.

Culbreath *et al.* (1993) and Buiel and Parlevleit (1996) stated that the resistant genotypes reduced the rate of epidemic development with considerable reduction in the incidence of GBNV. So, the genotypes showing high resistance or resistance response could be used as seed material after screening of genotypes further in different trials.

4.3.2 Severity of GBND in groundnut genotypes during *kharif* 2013

The data pertaining to severity of GBND in groundnut genotypes is presented in Table 4.9 and Figs. 4.10 to 4.14.

Table 4.9 Severity of GBND in groundnut genotypes during *kharif* 2013

S. No.	Genotype	Disease Severity* at				
		30DAS	45DAS	60DAS	75DAS	90DAS
1	ICGV 99058	1.66	1.66	2.66	2.66	2.66
2	ICGV 99072	1.00	1.67	2.00	2.33	3.00
3	ICGV 00162	2.01	2.67	3.01	3.01	3.34
4	ICGV 00187	1.00	1.67	2.00	2.00	2.00
5	ICGV 00189	1.32	1.66	1.99	1.99	2.99
6	ICGV 00191	1.33	1.66	1.66	2.00	2.00
7	ICGV 00201	0.99	1.33	1.99	1.99	1.99
8	ICGV 00202	1.00	1.67	2.00	2.00	2.00
9	ICGV 00203	1.32	1.65	1.65	2.99	2.99
10	ICGV 00206	1.00	1.66	2.00	2.00	2.00
11	ICGV 00211	1.00	1.00	1.66	2.00	2.00
12	ICGV 00213	1.00	1.33	1.67	2.00	2.00
13	ICGV 00241	1.67	2.33	2.33	2.33	2.33
14	ICGV 00246	1.68	2.34	2.68	2.68	2.68
15	ICGV 00247	1.32	1.66	1.66	1.99	1.99
16	ICGV 86590	2.01	2.34	2.67	2.67	2.67
17	ICGV 86699	1.67	1.67	2.01	2.01	2.01
18	ICGV 91114	2.01	2.67	3.01	3.34	3.34
19	ICGV 00308	1.99	2.66	2.99	2.99	3.99
20	ICGV 03042	1.68	2.68	3.02	3.02	3.02
21	ICGV 03057	2.33	2.67	2.67	2.67	2.67
22	ICGV 06100	2.01	2.01	2.34	2.34	2.34
23	ICGV 07222	1.68	1.68	2.01	2.01	2.01
24	ICGV 07220	1.34	2.00	2.00	2.00	2.00
25	ICGV 05155	2.02	2.02	2.35	2.35	2.35
26	ICGV 06146	1.33	1.99	1.99	1.99	1.99
27	ICGV 02266	1.34	1.67	2.67	2.67	2.67

Table 4.9 contd…						
28	ICGV 87846	2.00	2.00	2.00	2.00	2.00
29	ICGV 93468	1.99	2.99	3.65	4.32	4.32
30	ICGV 00348	2.01	2.01	2.67	3.01	3.34
31	ICGV 00350	1.66	1.99	2.66	2.66	2.99
32	ICGV 00351	1.33	2.00	2.33	2.67	2.67
33	ICGV 93260	1.99	2.65	2.99	3.65	3.65
34	ICGV 93261	2.00	2.33	2.66	2.66	2.66
35	ICGV 89280	1.67	2.01	2.34	3.01	4.01
36	ICGV 92195	1.66	1.99	2.66	2.99	3.33
37	ICGV 92035	1.67	2.00	2.33	3.00	3.00
38	ICGS 44	1.66	2.00	3.33	3.33	3.33
39	ICGS 76	1.67	2.34	2.34	2.67	2.67
40	ICR 48	1.34	2.01	2.01	2.01	2.01
41	ICGV 86031 (Resistant check)	1.66	1.99	2.33	2.33	2.33
42	JL 24 (susceptible check)	2.67	3.34	3.67	4.67	4.67
	Mean of all genotypes	1.61	2.04	2.40	2.60	2.71

Disease severity				
Effect	Num DF	Den DF	F Value	Pr > F
GEN	41	77.8	2.23	0.0012
TIME	4	331	50.85	<.0001
GEN*TIME	164	305	0.94	0.6549

*Mean of three replications

SAS analysis was performed and the values mentioned are angular transformed values

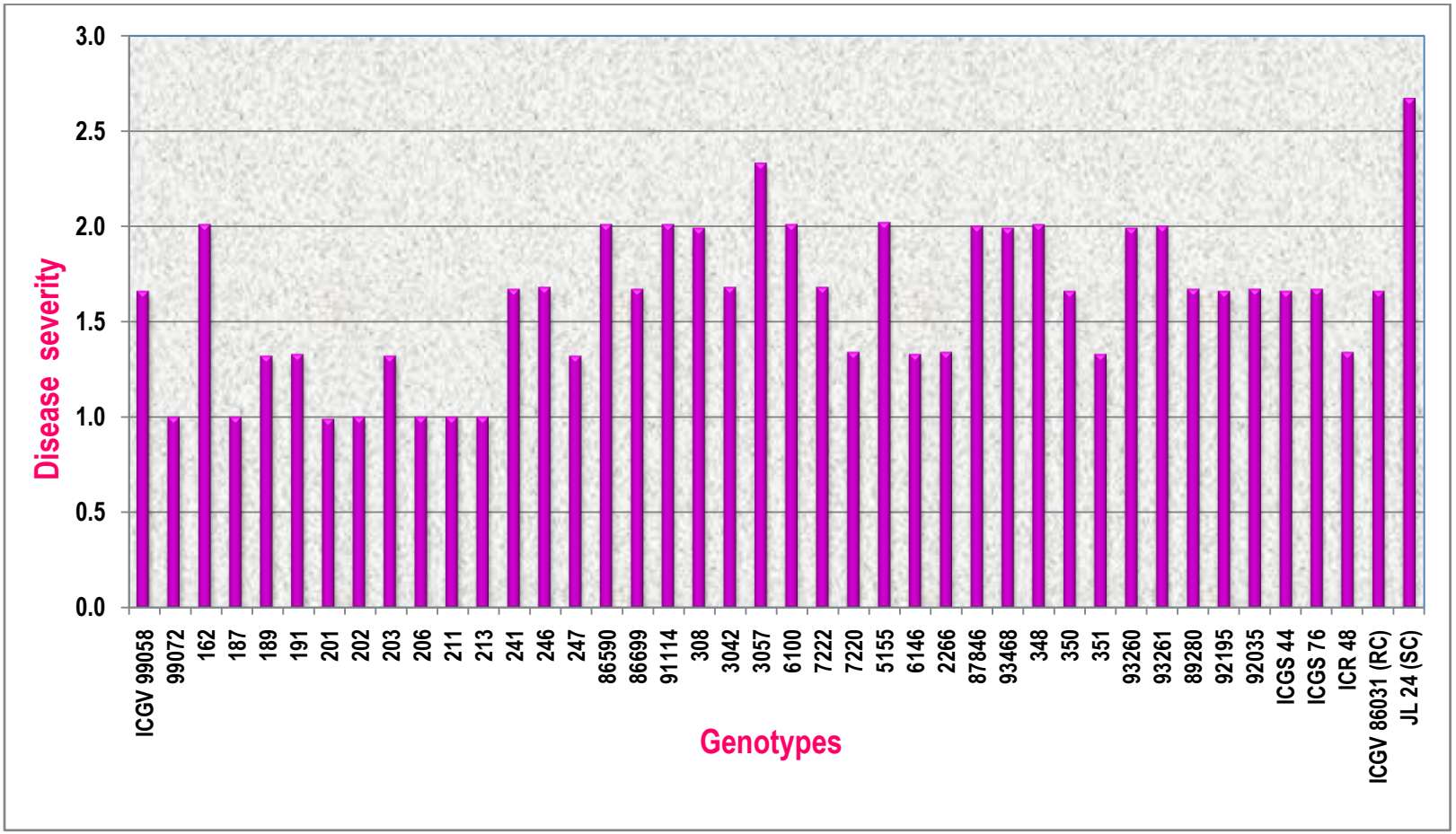


Figure 4.10 Severity of GBND in groundnut genotypes during *kharif* 2013 at 30 DAS

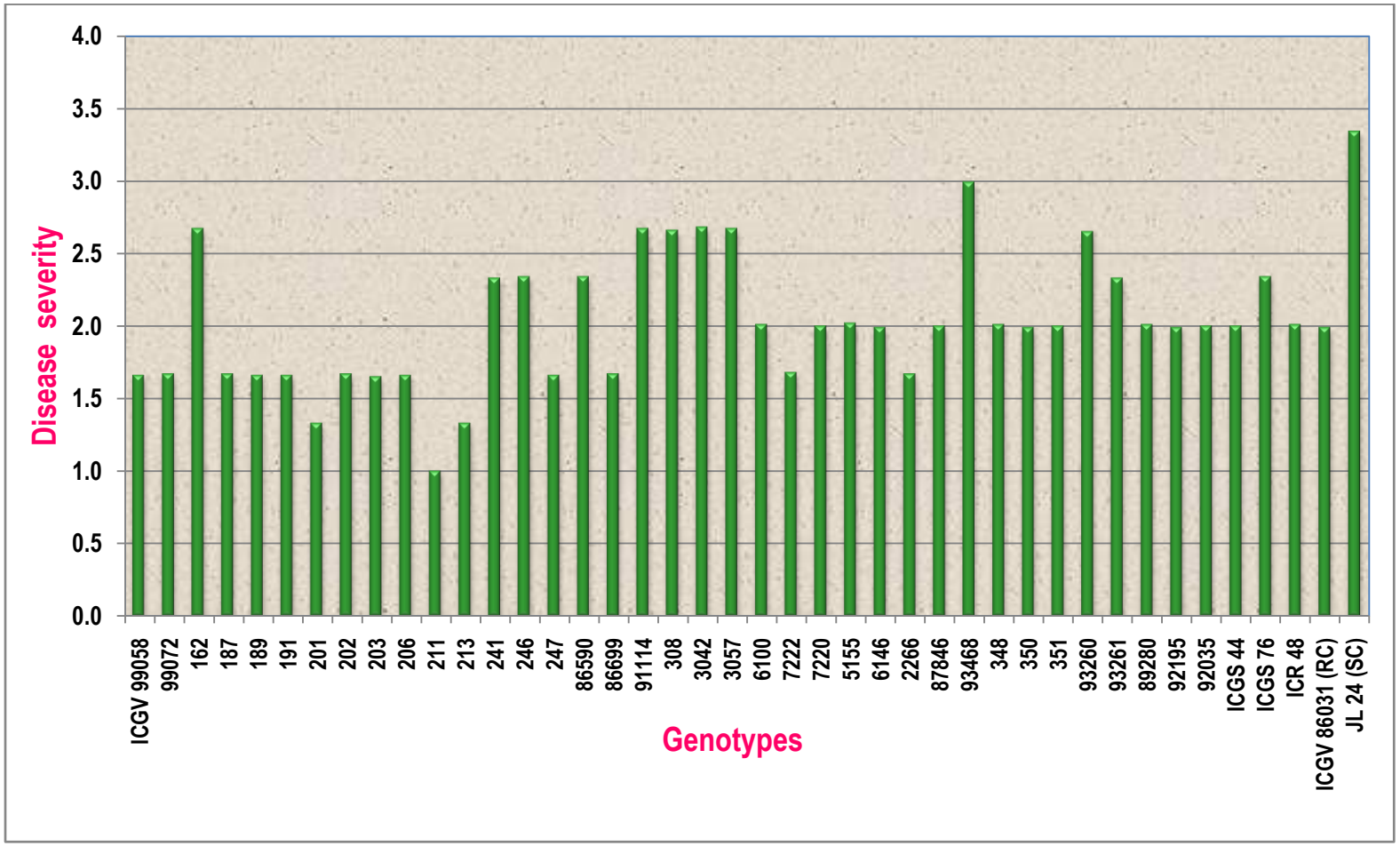


Figure 4.11 Severity of GBND in groundnut genotypes during *kharif* 2013 at 45 DAS

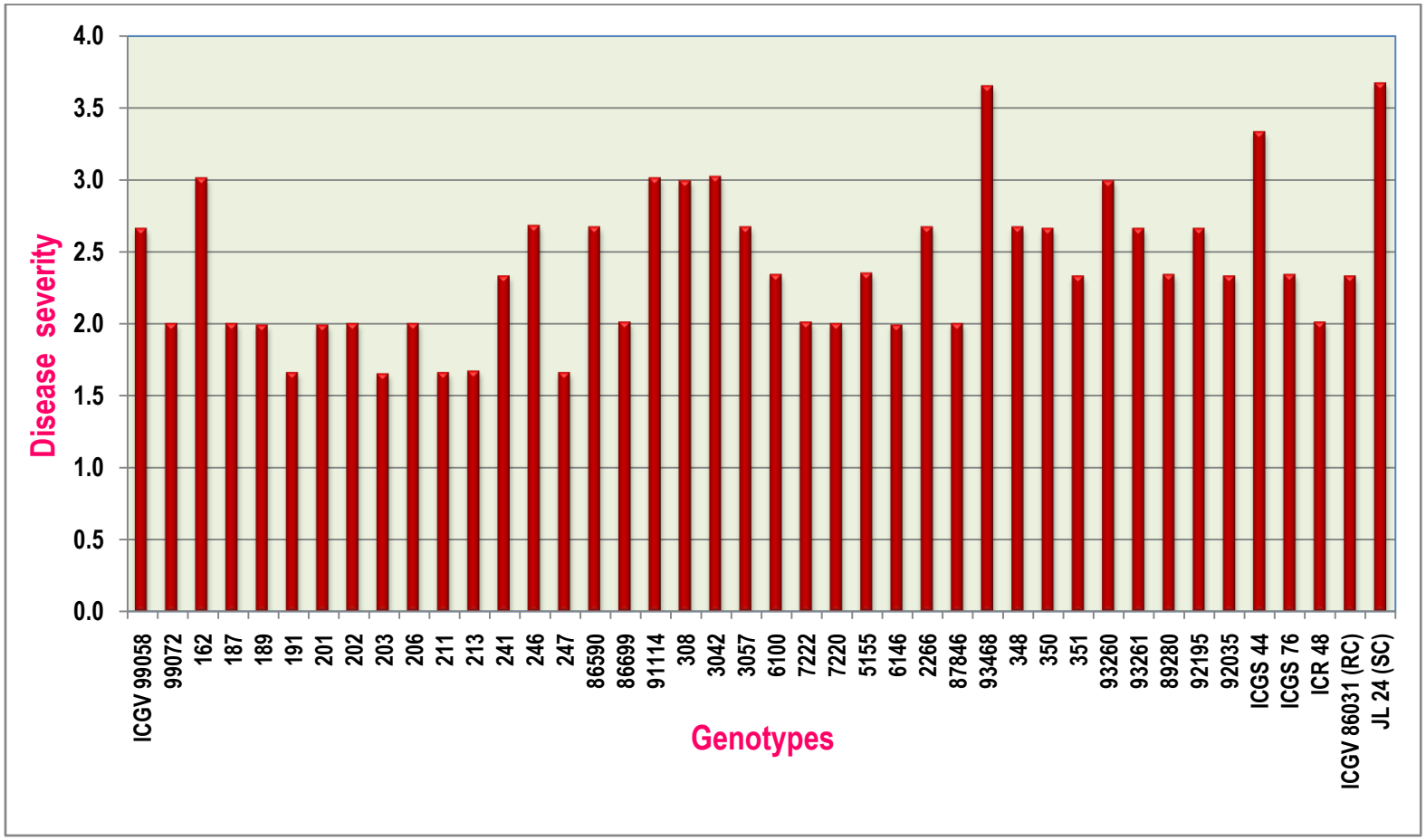


Figure 4.12 Severity of GBND in groundnut genotypes during *kharif* 2013 at 60 DAS

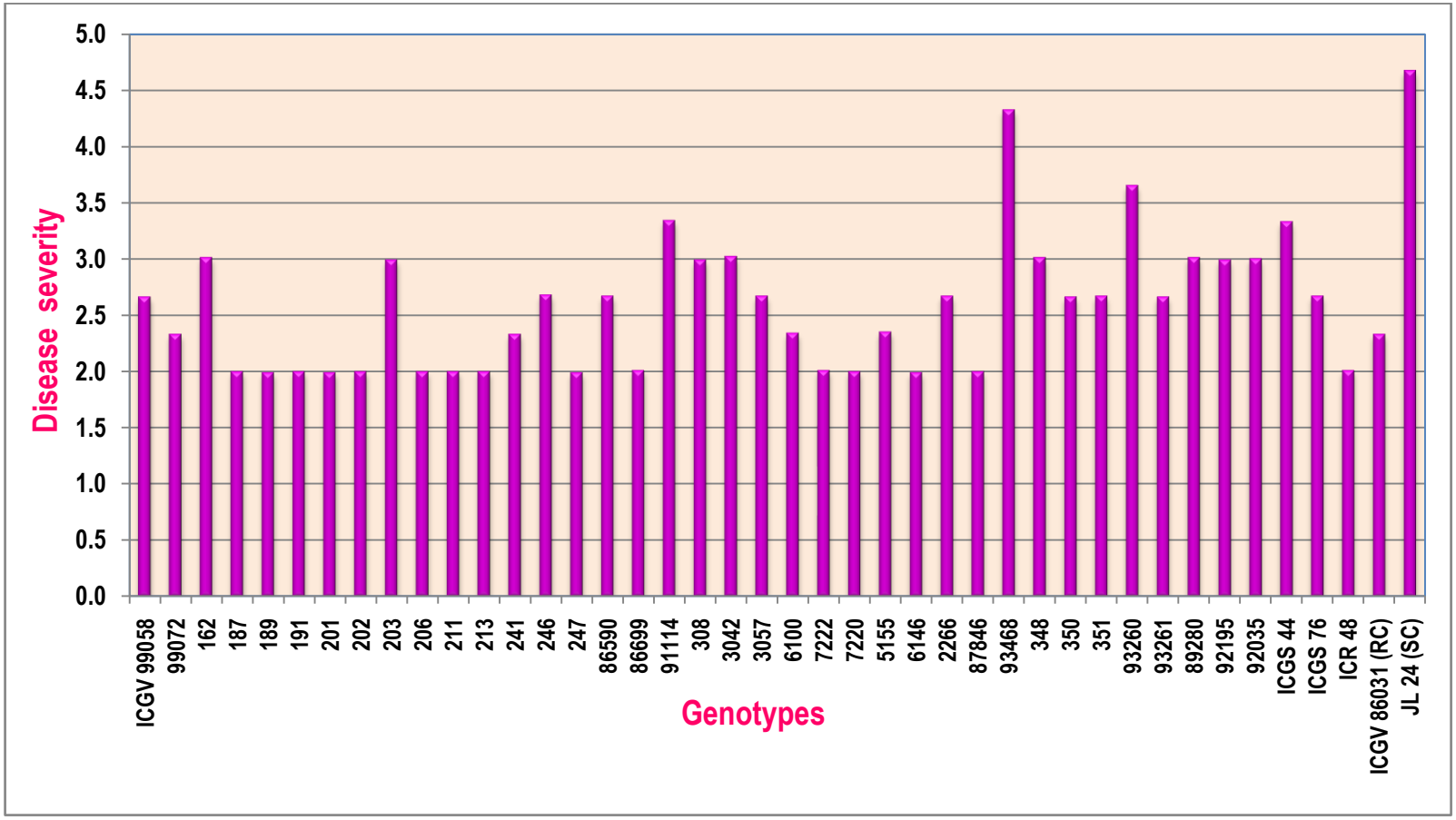


Figure 4.13 Severity of GBND in groundnut genotypes during *kharif* 2013 at 75 DAS

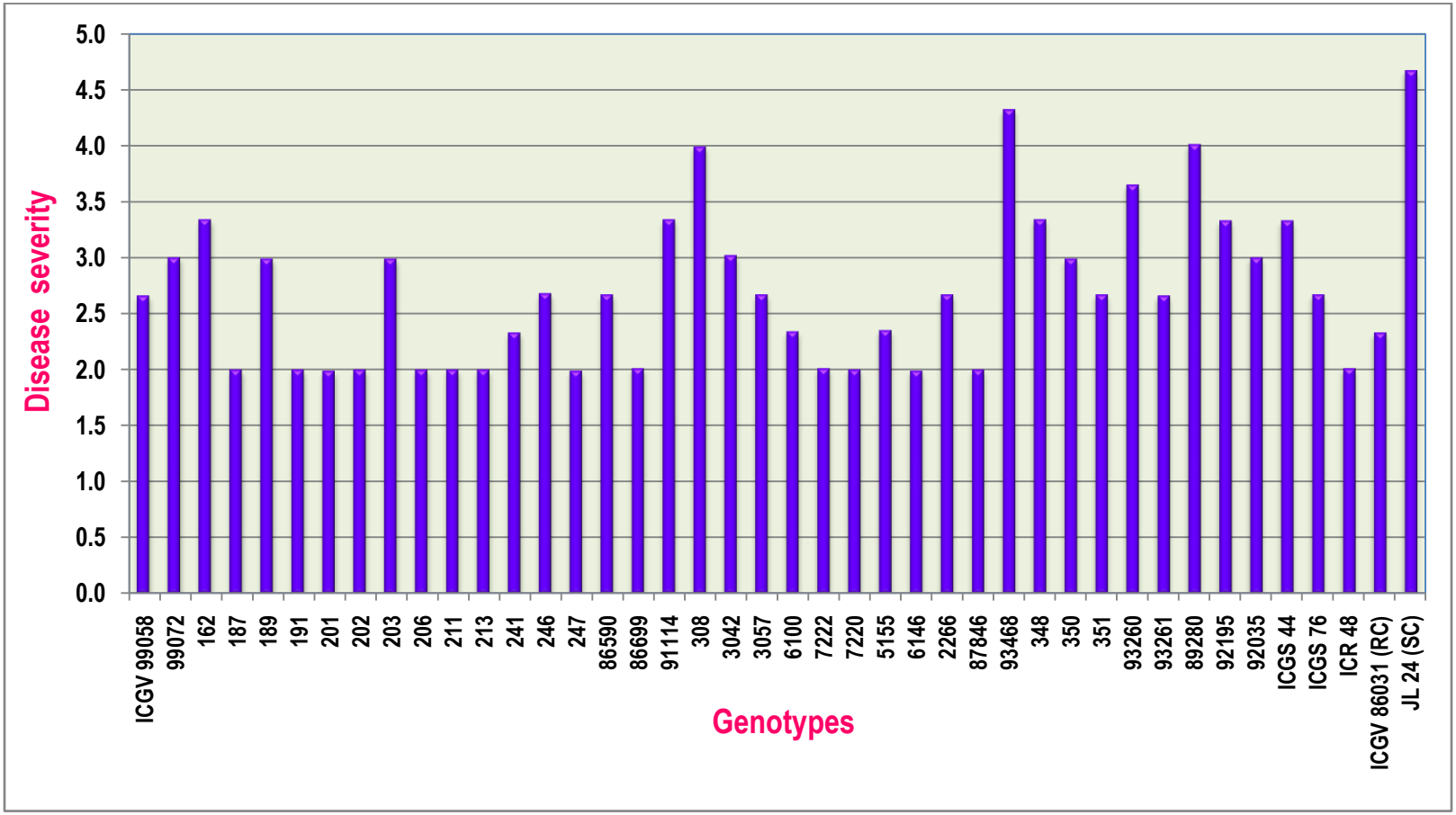


Figure 4.14 Severity of GBND in groundnut genotypes during *kharif* 2013 at 90 DAS

The average GBND disease severity in these genotypes ranged from 1.99 to 4.32 compared to 2.33 in ICGV 86031 (resistant check) and 4.67 in JL 24 (susceptible check).

The data revealed that the disease severity ranged from 0.99 to 2.67 at 30 DAS, 1.00 to 3.34 at 45 DAS, 1.65 to 3.67 at 60 DAS and 1.99 to 4.67 at 75 DAS and 90 DAS. There was progressive increase in disease severity from 1.61 (30 DAS) to 2.71 (90 DAS), considering the mean disease severity of all genotypes.

Significant difference in disease severity between genotypes was found at 30 DAS with 45 DAS, 60 DAS, 75 DAS and 90 DAS; 45 DAS with 60 DAS, 75 DAS and 90 DAS; 60 DAS with 90 DAS. No significant difference between genotypes was found between 60 DAS with 75 DAS and 75 DAS with 90 DAS.

The genotypes ICGV 00187 (2.00), ICGV 00191 (2.00), ICGV 00201 (1.99), ICGV 00202 (2.00), ICGV 00206 (2.00), ICGV 00211 (2.00), ICGV 00213 (2.00), ICGV 00247 (1.99), ICGV 86699 (2.01), ICGV 07222 (2.01), ICGV 07220 (2.00), ICGV 06146 (1.99) and ICGV 87846 (2.00) showed less disease severity compared to resistant check ICGV 86031 (2.33). Of all the genotypes tested, none of them showed high disease severity compared to susceptible check JL 24 (4.67) indicating the superiority of JL 24 as susceptible check.

The disease severity was in the range of 1.99 - 3.02 in resistant genotypes, 1.99 - 4.01 in moderately resistant genotypes and 2.66 - 4.32 in moderately susceptible genotypes. The resistant and susceptible genotypes could not be clearly differentiated by using disease severity scoring.

This was comparable with results obtained by Pensuk *et al.* (2002) and Buiel and Parlevleit (1996) who reported the disadvantage of using disease severity scoring due to the highly variable symptoms caused by GBNV that are not genotype specific.

Kesmala *et al.* (2006) reported that disease incidence is more advantageous than disease score because it is easy to evaluate. Moreover, field evaluation of lines is complicated initially by the non uniformity of disease distribution in the field resulting from random distribution of vectors.

4.3.3 Detection of GBNV through DAC-ELISA

Leaf samples of few genotypes showing resistant, moderately resistant and moderately susceptible disease reaction were randomly collected, along with resistant (ICGV 86031) and susceptible (JL 24) check and the samples were subjected to ELISA test for further confirmation of field reaction.

Details of DAC- ELISA test are furnished in Table 4.10.

The resistant genotypes *viz.,* ICGV 03042, ICGV 00350 and ICGV 00351 gave negative reaction to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.157 - 0.354 confirming their resistant reaction grade.

The moderately resistant genotypes *viz.,* ICGV 00187, ICGV 00189, ICGV 00213, ICGV 00241, ICGV 05155, ICGV 02266, ICGV 93261, ICGV 89280, ICGV 92195, ICGV 92035 and ICR 48 gave 16.66 to 66.66 per cent infection with GBNV antiserum and the absorbance values at 405 nm was in the range of 0.137 - 2.910 confirming their moderately resistant reaction.

The moderately susceptible genotypes *viz.,* ICGV 99058, ICGV 99072, ICGV 00162, ICGV 86590, ICGV 91114, ICGV 00308, ICGV 93468 and ICGS 44 gave 100 per cent infection with GBNV antiserum and the absorbance values was in the range of 1.669 - 3.427 confirming their moderately susceptible reaction.

The genotypes ICGV 86031 (resistant check) and JL 24 (susceptible check) gave zero and 100 per cent infection with GBNV antiserum which was in conformity with their disease reaction under field conditions.

Reddy *et al.* (2000) reported that of 83 accessions and one natural hybrid tested under field conditions, one accession of each of *A. benensis* (ICG 11551) and *A. cardenasii* (ICG 11564), two accessions each of *A. villosa* (ICG 13168 and ICG 8144) in the section *Arachis*, *A. appressipila* (ICG 8945 and ICG 8946) in the section Procumbentes, and one accession of *A. triseminata* (ICG 8131) in the section Triseminatae, were not infected by GBND under field conditions. These accessions showed zero per cent infection in field and ELISA test.

Govardhana *et al.* (2013) determined the serological properties of the virus infecting tomato fields with DAC-ELISA using polyclonal antibodies for different viruses

Table 4.10 Detection of virus causing GBND in groundnut samples collected from naturally infected field experiment during *kharif* -2013 through DAC-ELISA

S. No.	Genotype	No. of samples	GBNV antiserum	
			Absorbance Value (405nm) range	Per cent infection based on ELISA
1	ICGV 99058	6	1.669 - 2.601	100
2	ICGV 99072	6	2.332 - 3.176	100
3	ICGV 00162	6	1.937- 3.001	100
4	ICGV 00187	6	0.583 - 2.755	50
5	ICGV 00189	6	0.419 - 2.701	50
6	ICGV 00213	6	0.931 - 2.525	33.33
7	ICGV 00241	6	0.311 - 2.823	50
8	ICGV 86590	6	2.541 - 2.857	100
9	ICGV 91114	6	2.294 - 2.354	100
10	ICGV 00308	6	2.446 - 2.694	100
11	ICGV 03042	6	0.207 - 0.354	0
12	ICGV 05155	6	0.854 - 2.726	33.33
13	ICGV 02266	6	0.596 - 2.700	66.66
14	ICGV 93468	6	2.330 - 3.427	100
15	ICGV 00350	6	0.157 - 0.275	0
16	ICGV 00351	6	0.290 - 0.302	0
17	ICGV 93261	6	0.453 - 2.682	33.33
18	ICGV 89280	6	0.212 - 2.910	33.33
19	ICGV 92195	6	0.137 - 2.287	16.66
20	ICGV 92035	6	0.254 - 2.610	50
21	ICGS 44	6	2.217 - 3.277	100
22	ICR 48	6	0.195 - 2.283	66.66
23	ICGV 86031 (Resistant check)	6	0.268 - 0.338	0
24	JL 24 (Susceptible check)	6	2.667 - 2.853	100
	GBNV (+ ve control)	2	2.664 - 2.802	100
	Healthy (– ve control)	2	0.330 - 0.346	0
	Buffer	1	0.362	0

like *Tobacco streak virus* (TSV), *Cucumber mosaic virus* (CMV) and GBNV. Of the field samples tested, 11 samples showed positive reaction with DAC-ELISA.

4.4 SCREENING OF GROUNDNUT GENOTYPES FOR VIRUS RESISTANCE UNDER GREENHOUSE CONDITIONS

4.4.1 Incidence of GBND in groundnut genotypes

The data pertaining to incidence of GBND in groundnut genotypes at 1:10 virus concentration is presented in Table 4.11 and Figs. 4.15 to 4.17.

The average disease incidence at 1:10 virus concentration ranged from 64.71 to 100 per cent compared to 72.22 per cent in ICGV 86031 (resistant check) and 94.44 per cent in JL 24 (susceptible check) at 21 DAI.

The data revealed that the disease incidence ranged from 0 to 100 per cent at 7 DAI, 28.57 to 100 per cent at 14 DAI compared to 64.71 to 100 per cent at 21 DAI. There was progressive increase in disease incidence from 34.66 (7 DAI) to 88.79 (14 DAI) per cent, when the mean disease incidence of all genotypes were taken into consideration.

All the genotypes were highly susceptible to GBNV at higher virus concentration (1:10 dilution). Similar results were obtained by Rao *et al.* (2006) and Dwivedi *et al.* (1995).

The data pertaining to incidence of GBND in groundnut genotypes at 1:100 virus concentration is presented in Table 4.12 and Figs. 4.18 to 4.20.

The average disease incidence at 1:100 virus concentration ranged from 5.56 to 100 per cent compared to 26.67 in ICGV 86031 (resistant check) and 77.78 per cent in JL 24 (susceptible check).

The data revealed that the disease incidence ranged from 0.00 to 100 per cent at 7 and 14 DAI whereas 5.56 to 100 per cent at 21 DAI. There was progressive increase in mean disease incidence from 34.17 (7 DAI) to 54.21 (21 DAI) per cent among the genotypes. The data pertaining to grouping of groundnut genotypes for reaction to GBND at 1:100 virus concentration is presented in Table 4.13.

Table 4.11 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution

S. No.	Genotype	*GBND Incidence (%) at			Grade
		7 DAI	14 DAI	21 DAI	
1	ICGV 99058	46.15	92.31	92.31	HS
2	ICGV 99072	73.33	93.33	93.33	HS
3	ICGV 00162	50.00	94.44	94.44	HS
4	ICGV 00187	22.22	88.89	100.00	HS
5	ICGV 00189	50.00	100.00	100.00	HS
6	ICGV 00191	38.89	77.78	83.33	HS
7	ICGV 00201	44.44	83.33	83.33	HS
8	ICGV 00202	42.86	85.71	85.71	HS
9	ICGV 00203	16.67	77.78	88.89	HS
10	ICGV 00206	46.15	84.62	84.62	HS
11	ICGV 00211	53.33	73.33	80.00	HS
12	ICGV 00213	0.00	87.50	93.75	HS
13	ICGV 00241	50.00	81.25	87.50	HS
14	ICGV 00246	62.50	81.25	81.25	HS
15	ICGV 00247	37.50	100.00	100.00	HS
16	ICGV 86590	100.00	100.00	100.00	HS
17	ICGV 86699	64.71	64.71	64.71	HS
18	ICGV 91114	72.22	100.00	100.00	HS
19	ICGV 00308	77.78	94.44	94.44	HS
20	ICGV 03042	38.46	61.54	76.92	HS
21	ICGV 03057	0.00	66.67	66.67	HS
22	ICGV 06100	9.09	72.73	72.73	HS
23	ICGV 07222	14.29	28.57	85.71	HS
24	ICGV 07220	0.00	55.56	66.67	HS
25	ICGV 05155	6.25	81.25	87.50	HS
26	ICGV 06146	0.00	75.00	75.00	HS
27	ICGV 02266	50.00	50.00	100.00	HS

Table 4.11 contd..					
28	ICGV 87846	25.00	81.25	87.50	HS
29	ICGV 93468	27.78	94.44	94.44	HS
30	ICGV 00348	33.33	94.44	94.44	HS
31	ICGV 00350	23.53	100.00	100.00	HS
32	ICGV 00351	20.00	93.33	100.00	HS
33	ICGV 93260	66.67	66.67	77.78	HS
34	ICGV 93261	66.67	94.44	94.44	HS
35	ICGV 89280	11.11	94.44	94.44	HS
36	ICGV 92195	16.67	94.44	94.44	HS
37	ICGV 92035	5.88	100.00	100.00	HS
38	ICGS 44	6.25	93.75	93.75	HS
39	ICGS 76	0.00	92.31	92.31	HS
40	ICR 48	0.00	100.00	100.00	HS
41	ICGV 86031 (Resistant check)	33.33	72.22	72.22	HS
42	JL 24 (Susceptible check)	44.44	94.44	94.44	HS
	Mean of all genotypes	34.46	83.77	88.79	

*Mean of three replications

HS - Highly Susceptible

DAI - Days After Inoculation

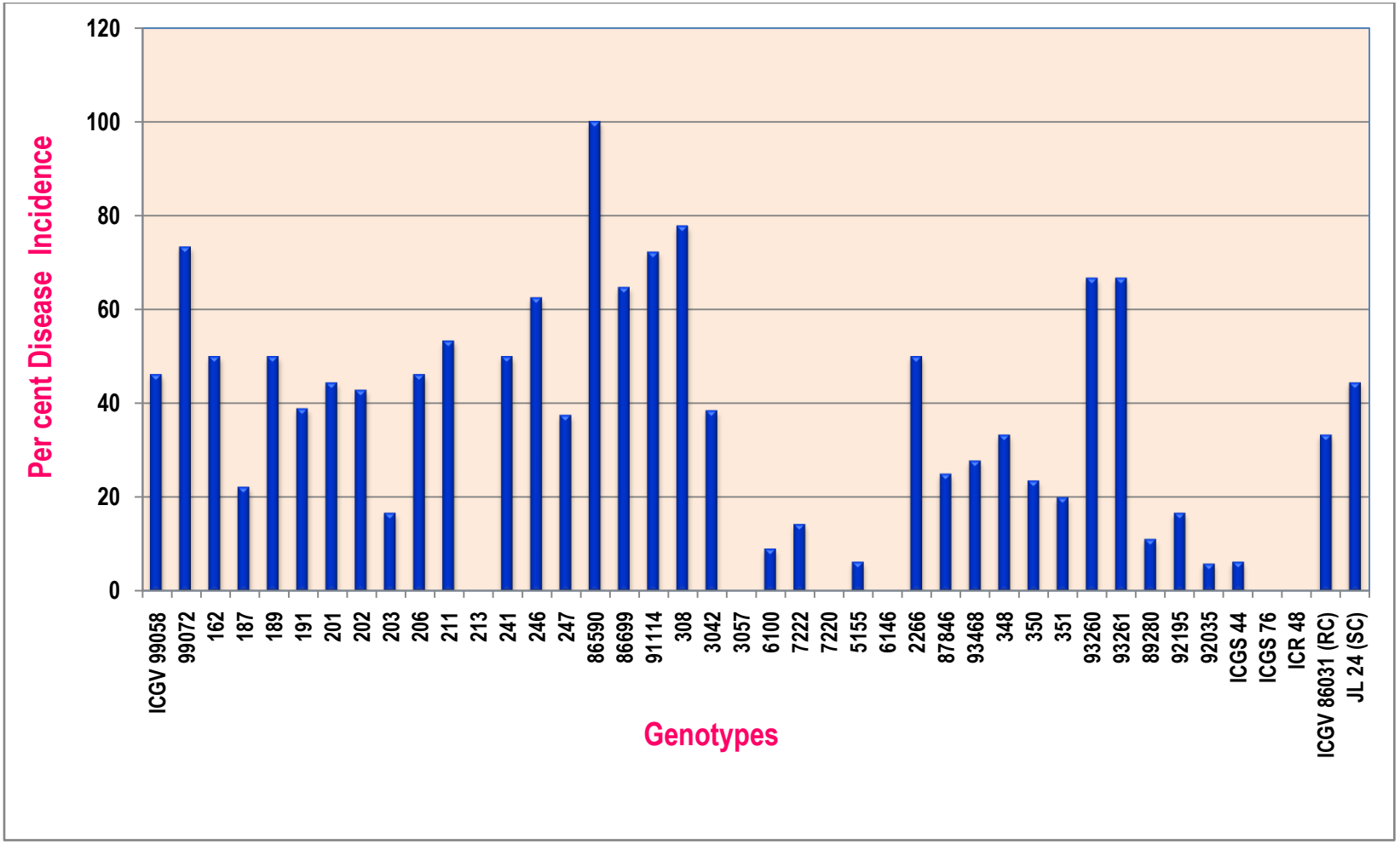


Figure 4.15 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution at 7 DAI

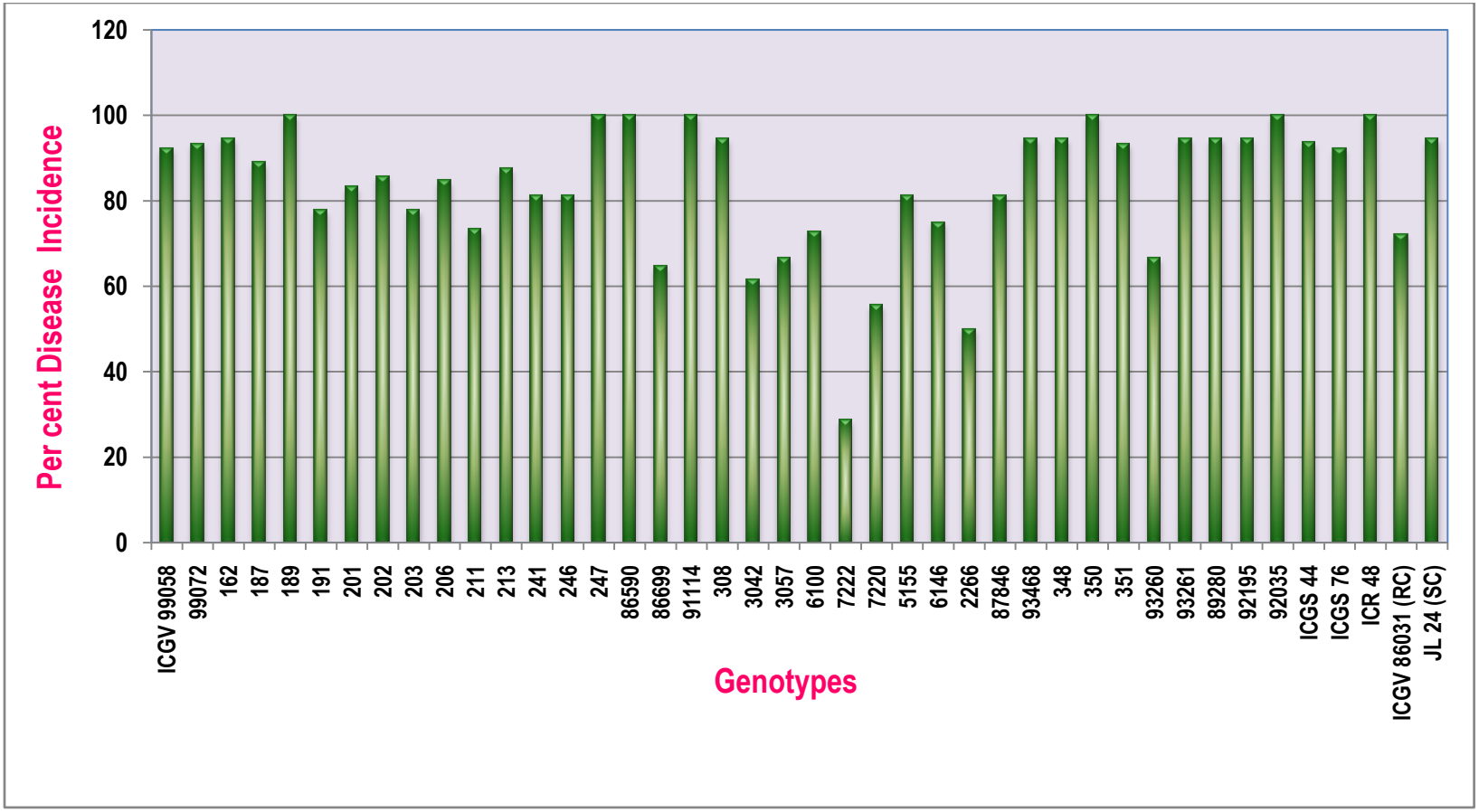


Figure 4.16 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution at 14 DAI

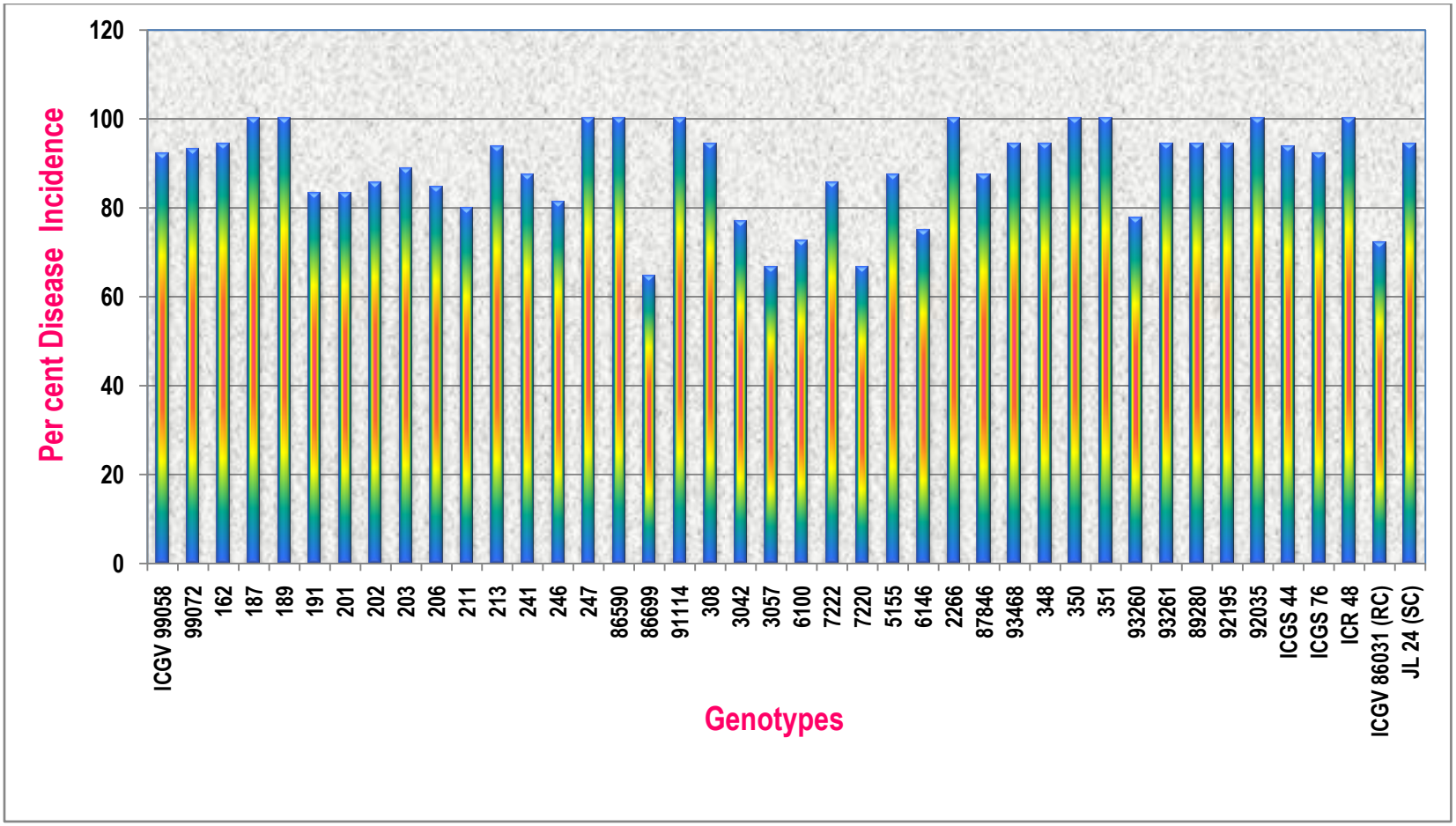


Figure 4.17 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution at 21 DAI

Table 4.12 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution

S. No.	Genotype	*GBND Incidence (%) at			Grade
		7 DAI	14 DAI	21 DAI	
1	ICGV 99058	50.00	58.33	58.33	HS
2	ICGV 99072	78.57	85.71	85.71	HS
3	ICGV 00162	60.00	60.00	73.33	HS
4	ICGV 00187	22.22	27.78	44.44	S
5	ICGV 00189	52.94	52.94	52.94	HS
6	ICGV 00191	38.89	38.89	38.89	S
7	ICGV 00201	47.06	47.06	52.94	HS
8	ICGV 00202	33.33	33.33	33.33	S
9	ICGV 00203	18.75	25.00	37.50	S
10	ICGV 00206	35.29	58.82	58.82	HS
11	ICGV 00211	47.06	52.94	52.94	HS
12	ICGV 00213	0.00	5.56	5.56	MR
13	ICGV 00241	57.14	64.29	64.29	HS
14	ICGV 00246	55.56	72.22	77.78	HS
15	ICGV 00247	40.00	46.67	53.33	HS
16	ICGV 86590	100.00	100.00	100.00	HS
17	ICGV 86699	70.59	88.24	88.24	HS
18	ICGV 91114	72.22	72.22	72.22	HS
19	ICGV 00308	82.35	82.35	82.35	HS
20	ICGV 03042	50.00	50.00	50.00	S
21	ICGV 03057	0.00	0.00	11.11	MS
22	ICGV 06100	9.09	27.27	36.36	S
23	ICGV 07222	8.33	25.00	25.00	MS
24	ICGV 07220	0.00	11.11	22.22	MS
25	ICGV 05155	6.25	25.00	37.50	S
26	ICGV 06146	0.00	0.00	7.14	MR

Table 4.12 contd..					
27	ICGV 02266	50.00	50.00	100.00	HS
28	ICGV 87846	25.00	56.25	56.25	HS
29	ICGV 93468	27.78	55.56	66.67	HS
30	ICGV 00348	33.33	55.56	55.56	HS
31	ICGV 00350	22.22	61.11	77.78	HS
32	ICGV 00351	16.67	61.11	61.11	HS
33	ICGV 93260	44.44	44.44	50.00	S
34	ICGV 93261	66.67	72.22	72.22	HS
35	ICGV 89280	11.11	55.56	72.22	HS
36	ICGV 92195	16.67	61.11	61.11	HS
37	ICGV 92035	5.56	72.22	72.22	HS
38	ICGS 44	6.67	46.67	46.67	S
39	ICGS 76	0.00	25.00	25.00	MS
40	ICR 48	0.00	25.00	33.33	S
41	ICGV 86031 (Resistant check)	6.67	13.33	26.67	S
42	JL 24 (Susceptible check)	66.67	77.78	77.78	HS
	Mean of all genotypes	34.17	48.66	54.21	

*Mean of three replications

DAI Days After Inoculation

MR- Moderately Resistant; MS- Moderately Susceptible; S- Susceptible; HS- Highly Susceptible

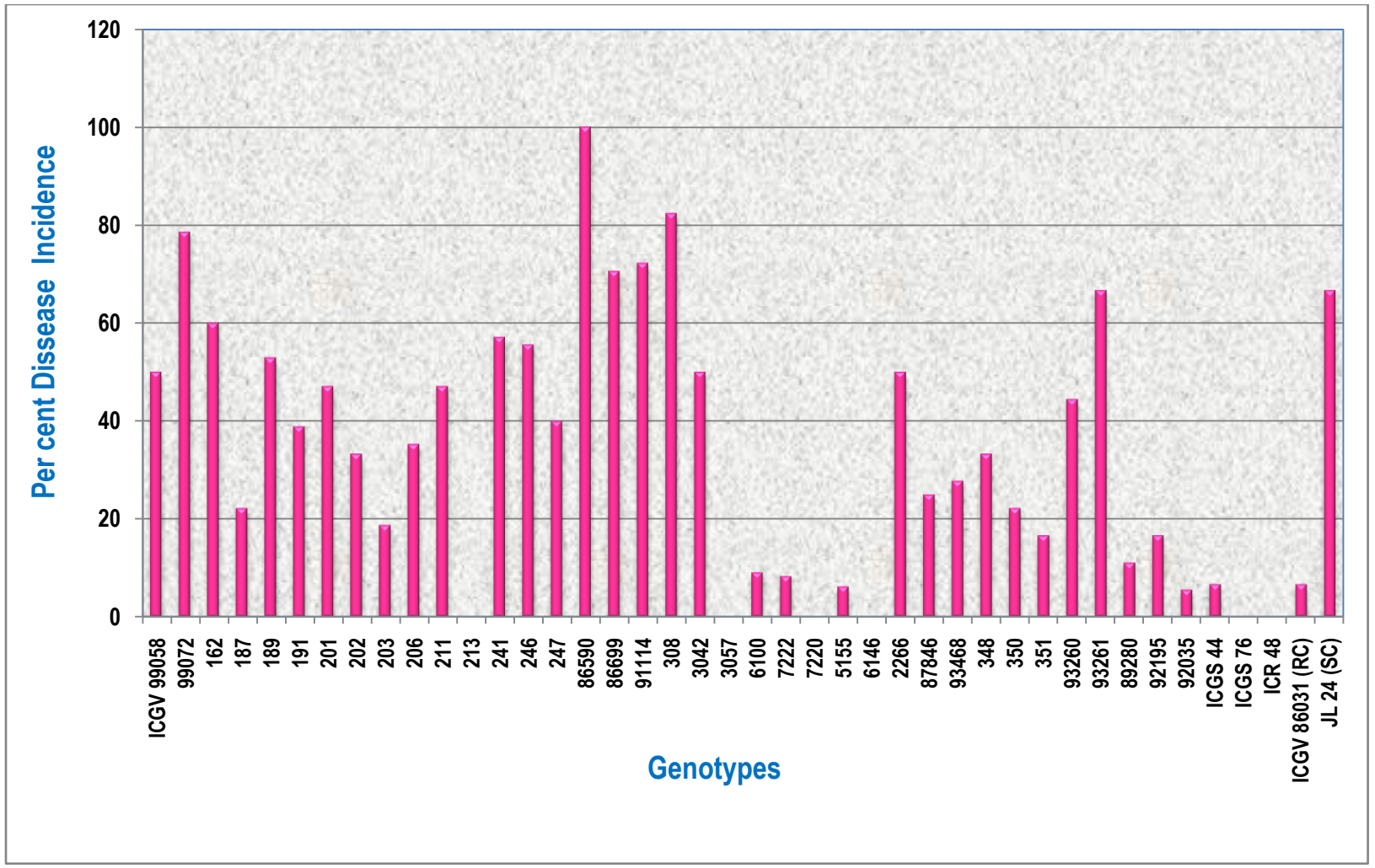


Figure 4.18 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution at 7 DAI

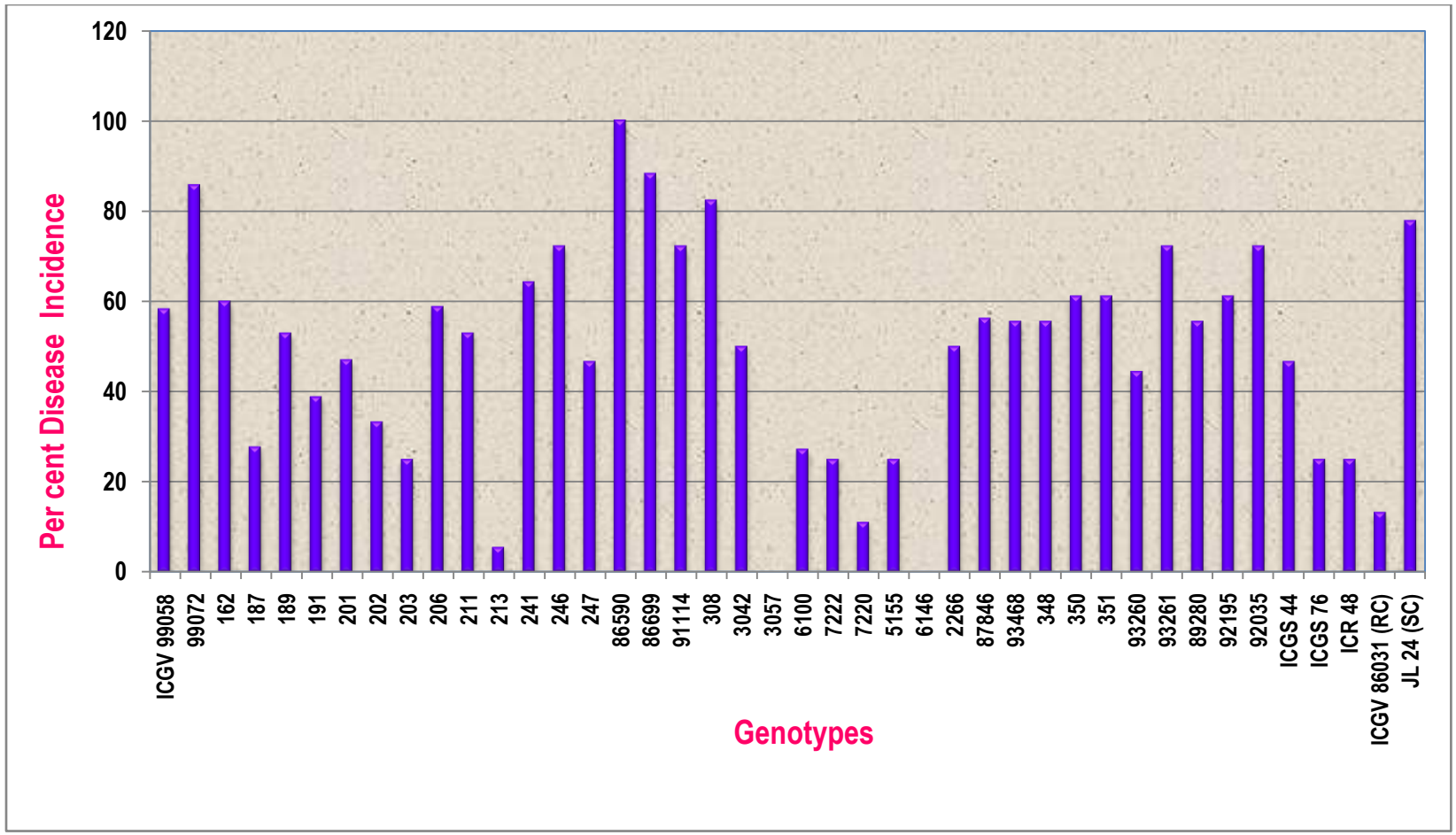


Figure 4.19 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution at 14 DAI

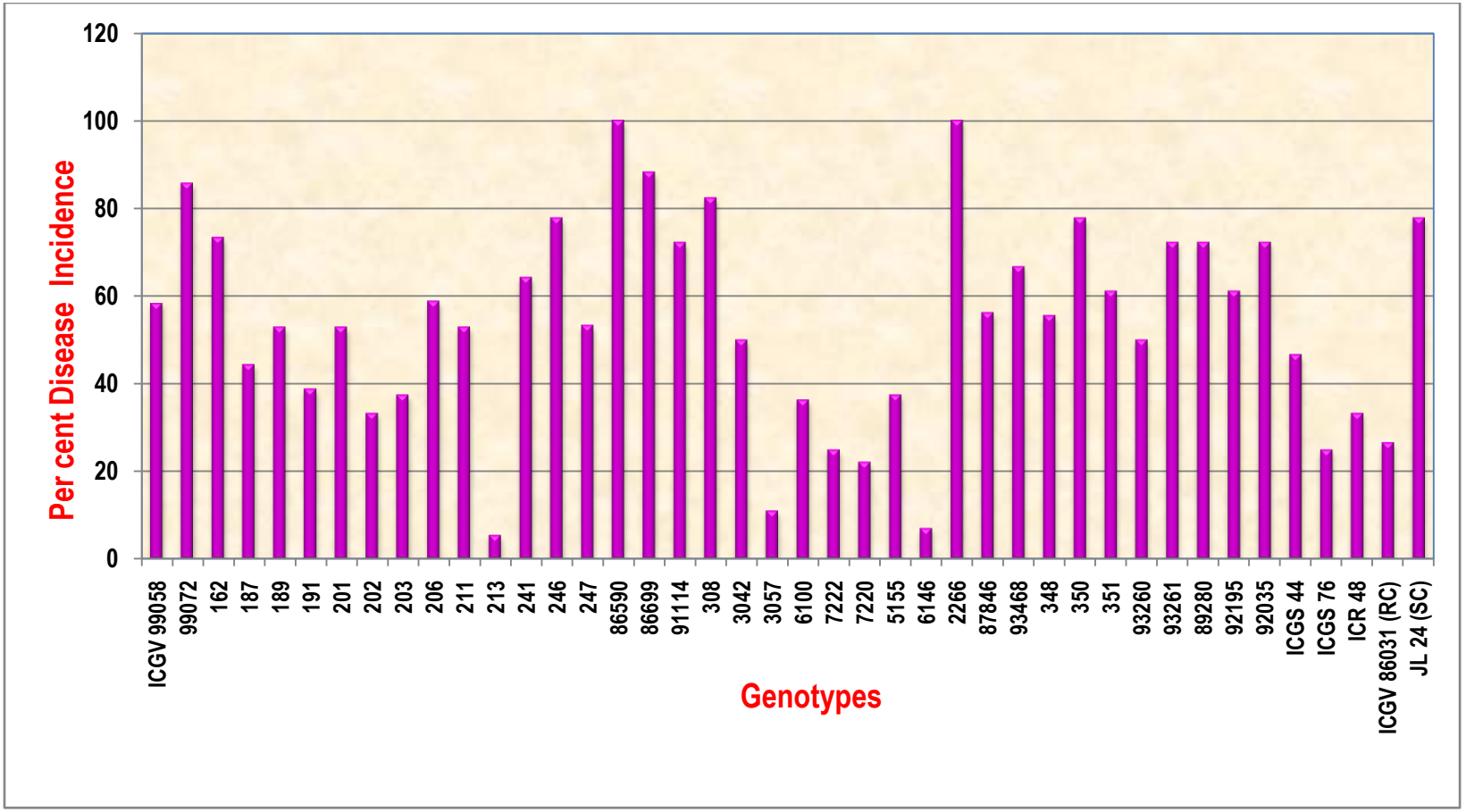


Figure 4.20 Incidence of GBND in groundnut genotypes upon mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution at 21 DAI

Table 4.13 Grouping of groundnut genotypes based on reaction to bud necrosis virus at 1: 100 dilution

Scale	Disease Incidence (%)	Grade	Number of entries	Genotypes
0	0-1.0	Highly Resistant	0	Nil
1	1.1-5.0	Resistant	0	Nil
2	5.1-10.0	Moderately Resistant	2	ICGV 00213, 06146
3	10.1 - 25.0	Moderately susceptible	4	ICGV 03057, 07222, 07220,ICGS 76
4	25.1- 50.0	Susceptible	10	ICGV 00187, 00191, 00202, 00203,03042, 06100,05155, 93260, ICGS 44, ICR 48
5	50.1 and above	Highly susceptible	24	ICGV 99058, 99072, 00162, 00189, 00201,00206,00211, 00241, 00246, 00247, 86590, 86699, 91114, 00308, 02266, 87846, 93468, 00348, 00350, 00351, 93261, 89280, 92195, 92035

The data revealed that out of the 40 genotypes tested, two genotypes *viz.,* ICGV 00213 (Plate 4.25), 06146 (Plate 4.26) were moderately resistant (disease incidence of 5.56 and 7.14 per cent), four genotypes *viz.,* ICGV 03057, ICGS 76 (Plate 4.27) ICGV 07220 (Plate 4.28) and ICGV 07222 were moderately susceptible (11.11 – 25 per cent), ten genotypes *viz.,* ICGV 00187, ICGV 00191, ICGV 00202, ICGV 00203, ICGV 03042, ICGV 06100 (Plate 4.29), ICGV 05155, ICGV 93260, ICGS 44 and ICR 48 (Plate 4.30) were susceptible (26.67 – 50 per cent) and twenty four genotype *viz.*, ICGV 99058, ICGV 99072, ICGV 00162, ICGV 00189, ICGV 00201, ICGV 00206, ICGV 00211, ICGV 00241, ICGV 00246, ICGV 00247, ICGV 86590, ICGV 86699, ICGV 91114, ICGV 00308, ICGV 02266, ICGV 87846, ICGV 93468, ICGV 00348, ICGV 00350, ICGV 00351, ICGV 93261 (Plate 4.31), ICGV 89280, ICGV 92195 and 92035 were highly susceptible (52.94 – 100 per cent). There were no genotypes pertaining to highly resistant and resistant disease reaction grade.

The genotypes ICGV 00213, ICGV 03057, ICGV 07220, ICGV 06146, ICGS 76 and ICR 48 showed no disease incidence at 7 DAI for both 1:10 and 1:100 virus concentrations indicating their longer incubation period. At 1:10 virus concentration, due to high disease pressure these genotypes showed highly susceptible disease reaction at 21 DAI. At 1:100 virus concentration, these genotypes showed moderately resistant and moderately susceptible disease reaction except ICR 48 which showed susceptible disease reaction.

The above results indicate longer incubation period of virus inside the host plant which may be due to unsuitable environment in the host plant or may be due to block in movement of virus inside the plant due to host defense response.

Buiel and Parlevleit (1996) reported that young tissue and young plants are more susceptible while mature tissue and plants are highly resistant to GBNV. Disease incidence decreased and incubation period increased with the age of plants and leaves. This type of resistance (mature plant and tissue) occurs irrespective of the susceptibility level of the genotype to GBNV. However, this type of resistance develops earlier in the resistant than in the susceptible genotype.

Dwivedi *et al.* (1995) screened forty two groundnut genotypes for resistance to GBNV. All the genotypes were highly susceptible to GBNV at higher virus concentration

Plate 4.25 Moderately resistant genotype ICGV 00213, compared with resistant (ICGV 86031) and susceptible (JL 24) check

Plate 4.26 Moderately resistant genotype ICGV 006146, compared with resistant (ICGV 86031) and susceptible (JL 24) check

Plate 4.27 Moderately susceptible genotype ICGS 76, compared with resistant (ICGV 86031) and susceptible (JL 24) check

Plate 4.28 Moderately susceptible genotype ICGV 07220, compared with resistant (ICGV 86031) and susceptible (JL 24) check

Plate 4.29 Susceptible genotype ICGV 06100, compared with resistant (ICGV 86031) and susceptible (JL 24) check

Plate 4.30 Susceptible genotype ICR 48, compared with resistant (ICGV 86031) and susceptible (JL 24) check

Plate 4.31 Highly susceptible genotype ICGV 93261, compared with resistant (ICGV 86031) and susceptible (JL 24) check

of 1:10 dilution. At lower virus concentration of 1:100 dilution, three genotypes ICGV 86388, ICGV 91239 and ICGV 91245 showed resistance to the virus while others were highly susceptible.

Rao *et al.* (2006) challenged progeny of groundnut transgenic plants at two levels of concentration i.e at 1:100 and 1:50. At 1:100 concentration, 24 of 36 transgenic plants

tested did not exhibit any symptoms and did not acquired the virus. However, at 1:50 concentration all the 24 lines acquired the virus.

In the present study, none of the groundnut genotypes screened under artificial inoculated conditions using sap of the virus were highly resistant or resistant to the GBND. This could be attributed to the high inoculum pressure of the virus. However, the reaction of these genotypes may change, if the screening is attempted with lower virus concentration of 1:100 or 1:1000 (Rao *et al.*, 2003b; Kalyani *et al.*, 2005).

4.4.2 Severity of GBND in groundnut genotypes

The data pertaining to severity of GBND in groundnut genotypes at 1:10 and 1:100 virus concentrations is presented in Table 4.14 and Figs. 4.21 to 4.26.

The average GBND disease severity in these genotypes at 1:10 virus concentration ranged from 2 to 5 compared to 4 in ICGV 86031 (resistant check) and 5 in JL 24 (susceptible check). At 1:100 virus concentration disease severity ranged from 2 to 4 compared to 2 in ICGV 86031 (resistant check) and 4 in JL 24 (susceptible check).

The data revealed that the disease severity at 1:10 virus concentration ranged from 1 to 4 at 7 DAI, 2 to 4 at 14 DAI and 2 to 5 at 21 DAI. There was progressive increase in disease severity from 2.33 (7 DAI) to 3.86 (21 DAI), when we consider the mean disease severity of all genotypes. At 1:100 virus concentration disease severity, ranged from 1 to 3 at 7 DAI, 1 to 4 at 14 DAI and 2 to 4 at 21 DAI. There was progressive increase in disease severity from 2.07 (7 DAI) to 3.05 (21 DAI), when we consider the mean disease severity of all genotypes.

At 1:10 virus concentration, the highly susceptible group has 2 - 5 as their severity. While, at 1:100 virus concentration, the moderately resistant and moderately susceptible reaction grade genotypes have 2 as their severity, the susceptible and highly susceptible

Table 4.14 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution and 1:100 dilution

S. No.	Genotype	Severity* at 1:10 dilution			Severity* at 1:100 dilution		
		7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI
1	ICGV 99058	4	4	4	3	3	4
2	ICGV 99072	4	4	4	3	3	4
3	ICGV 00162	3	4	4	3	3	4
4	ICGV 00187	2	3	3	3	3	3
5	ICGV 00189	3	3	3	2	2	2
6	ICGV 00191	3	3	3	2	3	3
7	ICGV 00201	3	3	4	2	3	3
8	ICGV 00202	3	3	3	2	3	3
9	ICGV 00203	3	3	4	2	3	3
10	ICGV 00206	3	3	4	2	3	3
11	ICGV 00211	2	3	4	2	3	3
12	ICGV 00213	1	3	4	1	2	2
13	ICGV 00241	3	4	4	3	4	4
14	ICGV 00246	3	4	4	3	3	4
15	ICGV 00247	2	3	3	2	2	3
16	ICGV 86590	4	4	4	3	4	4
17	ICGV 86699	4	4	5	3	4	4
18	ICGV 91114	3	4	4	2	3	3
19	ICGV 00308	3	3	3	3	3	3
20	ICGV 03042	3	3	3	2	3	3
21	ICGV 03057	1	2	2	1	1	2
22	ICGV 06100	2	3	4	2	3	3
23	ICGV 07222	2	2	3	2	2	2
24	ICGV 07220	1	2	4	1	2	2
25	ICGV 05155	2	3	4	2	2	3

Table 4.14 contd..							
26	ICGV 06146	1	3	4	1	1	2
27	ICGV 02266	2	2	2	2	2	2
28	ICGV 87846	2	4	5	2	2	3
29	ICGV 93468	2	3	4	2	2	3
30	ICGV 00348	2	3	5	2	3	4
31	ICGV 00350	2	3	4	2	2	3
32	ICGV 00351	2	3	4	2	3	3
33	ICGV 93260	2	2	3	2	2	3
34	ICGV 93261	2	3	3	2	2	2
35	ICGV 89280	2	3	3	2	2	3
36	ICGV 92195	2	3	5	2	3	4
37	ICGV 92035	2	3	5	2	3	4
38	ICGS 44	2	3	5	2	3	4
39	ICGS 76	1	3	5	1	2	2
40	ICR 48	1	4	5	1	2	3
41	ICGV 86031 (Resistant check)	2	3	4	2	2	2
42	JL 24 (Susceptible check)	2	4	5	2	3	4
	Mean of all genotypes	2.33	3.14	3.86	2.07	2.60	3.05

*Mean of three replications

DAI- Days after Inoculation

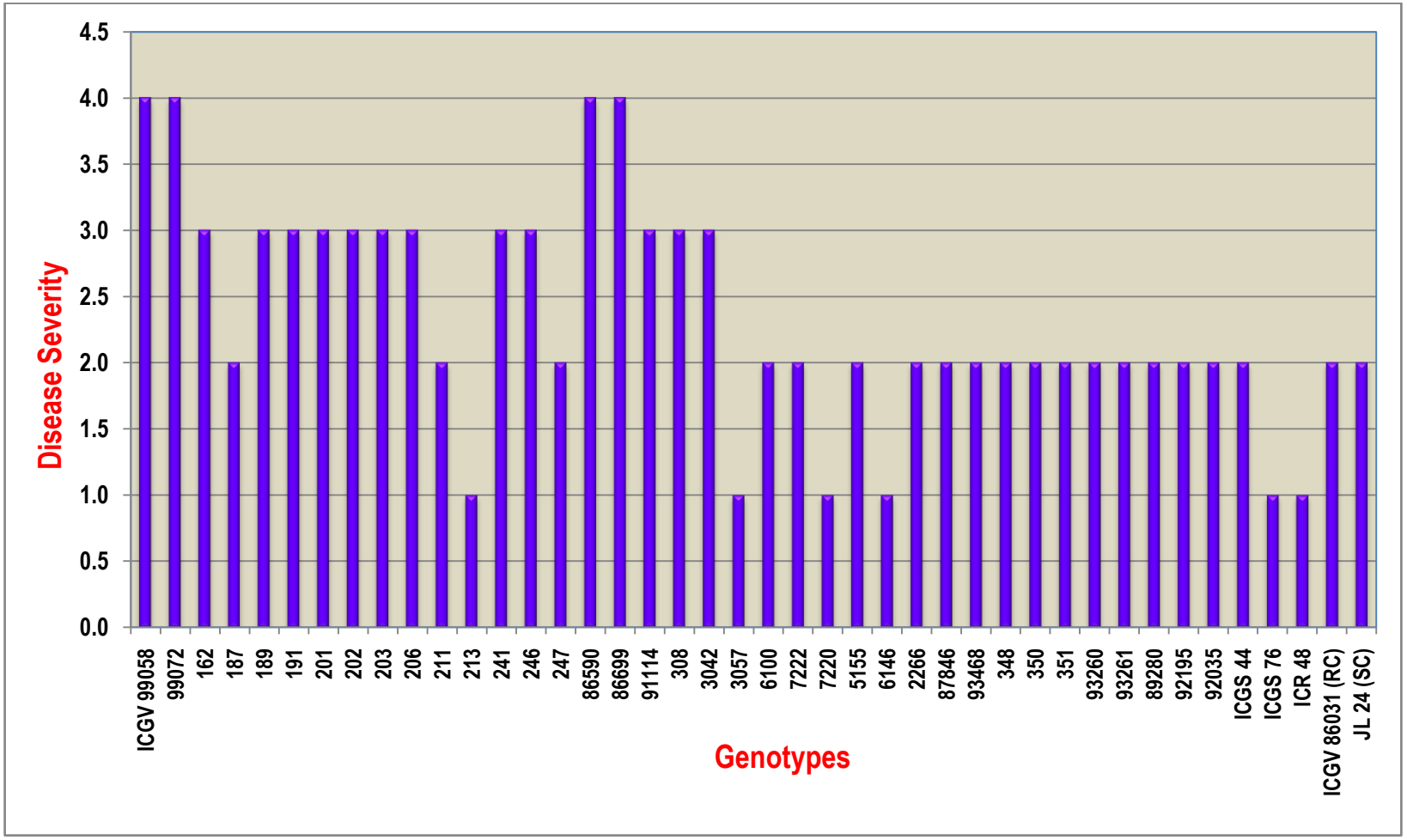


Figure 4.21 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution at 7 DAI

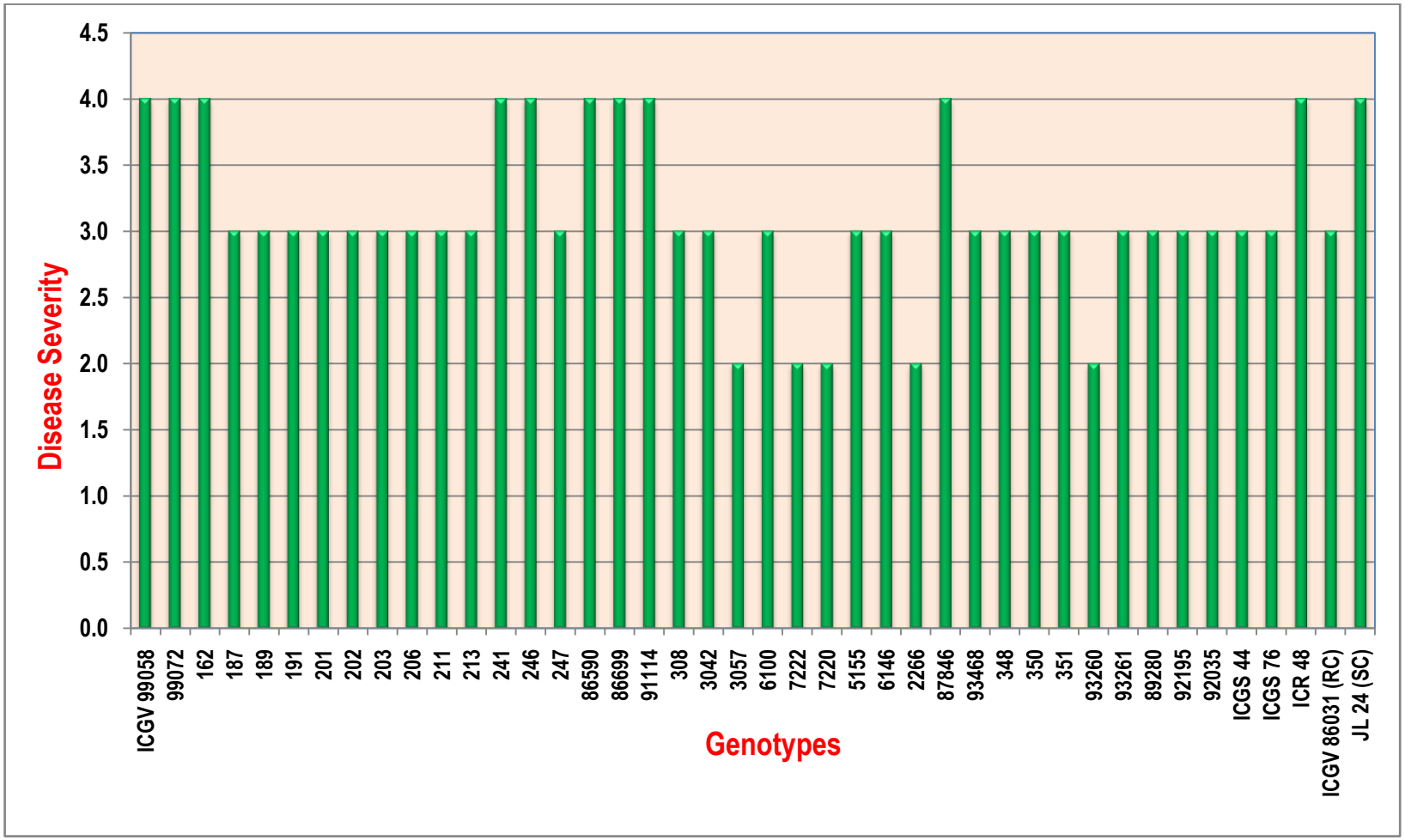


Figure 4.22 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution at 14 DAI

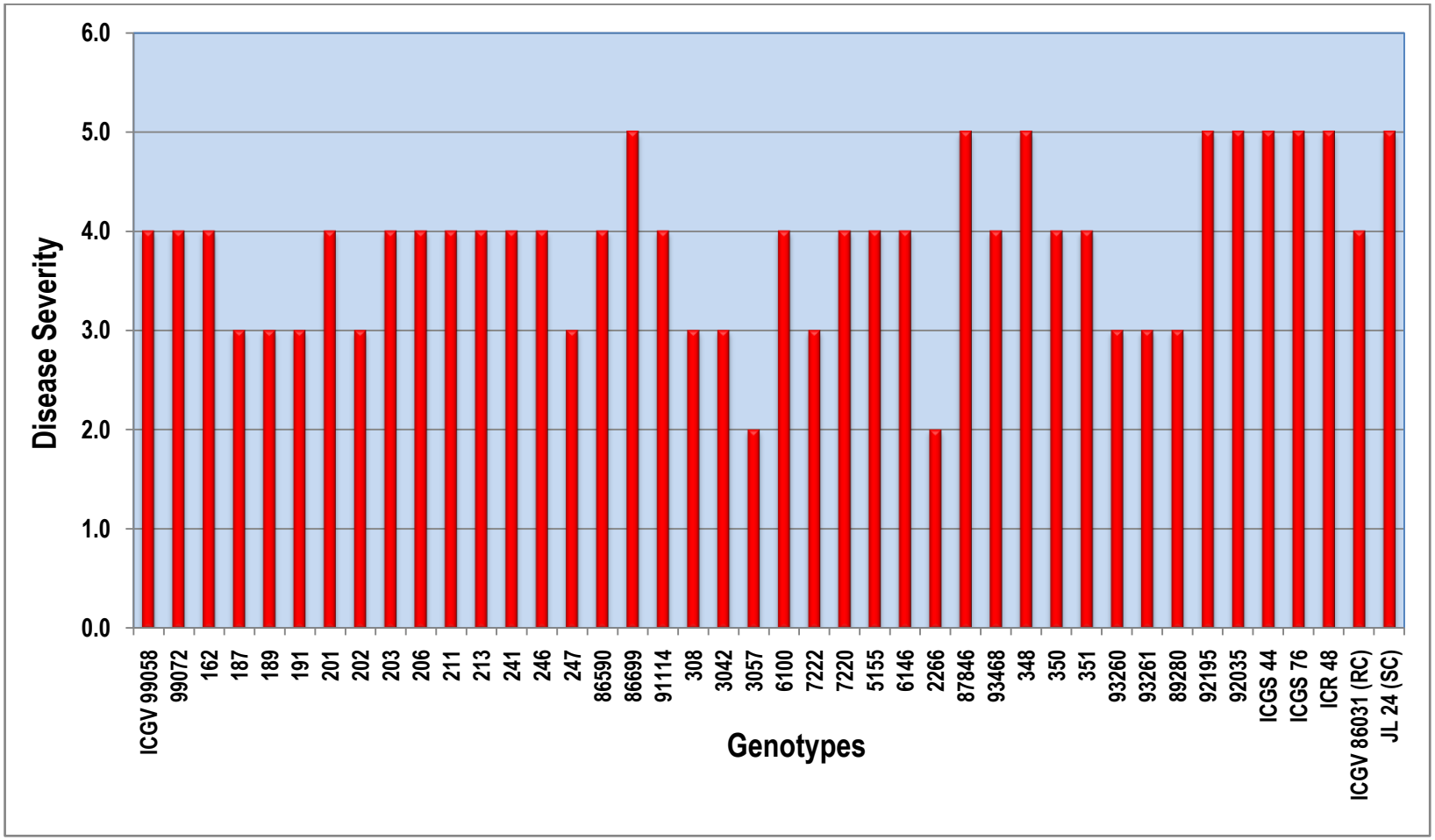


Figure 4.23 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:10 dilution at 21 DAI

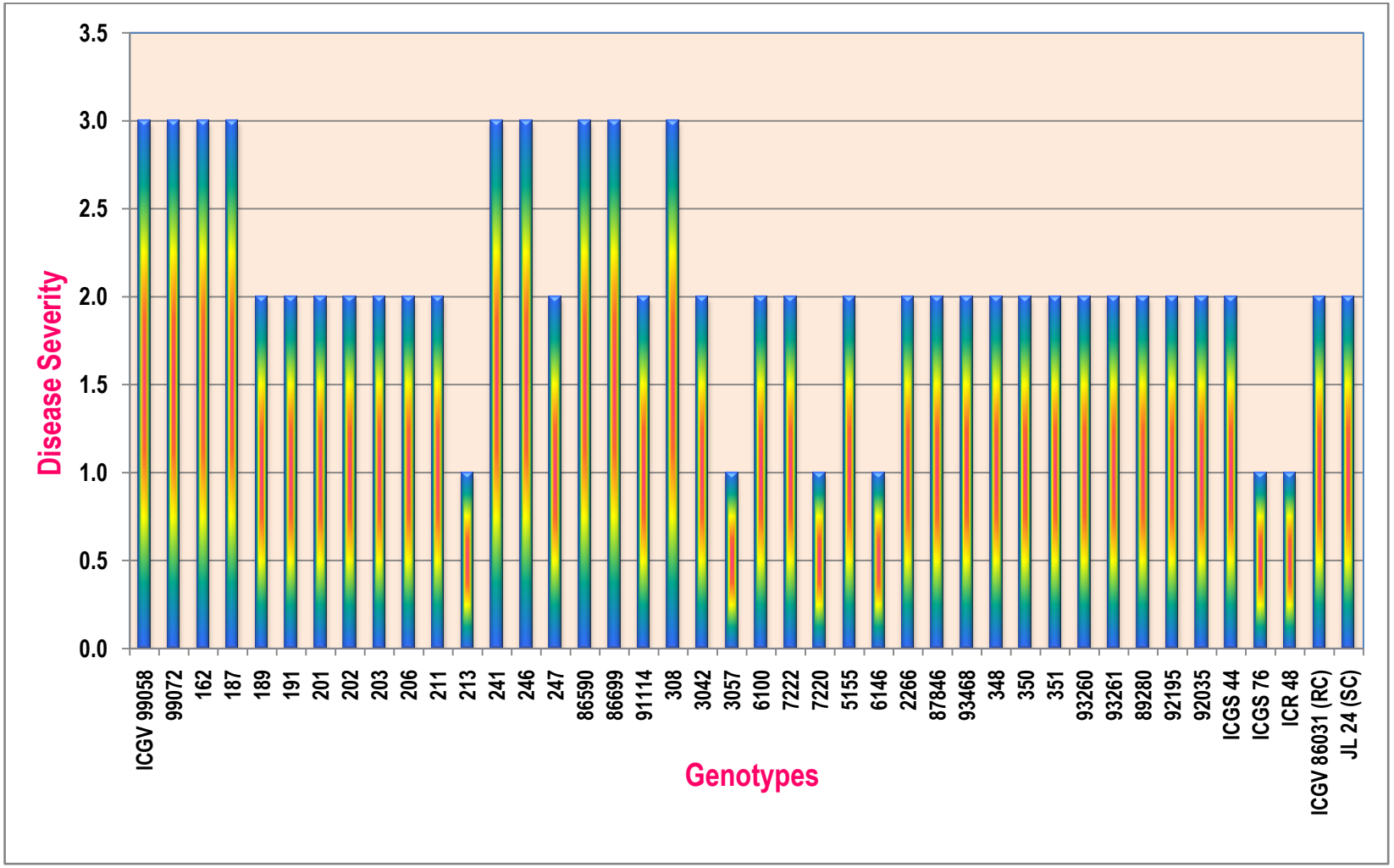


Figure 4.24 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution at 7 DAI

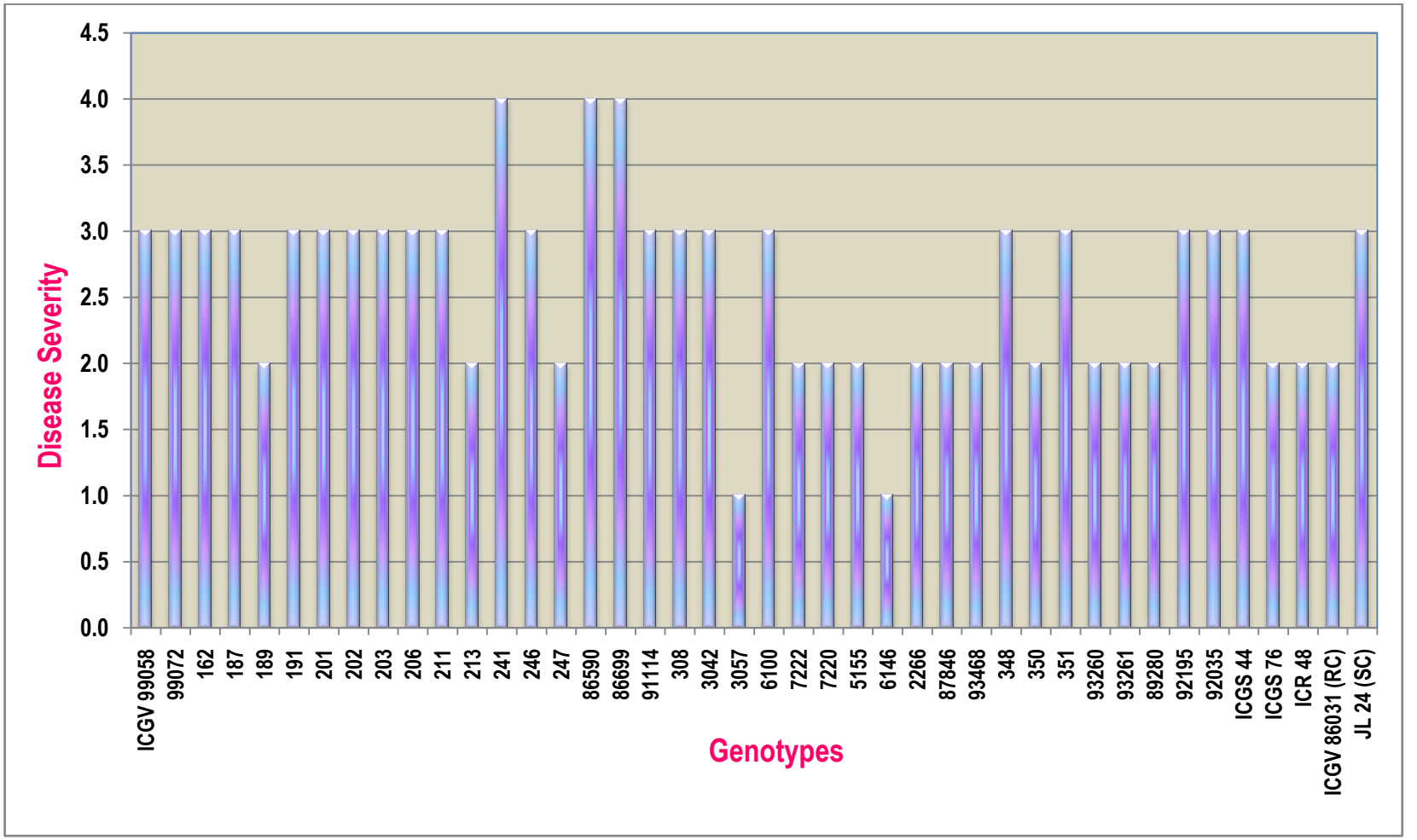


Figure 4.25 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution at 14 DAI

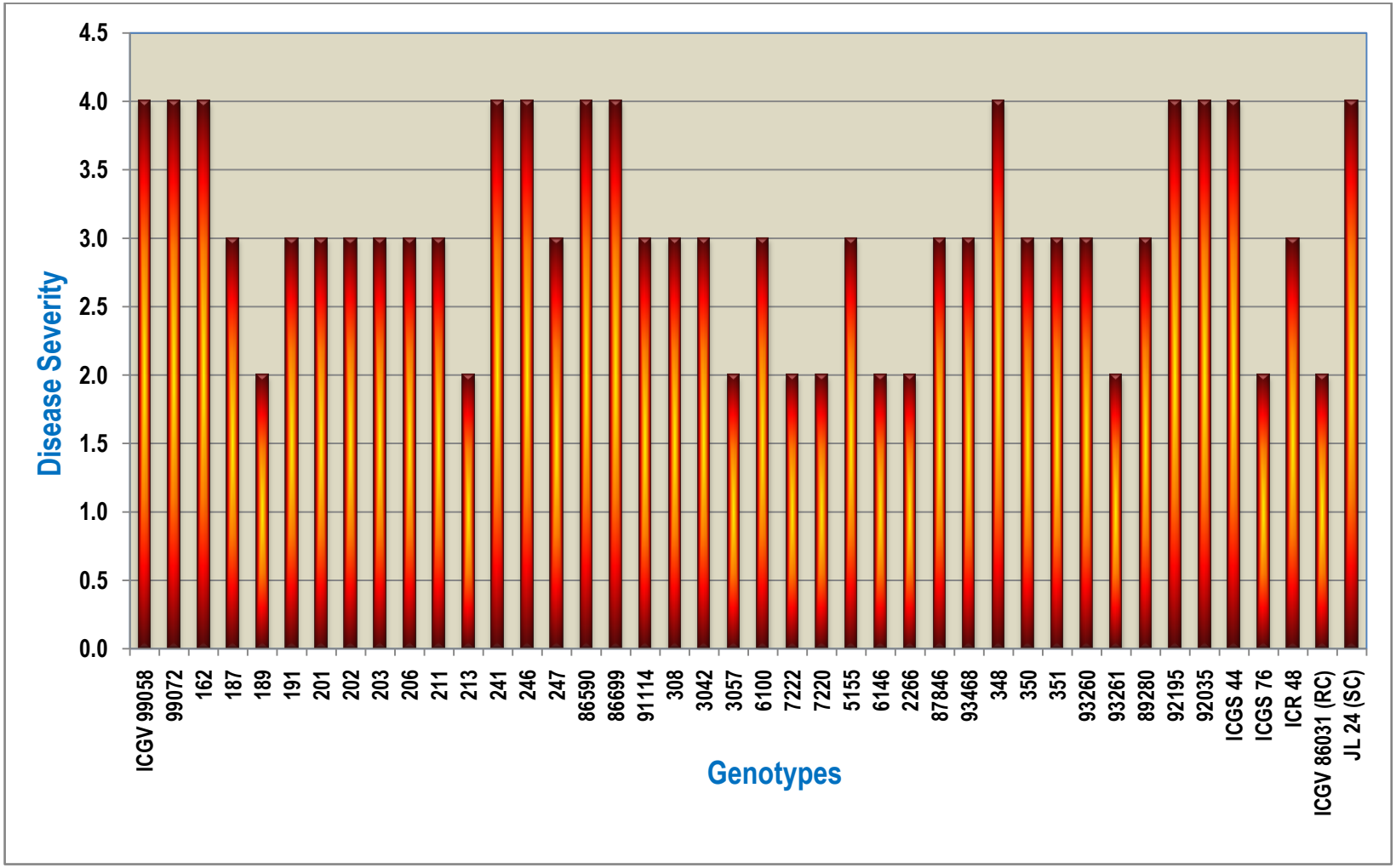


Figure 4.26 Disease severity of groundnut genotypes to mechanical inoculation of groundnut bud necrosis virus at 1:100 dilution at 21 DAI

reaction grade has 2 - 4 as their severity. This clearly shows the drawback in using disease severity as a parameter to measure the disease.

4.4.3 Detection of GBNV through DAC-ELISA

The genotypes showing moderately resistant, moderately susceptible and susceptible reaction at 1:100 dilution of virus concentration were selected for ELISA test. ICGV 86031 (resistant check) and JL 24 (susceptible check) at 1:10 and 1:100 dilution of virus concentration were also tested by ELISA.

The data pertaining to DAC-ELISA is presented in Table 4.15 and Plate 4.32.

The moderately resistant genotypes *viz.,* ICGV 00213 and 06146 gave positive reaction with 6.11 and 28.57 per cent infection with GBNV antiserum and the absorbance values at 405 nm was in the range of 0.090 – 1.624 confirming their moderately resistant reaction grade.

The moderately susceptible genotypes *viz.,* ICGV 03057, ICGV 07222, ICGV 07220 and ICGS 76 gave positive reaction with 12.5 - 50 per cent infection with GBNV antiserum and the absorbance values at 405 nm was in the range of 0.100 – 1.841 confirming their moderately susceptible reaction grade.

The susceptible genotypes *viz.,* ICGV 00187, ICGV 00191, ICGV 00202, ICGV 00203, ICGV 03042, ICGV 06100, ICGV 05155, ICGV 93260, ICGS 44 and ICR 48 gave positive reaction with 73.33 – 93.75 per cent incidence to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.094 – 1.941 confirming their susceptible reaction grade.

The resistant check ICGV 86031 at 1:10 virus concentration and 1:100 virus concentration gave positive reaction with 93.33 and 38.09 per cent infection to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.407 - 2.559 and 0.088 - 1.820 respectively.

The susceptible check JL 24 at 1:10 virus concentration and 1:100 virus concentration gave positive reaction with 100 and 85.71 per cent infection to GBNV antiserum and the absorbance values at 405 nm was in the range of 0.593 - 2.218 and 0.397 – 2.129 respectively.

Table 4.15 Detection of virus causing GBND in groundnut samples collected from greenhouse experiment through DAC-ELISA

S. No.	Genotype	Virus Concentration	No. of samples	Absorbance Value (405nm) range	Per cent Infection based on ELISA
1	ICGV 00187	10^{-2}	15	0.228 – 1.941	73.33
2	ICGV 00191	10^{-2}	15	0.315 – 1.687	80
3	ICGV 00202	10^{-2}	15	0.413 – 1.836	80
4	ICGV 00203	10^{-2}	15	0.409 - 1.663	93.33
5	ICGV 00213	10^{-2}	18	0.110 – 1.268	6.11
6	ICGV 03042	10^{-2}	16	0.094 – 1.076	93.75
7	ICGV 03057	10^{-2}	15	0.100 – 1.369	12.5
8	ICGV 06100	10^{-2}	15	0.297 – 1.814	93.33
9	ICGV 07222	10^{-2}	15	0.404 – 1.189	46.67
10	ICGV 07220	10^{-2}	16	0.101 – 1.776	50
11	ICGV 05155	10^{-2}	15	0.480 – 1.761	93.33
12	ICGV 06146	10^{-2}	14	0.090 – 1.624	28.57
13	ICGV 93260	10^{-2}	15	0.132 – 1.521	86.67
14	ICGS 44	10^{-2}	15	0.106 – 1.923	93.33
15	ICGS 76	10^{-2}	15	0.398 – 1.841	40
16	ICR 48	10^{-2}	15	0.248 – 1.315	86.67
17	ICGV 86031 (Resistant check)	10^{-1}	15	0.407 – 2.559	93.33
18	ICGV 86031 (Resistant check)	10^{-2}	21	0.088 - 1.820	38.09
19	JL 24 (Susceptible check)	10^{-1}	12	0.593 – 2.218	100
20	JL 24 (Susceptible check)	10^{-2}	14	0.397 - 2.129	85.71
21	GBNV (+ ve control)		6	1.924 – 2.217	100
22	Healthy(- ve control)		5	0.101- 0.119	0
23	Buffer		7	0.146 - 0.295	0

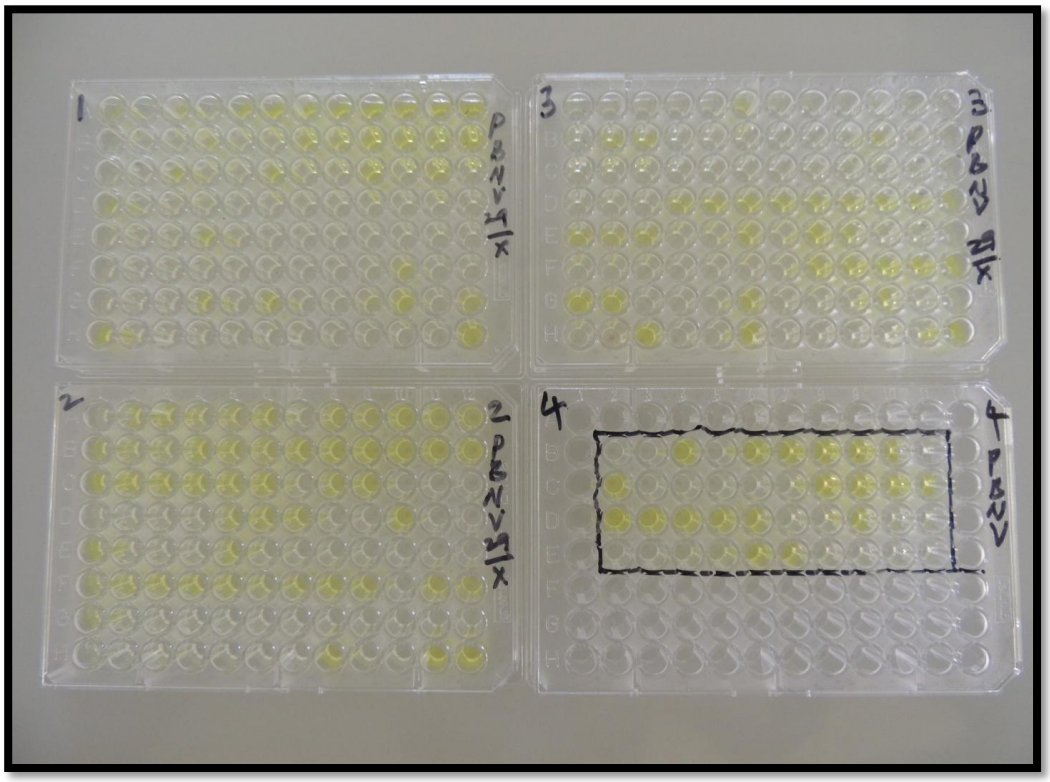


Plate 4.32 Detection of GBNV in groundnut samples collected from greenhouse by DAC-ELISA

The per cent infection to GBNV antiserum and the absorbance values at 405nm clearly differentiated the resistant and susceptible check at 1:10 virus concentration and 1:100 virus concentrations.

The ICGV 86031 (resistant check) showed 93.33 per cent susceptibility with 1:10 dilution of virus concentration and positive reaction with ELISA. This might be due to the high disease pressure applied.

Reddy *et al.* (2000) reported that genotypes ICGV 86031 and ICGV 86388 cause substantial losses to the crop under high disease pressure.

Reddy *et al.* (2000) evaluated seven accession of groundnut showing field resistance for virus resistance by sap inoculation. Of all the accessions tested, one accession of *Arachis cardenasii* (ICG 11564) and two accessions of *A. villosa* (ICGs 13168 and 8144) were free from systemic infection even after repeated sap inoculation. These accessions showed virus replication but systemic leaves were free from infection as detected by ELISA. This might be due to block in systemic virus movement.

4.5 IDENTIFICATION OF VECTOR (FIELD) AND VIRUS (GREENHOUSE) RESISTANT GENOTYPES

The genotype ICGV 06146 showed resistant reaction in field and moderately resistant reaction in greenhouse screening. ICGV 00213 showed moderately resistant reaction in both field and greenhouse screening. The genotypes *viz.*, ICGV 07222, ICGV 03057 and ICGS 76 showed moderately resistant reaction in field and moderately susceptible reaction in greenhouse. ICGV 00187, ICGV 00191, ICGV 00202, ICGV 00203, ICGV 06100, ICGV 93260, ICGV 05155 and ICR 48 gave moderately resistant reaction in field and susceptible reaction in greenhouse. ICGV 03042 showed resistant reaction in field and susceptible reaction in greenhouse. ICGV 07220 showed resistant reaction in field and moderately susceptible reaction in greenhouse. ICGS 76 showed moderately resistant reaction in field and moderately susceptible reaction in greenhouse.

The genotypic differences may be due to inherent response for resistance and susceptibility to GBNV. The genotypes mentioned above that showed variable degree of resistance under field and greenhouse conditions had Spanish bunch growth habit except ICGS 76 and ICR 48 which had Virginia bunch growth habit.

The genotypes *viz.,* ICGV 00187, ICGV 00191, ICGV 00202, ICGV 00203, ICGV 00213, ICGV 06146 and ICGV 93260 were also reported as resistant for foliar diseases whereas, the genotypes *viz.,* ICGV 03057, ICGV 07222, ICGV 07220, ICGV 05155 and ICR 48 were drought resistant.

The resistant check ICGV 86031 showed resistant reaction in field and susceptible reaction in greenhouse whereas, susceptible check JL 24, showed susceptible reaction in field and highly susceptible reaction in greenhouse. This implies that ICGV 86031 is resistant to vector *Thrips palmi* and susceptible to GBNV whereas, JL 24 is susceptible to both vector and virus.

The resistance showed by above genotypes could be associated with non preference of the vector or slower multiplication of virus in the host plant. In any case both the characters are of good value for a resistant genotype.

Future line of work

The present study indicates the need of future work in the following lines

1. Systematic survey for the incidence and severity of GBND in other groundnut growing areas of Andhra Pradesh season wise, so as to document the natural disease incidence in the backdrop of different agro climatic regions of the states.

2. Further screening of advanced breeding lines in multi location trails will help in direct release of these genotypes as promising varieties in hot spot locations of the country.

Chapter V

SUMMARY AND CONCLUSIONS

Chapter V

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Groundnut Bud Necrosis disease (GBND) caused by *Groundnut Bud Necrosis Virus* (GBNV) is widely distributed and endemic in many states such as Andhra Pradesh, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal.

Survey carried out in groundnut growing areas of Anantapur district of Andhra Pradesh during *kharif* and *rabi* of 2013-14 and in Karimnagar and Warangal districts during 2013-14 *rabi* season revealed the natural occurrence of GBND in different villages and mandals of these districts. In all, disease incidence ranged from 0 to 20 per cent, with a maximum mean incidence of 8.50 per cent in Anantapur district followed by Karimnagar (0.97 per cent) and Warangal (0.94 per cent) district. Different types of symptoms including, chlorotic spots, general chlorosis of top leaves, severe leaf distortion and severe necrosis with stunting were observed in the fields surveyed.

GBND incidence ranged from 0-20 per cent during *rabi* 2013-14 in Anantapur district. In which, maximum mean incidence (13 per cent) was recorded in Mulappagaripalli village of Nallamada mandals and minimum mean incidence (2.5 per cent) was recorded in Gachiguntapalli village of Obuladevaracheruvu mandal. In Warangal district, maximum disease incidence was recorded in Mogilicherla village (3.75 per cent) of Kuravi mandal and it was nil in Rajole, Narayanapur villages of Kuravi mandal and in Laxmipur, Reddial, Ammangal villages of Mahabubabad mandal. In Karimnagar district, Raghavapeta village of Mallapur mandal recorded maximum (5 per cent) and Muthampet, Mallapur villages of Mallapur mandal, Regunta of Metpalle mandal, and in Joganpalle, Venkatapur villages of Korutla mandal were free from the disease (zero incidence) during 2013-14. Samples collected from Karimnagar and Warangal district did not showed positive reaction for GBNV in DAC-ELISA. In Anantapur district, ELISA results of collected samples were in correlation with observed symptoms with a few exceptions.

Of the fifteen common weed species found in and around the surveyed groundnut fields, *P. hysterophorus, Celosia argentea, Tridax procumbens, Achyranthus aspera* and

Cynodon dactylon were more predominant and found in all the fields surveyed during *rabi* 2013-14, indicating their probable role in survival of the virus during off season.

Evaluation of forty groundnut genotypes for vector resistant sources under natural field conditions during *kharif* 2013-14 revealed GBND incidence in these genotypes ranging from 2.57 to 22.71 per cent compared to 4.04 per cent in ICGV 86031 (resistant check) and 25.45 per cent in JL 24 (susceptible check). There was progressive increase in mean disease incidence from 2.51 (30 DAS) to 7.81 (90 DAS) per cent for all genotypes. The data revealed that out of the 40 genotypes tested, eight genotypes *viz.*, ICGV 00201, 00211, 86699, 03042, 07220, 06146, 00350 and ICGV 00351were resistant (disease incidence of 2.57 - 4.99 per cent). 24 genotypes *viz.*, ICGV 00187, 00189, 00191, 00202, 00203, 00206, 00213, 00241, 00246, 00247, 03057, 06100, 07222, 05155, 02266, 87846, 00348, 93260, 93261, 89280, 92195, 92035, ICGS 76 and ICR 48 were moderately resistant (5.13 – 9.93 per cent). Eight genotypes *viz.*, ICGV 99058, 99072, 00162, 86590, 91114, 00308, 93468 and ICGS 44, were moderately susceptible (10.21 – 22.71 per cent). Four genotypes *viz.*, ICGV 07220 (2.57 %), ICGV 00350 (2.64 %), ICGV 00351(3.36 %), ICGV 00211 (4.02 %) were significantly superior compared to the resistant check ICGV 86031 (4.04 %). None of the genotypes showed highly resistant, susceptible and highly susceptible disease reaction grade.

The average GBND disease severity in these genotypes under field conditions ranged from 1.99 to 4.32 compared to 2.33 in ICGV 86031 (resistant check) and 4.67 in JL 24 (susceptible check). There was progressive increase in disease severity from 1.61 (30 DAS) to 2.71 (90 DAS), when the mean disease severity of all genotypes were taken into consideration. Resistant genotypes recorded disease severity (1.99 – 3.02) followed by moderately resistant genotypes (1.99 - 4.01) and moderately susceptible genotypes (2.66 - 4.32). Resistant and susceptible genotypes could not be clearly differentiated by utilizing disease severity scoring because there was overlapping of disease severity. Genotypes showing resistant, moderately resistant and moderately susceptible disease reaction were randomly selected. These genotypes along with resistant (ICGV 86031) and susceptible (JL 24) check were subjected to ELISA test and results were in confirmation to the reaction group.

Screening of groundnut genotypes for virus resistant sources under greenhouse conditions by mechanical inoculation revealed mean disease incidence at 1:10 dilution of standard extract ranging from 64.71 to 100 per cent compared to 72.22 per cent in ICGV 86031 (resistant check) and 94.44 per cent in JL 24 (susceptible check). There was progressive increase in disease incidence from 34.66 (7 DAI) to 88.79 (21 DAI) per cent, when we consider the mean disease incidence of all genotypes. All the genotypes were highly susceptible to GBNV at higher virus concentration (1:10 dilution). The mean disease incidence at 1:100 virus concentration ranged from 5.56 to 100 per cent compared to 26.67 in ICGV 86031 (resistant check) and 77.78 per cent in JL 24 (susceptible check) at 21 days after inoculation.

The data revealed that out of the genotypes tested, at 1:100 virus concentration two genotypes *viz.*, ICGV 00213 and ICGV 06146 were moderately resistant (disease incidence of 5.56 and 7.14 per cent), four genotypes *viz.*, ICGV 03057, 07222, 07220 and ICGS 76 were moderately susceptible (11.11 – 25 per cent), ten genotypes *viz.*, ICGV 00187, 00191, 00202, 00203, 03042, 06100, 05155, 93260, ICGS 44 and ICR 48 were susceptible (26.67 – 50 per cent) and 24 genotype *viz.*, ICGV 99058, 99072, 00162, 00189, 00201, 00206, 00211, 00241, 00246, 00247, 86590, 86699, 91114, 00308, 02266, 87846, 93468, 00348, 00350, 00351, 93261, 89280, 92195 and ICGV 92035 were highly susceptible (52.94 – 100 per cent). There were no genotypes pertaining to highly resistant and resistant disease reaction grade.

The genotypes ICGV 00213, 03057, 07220, 06146, ICGS 76 and ICR 48 showed no disease incidence at 7 DAI for both 1:10 and 1:100 virus concentrations indicating their longer incubation period for expression of symptoms. At 1:10 virus concentration, due to high disease pressure these genotypes showed highly susceptible disease reaction at 21 DAI. At 1:100 virus concentration, these genotypes could be differentiated into moderately resistant and moderately susceptible disease reaction group except ICR 48 which showed susceptible disease reaction.

The mean GBND disease severity in these genotypes under greenhouse conditions, at 1:10 virus concentration, ranged from 2 to 5 compared to 4 in ICGV 86031 (resistant check) and 5 in JL 24 (susceptible check). At 1:100 virus concentration, disease severity

ranged from 2 to 4 compared to 2 in ICGV 86031 (resistant check) and 4 in JL 24 (susceptible check).

The genotypes showing moderately resistant, moderately susceptible and susceptible reaction at 1:100 dilution of virus concentration under greenhouse conditions were selected for ELISA test. ICGV 86031 (resistant check) and JL 24 (susceptible check) at 1:10 and 1:100 dilution of virus concentration were also tested by ELISA. The per cent incidence to GBNV antiserum and the absorbance values at 405 nm clearly differentiated the resistant and susceptible check at 1:10 virus concentration and 1:100 virus concentration.

The genotype ICGV 06146 showed resistant reaction in field and moderately resistant reaction in greenhouse screening. ICGV 00213 showed moderately resistant reaction in both field and greenhouse screening. The genotypes *viz.,* ICGV 07222, 03057 and ICGS 76 showed moderately resistant reaction in field and moderately susceptible reaction in greenhouse. The genotypes *viz.,* ICGV 00187, 00191, 00202, 00203, 00213, 06146 and ICGV 93260 were also reported as resistant for foliar diseases whereas, the genotypes *viz.,* ICGV 03057, 07222, 07220, 05155 and ICR 48 were drought resistant.

The present study revealed that the genotypes which were resistant or moderately resistant to the vector under field conditions showed relative degree of susceptibility under high disease pressure in greenhouse conditions. The genotypes ICGV 06146, 00213, 07222, 03057, 00187, 00191, 00202, 00203, 06100, 93260, 05155, ICGS 76 and ICR 48 which were found promising with combined resistance to the vector and GBNV can be further evaluated and genotypes showing consistency in field resistance can be used in resistance breeding programme.

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The pattern of 'Literature cited' presented above is in accordance with the guidelines for thesis presentation for Acharya N.G Ranga Agricultural University, Hyderabad.

* Original not seen

APPENDICES

Appendix A. Standard week wise weather data recorded at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) , during *kharif* 2013

Std Week.	Dates	Temperature (^{O}C)		Humidity (%)		Rainfall (mm)	Wind speed (kmph)
		Maximum	Minimum	Morning	Evening		
32	04th Aug -10th	28.27	21.27	91.42	71.71	52.6	8.21
33	11th -17th	27.85	21.42	94.85	77.28	87.8	8.67
34	18th -24th	28.59	20.65	88.14	65.14	3.6	10.28
35	25th -31st	29.27	21.48	92.71	70.28	0.8	5.72
36	01st -07th Sep	30.09	21.01	93.28	63.14	51.1	5.9
37	08th -14th	30.18	21.77	95.42	72.14	48.89	3.84
38	15th -21st	29.38	20.97	94.00	73.00	177.59	6.68
39	22nd -28th	30.82	20.91	89.71	59.14	0	6.58
40	29th -05th Oct	29.61	21.44	93.14	68.28	24.39	6.4
41	06th -12th	29.92	21.00	94.71	69.14	69.4	5.4
42	13th -19th	31.21	19.88	91.71	51.00	5.79	3.31
43	20th -26th	26.8	20.82	96.85	80.00	107.59	4.48
44	27th -02nd Nov	29.87	18.45	94.14	54.42	0	3.42
45	03rd -09th	28.54	15.17	92.71	50.14	0	3.57
46	10th -16th	27.95	13.02	90.28	37.71	0	3.54
47	17th -23rd	28.02	16.18	93	55.42	18.69	4.24
48	24th -30th	28.44	16.05	93.85	53.28	2	4.08
49	1st Dec-7th	27.62	12.18	95.28	45.28	0	3.81
50	8th-14th	28.61	8.31	94.28	30.00	0	2.67
51	15th-21st	28.09	10.82	92.57	36.00	0	4.32

Appendix B. Standard week wise weather data recorded at Agricultural Research Station, Kadiri, Anantapur, during *kharif* 2013

Std Week.	Dates	Temperature (^{O}C)		Humidity (%)		Rainfall (mm)	Wind speed (kmph)
		Maximum	Minimum	Morning	Evening		
24	June 09th-15th	32.7	24.3	73.3	37.4	0.6	11.0
25	16th -22nd	33.8	24.4	68.9	34.7	0.3	10.6
26	23rd -29th	32.9	24.4	73.3	37.9	0.3	11.2
27	30th -06st July	32.4	24.5	69.3	38.6	0.1	10.4
28	07th -13th	29.6	22.9	85.3	51.7	8.6	9.2
29	14th -20th	30.9	23.8	81.4	46.4	12.2	11.1
30	21st -27th	30.3	23.6	75.9	49.4	1.0	10.5
31	28th -03rd Aug	31.0	23.6	76.9	45.1	0.8	12.1
32	04th -10th	31.8	23.7	76.4	41.1	4.3	9.8
33	11th -17th	28.6	22.8	85.9	57.0	41.1	17.9
34	18th -24th	31.1	23.1	80.9	45.1	5.2	10.8
35	25th -31st	32.6	23.3	76.3	39.0	68.3	7.8
36	01st -07th Sep	30.1	22.4	89.4	51.0	42.2	7.3
37	08th -14th	26.3	20.9	90.4	67.9	18.6	6.3
38	15th -21st	29.5	22.5	84.8	51.8	1.3	6.8
39	22nd -28th	31.5	22.4	83.1	42.7	2.2	6.8
40	29th -05th Oct	30.3	22.2	85.7	49.9	37.1	6.7
41	06th -12th	30.3	22.7	86.6	51.0	20.2	6.9
42	13th -19th	30.5	22.8	87.7	51.0	3.1	6.1
43	20th -26th	27.3	21.8	93.7	67.1	100.8	6.5
44	27th -02nd Nov	29.5	21.4	91.7	51.6	2.4	6.4
45	03rd -09th	28.2	20.7	91.3	50.1	18.4	6.5
46	10th -16th	27.1	17.8	91.6	45.3	0.9	5.5
47	17th -23rd	28.3	18.2	92.6	51.7	1.0	5.4
48	24th -30th	29.4	20.5	90.4	41.7	0.3	6.1

Appendix C. Standard week wise weather data recorded at Agricultural Research Station, Kadiri, Anantapur, during *rabi* 2013 14

Std Week.	Dates	Temperature (^{O}C)		Humidity (%)		Rainfall (mm)	Wind speed (kmph)
		Maximum	Minimum	Morning	Evening		
49	1st Dec -7th	27.7	18.0	90.0	44.4	0.0	6.6
50	8th-14th	27.9	16.0	86.0	39.0	0.0	6.8
51	15th-21st	27.7	14.2	90.7	31.6	0.0	5.6
1	22nd-28th	27.1	16.2	88.9	39.1	0.0	7.2
2	29th- 4th Jan	26.3	16.5	89.1	39.4	0.0	6.7
3	5th-11th	29.1	16.4	91.7	32.0	0.0	6.1
4	12th-18th	29.6	17.4	88.6	28.4	0.0	7.0
5	19th-25th	28.4	17.3	84.6	35.7	0.0	7.6
6	26th-1stFeb	28.4	16.4	84.4	34.7	0.0	7.9
7	2nd-8th	31.5	16.8	69.3	18.6	0.0	6.6
8	9th-15th	32.2	19.6	64.7	22.9	0.0	6.3
9	16th-22nd	31.0	20.5	79.1	32.4	0.0	6.8
10	23rd-1stMar	31.1	20.0	78.7	29.7	0.0	7.5
11	2nd-8th	30.8	20.7	83.7	39.1	2.9	7.8
12	9th-15th	31.8	20.4	72.0	24.4	0.0	7.6
13	16th-22nd	35.2	20.8	54.7	13.9	0.0	7.5
14	23rd-29th	34.9	20.6	50.7	14.0	0.0	6.2

Appendix D. Standard week wise weather data recorded at Regional Agricultural Research Station (RARS), Jagtial, Karimnagar, during *rabi* 2013-14

Std Week.	Dates	Temperature (^{O}C)		Humidity (%)		Rainfall (mm)	Wind speed (kmph)
		Maximum	Minimum	Morning	Evening		
38	17th Sep-23rd	29.7	23.3	85.7	64.0	59.2	4.3
39	24rd-30th	33.0	24.2	77.9	58.6	0.0	3.2
40	1st Oct-7th Dec	32.5	23.4	84.6	70.0	137.4	3.6
41	8th-14th	31.9	23.2	89.3	70.0	33.8	3.2
42	15th-21st	33.2	21.8	82.7	62.9	0.2	1.4
43	22nd-28th	29.5	22.7	89.9	71.0	50.1	1.1
44	29th-4th Nov	31.5	18.6	84.0	50.9	0.0	0.6
45	5th-11th	30.1	16.8	84.7	51.4	0.0	1.0
46	12th-18th	28.7	14.5	82.7	45.6	0.0	1.3
47	19th-25th	30.6	16.2	84.9	50.9	1.8	1.7
48	26th -2nd Dec	30.8	18.0	82.0	51.4	0.0	1.5
49	3rd -9th	29.9	13.8	74.3	32.4	0.0	1.9
50	10th-16th	30.1	10.2	69.4	24.7	0.0	1.7
51	17th-23rd	30.0	11.5	72.0	32.7	0.0	1.5
52	24th-31st	29.3	13.8	80.9	38.5	0.0	1.8
1	1st Jan-7th	30.1	15.0	79.9	39.6	0.0	1.4
2	8th -14th	30.3	12.8	82.0	43.7	0.0	2.7
3	15th- 21st	30.3	11.7	79.0	44.1	0.0	3.8
4	22nd-28th	29.4	14.1	80.7	41.9	0.0	2.2

Appendix E. Standard week wise weather data recorded at Regional Agricultural Research Station (RARS), Warangal during *rabi* 2013-14

Std Week.	Dates	Temperature (^{O}C)		Humidity (%)		Rainfall (mm)
		Maximum	Minimum	Morning	Evening	
38	17th Sep-23rd	29.0	22.6	87.7	68.3	38.2
39	24rd-30th	30.3	22.7	86.7	62.1	63.2
40	1st Oct-7th Dec	30.0	23.5	90.3	63.7	16.2
41	8th-14th	29.4	23.3	89.7	64.9	36.0
42	15th-21st	30.6	22.4	90.6	62.1	0.0
43	22nd-28th	26.7	21.9	87.4	66.4	183.0
44	29th-4th Nov	28.3	20.6	89.7	59.6	0.0
45	5th-11th	27.0	18.8	91.1	63.6	0.0
46	12th-18th	25.7	14.6	85.4	59.7	0.0
47	19th-25th	26.7	17.5	86.0	60.6	7.2
48	26th -2nd Dec	29.0	18.0	86.8	60.7	1.4
49	3rd -9th	27.7	14.7	84.9	43.6	0.0
50	10th-16th	27.3	12.8	87.9	45.9	0.0
51	17th-23rd	26.2	14.5	86.9	54.1	0.0
52	24th-31st	26.6	15.8	89.1	58.1	0.0
1	1st Jan-7th	27.6	15.4	80.6	56.1	0.0
2	8th -14th	27.7	18.5	86.4	58.9	0.0
3	15th- 21st	28.7	17.9	82.3	59.4	0.0
4	22nd-28th	28.1	18.3	51.3	70.1	0.0