INTRODUCTION
1. INTRODUCTION

Groundnut (Arachis hypogaea L.) is a member of Papilionaceae subfamily of the Fabaceae family which comprises important edible oil seed crops in the world. India, China, Nigeria, Senegal, Sudan, Burma and the USA are the major groundnut-producing countries of the world. These countries together accounts for a total area of 18.9 million hectares and a production of 17.8 million tonnes i.e. 69% of the area and 70% of the production. In terms of cultivated area and production in the world, India ranks second. In India about 5.5 million hectares area is under cultivation annually and the production is about 6 million tonnes. About 80% of the total area lies in the five states of India viz., Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra, which together account for 84% of the total production. The productivity level of groundnut in India is less than a half of the major groundnut growing countries and nearly one-third of the world levels.

Owing to its high content of digestible proteins (22-30%), vitamins (E, K & B group), minerals (phosphorus, calcium, magnesium and potassium) and phytosterols (Savage and Keenan, 1994) groundnut has gained importance as a food crop in the recent years. The oil content of the seed ranges between 44% and 50%, depending upon the varieties and agronomic conditions. Groundnut oil is extensively used as a cooking medium both as refined oil and Vanaspati Ghee in addition to its use in manufacturing cosmetics, soap making and lubricants, olein stearin and their salts. Raw, roasted or sweetened kernels are directly used for consumption which can provide a calorific value of 349 per 100 grams. Groundnut kernels are exported for confectionary purposes. The residual oilcake after extraction of oil contains 7% to 8% of N, 1.5% of P₂O₅ and 1.2% of K₂O making it useful as a fertilizer (http://agroguide.weebly.com/uploads/5/0/3/1/5031454/oil_seed.pdf). It is an important protein supplement in cattle and poultry feeds as well. The oil-cake can also be used for manufacturing artificial fiber. The haulms are used as fodder for livestock. The shell is used as fuel and in manufacturing coarse boards, cork substitutes etc. Groundnut is a modulating legume with symbiotic nitrogen fixation in root nodules improving the soil fertility which makes it valuable for rotation crop.

On the basis of branching pattern cultivated groundnut can be classified into two subspecies, hypogaea with alternate branching and subspecies fastigiata with
sequential branching. Each subspecies is further divided into two botanical varieties; subsp. *hypogaea* into var. *hypogaea* (Virginia) and var. *hirsuta*; and subsp. *fastigiata* into var. *fastigiata* (Valencia), var. *vulgaris* (Spanish), var. *peruviana*, and var. *aequatoriana*. The flowers are borne axillary mostly near the base of plant. Flowers are self pollinated and stalk of ovary elongates to a peg which carries fertilized ovules at the tip. The peg grows positively geotropic and the tip becomes diageotropic until it penetrates soil to some depth (about 7 cm) and ovary starts developing into a pod which contains seeds which matures generally in about 60 days from fertilization.

### 1.1 CLIMATE AND SOIL

Groundnut is cultivated throughout the tropics which are extended to the subtropical countries lying between 45 °N and 35 °S latitude and up to an altitude of 1000 metres. Places with an annual rainfall of 1,250 mm dwell a successful growth of groundnut. During the flowering and pegging of the crop the distribution of the rainfall should be abundant. The total amount of rainfall required is 100 mm, 150 mm and 400-500 mm for pre-sowing operations (preparatory cultivation), sowing, and for flowering and pod development respectively. The groundnut crop is sensitive to frost, long and severe drought or water stagnation. Even though groundnut is grown on a wide variety of soil types, the crop is more suitable for sandy loam and loamy soils and in the black soils with good drainage.

### 1.2 PRODUCTION CONSTRAINTS

Groundnut yield has become very low in most Asian countries, owing to a number of biotic and abiotic stresses, apart from its cultivation on marginal lands. Moisture stress and frequent droughts, disease and pest attacks, low input use, etc are major production constraints. In addition, low output prices reduce incentives for farmers to invest in productivity enhancing technologies such as improved seeds, fertilizers and pesticides. Groundnut being a rainfed crop, its yield is largely determined by the quantum and temporal distribution of rainfall, in spite of which it performs well under low rainfall conditions if the rainfall is evenly distributed during the growing period. Moisture stress at critical growth stages can reduce yield substantially. The total production losses in groundnut due to all constraints at their moderate severity were estimated about 12.74 lakh tones, which amounts to an
economic loss of Rs. 1783.34 crores (Dhandhalya and Shiyani, 2009). Irrigation is limited to a very small proportion of the total groundnut area. Hence, groundnut yield is uncertain and production is riskier, discouraging farmers from investing in technology, inputs and irrigation. One of the main factors limiting improvement in groundnut yield is the lack of adoption of improved technologies. A majority of farmers grows traditional varieties that are adapted to local agro-ecological conditions, but have low genetic yield potential. In recent years, a number of new varieties with higher yield, better tolerance to drought and higher resistance to insect pests and diseases have been released by national and international agricultural research systems. However, their adoption is constrained by a lack of access to seed (seed requirement of groundnut is as high as 80 kg /ha 120 kg /ha) and outturn is low and uncertain. Poor storage conditions and low use of seed treatment chemicals reduce seed quality. Seed multiplication and delivery systems too are poor. Private sector participation in seed multiplication and delivery is limited because of high seed requirement and a low multiplication factor.

Groundnut is susceptible to a large number of fungal, viral and bacterial diseases, though all of them may not be economically important (Roy and Shiyani 2000, Dhandhalya and Shiyani, 2009). Diseases such as rust, early leaf spot, late leaf spot and bacterial wilt can cause considerable yield loss. The tobacco caterpillar, gram pod borer and leaf miner are some of main insect pests responsible for reducing groundnut yield. Aflatoxin contamination (by the fungi Aspergillus flavus and Aspergillus parasiticus) is an important constraint affecting groundnut quality in most Asian countries. It is a major health risk to both humans and animals and importing countries place strict restrictions on acceptable aflatoxin levels. In addition to biophysical constraints, domestic and international trade policies have acted as disincentives to groundnut production.

One of the major constraints in production of groundnut is reduction in yield caused by the various diseases caused by viruses. Although natural infection of more than 30 plant viruses representing 14 groups have been recorded on groundnut in different countries (Sreenivasulu, 2005), Peanut bud necrosis virus (PBNV), Tobacco streak virus (TSV), Peanut mottle virus (PeMoV) and Indian peanut clump virus (ICPV) are the main viruses which attack the groundnut crop in India. Among viral diseases Bud necrosis is the most destructive disease of groundnut in many groundnut growing areas of India and is potentially damaging in other countries.
1.3 **PEANUT BUD NECROSIS DISEASE**

The incidence of peanut or groundnut bud necrosis disease (PBND) was reported in the annual report of Indian Agricultural Research Institute (IARI), India in 1949 and later by Chohan (1972, 1974); Ghanekar et al., (1979) and the name given ‘Bud Necrosis’ by Reddy et al., (1968). The disease is reported to occur in all prominent groundnut-growing areas of India. In Karnataka, the disease was first reported from Dharwar (Siddramaiah et al., 1977). The incidence of bud necrosis of groundnut ranges from 5 to 80% in different parts of the Indian subcontinent. Worldwide it causes a loss of over one billion dollars annually (Goldbach and Peters, 1994; Moyer, 1999; Prins et al., 1995). Losses due to PBND have been estimated at over 89 million US $ per annum (Anonymous, 1992). The yield loss due to PBNV in India was estimated to be more than 80% (Das Gupta et al., 2003). PBNV is the type species of genus *Tospovirus* and family *Bunyaviridae*. *Tospovirus* constitute the only genus of plant-infecting viruses in the family *Bunyaviridae* (Fauquet et al., 2005). The virus is vectored by thrips, *Frankliniella occidentalis*, *F. schultzei*, *F. fusca*, *Thrips tabaci* and *Scirtothrips dorsalis* which infest over 800 plant species, both dicots and monocots, in more than 80 plant families. The largest numbers of susceptible plant species were reported from the families *Solanaceae* and *Compositae* families (Prins, 1996).

1.4 **SYMPTOMS**

Symptoms produced by peanut bud necrosis virus (PBNV) in groundnut are difficult to distinguish from those caused by tomato spotted wilt virus (TS WV). Initial symptoms appear on young quadrifoliolates as mild chlorotic mottle or spots, which develop into necrotic and chlorotic rings and streaks. Necrosis of the terminal bud, a characteristic symptom, occurs on crops grown in the rainy and post rainy seasons, when ambient temperatures are relatively high. Secondary symptoms are stunting, axillary shoot proliferation, and malformation of leaflets. If plants are infected early, they are stunted and bushy. If plants older than one month are infected, the symptoms may be restricted to a few branches or to the apical parts of the plants. Due to the severity of the symptoms, the virus causes severe losses to the groundnut crop, especially when plants are infected before they are a month old. Seeds from such plants are small, shriveled, mottled, and discolored. Late-infected plants may produce seed of normal size. However, testae on such seed are often mottled and cracked.
1.5 **CAUSAL VIRUS**

Until 1990, PBND in India was reported to be caused by TSWV (Reddy *et al.*, 1991). High-quality antisera became available for the detection of tospoviruses, to which the group TSWV belongs, only during the late 1980s. Data from serological comparisons and subsequently from sequencing of nucleic acids revealed the existence of several distinct tospoviruses (German *et al.*, 1992; de Avila *et al.*, 1993). In 1992, the virus causing PBND was identified as a distinct tospovirus and named PBNV. With ELISA as well as Western blots, PBNV was shown to be serologically distinct from TSWV and *Impatiens necrotic spot virus* (INSV) (Reddy *et al.*, 1992). PBNV contains three RNA species of about 9.0 kb (1RNA), 5.0 kb (mRNA), and 3.0 kb (sRNA) (Gowda *et al.*, 1998).

1.6 **TRANSMISSION**

Amin *et al.*, (1981) reported that the virus causing PBND in India is transmitted by *Frankliniella schultzei* and *Scirtothrips dorsalis*. Subsequent investigations, which involved accurate identification of thrips, showed that in fact *Thrips palmi* transmits PBNV, and not *F. schultzei* or *S. dorsalis*, which are also present on the plants. Further experiments showed that *T. palmi* could acquire PBNV as larvae and transmit it as adults. Maximum transmission (100%) was obtained when there were 10 adults per plant. The majority of individual adult thrips transmitted the virus for more than half of their life period, indicating the degree of erratic transmission. Cowpea was found to be the best host for rearing and multiplying *T. palmi* under laboratory conditions (Vijaya Lakshmi, 1994; Wightman *et al.*, 1995).

1.7 **MANAGEMENT OF PBND**

Strategies for management of viral diseases normally include control of vector population using insecticides and resistant cultivars. Several cultural practices such as adjustments to sowing dates, sowing at the recommended rate, adopting measures to maintain plant population, intercropping with fast-growing cereal crops such as maize and pearl millet can reduce the incidence of PBND. These practices have been shown to reduce infestation by *T. palmi*. Roguing of infected plants, especially during early stages of plant growth, should be avoided because this practice creates gaps in the field and can increase PBND incidence. Considerable progress has been made in identifying the sources of field resistance to PBND (Reddy *et al.*, 1995).
When compared with susceptible genotypes, some of the field resistant genotypes such as ICGV 86388, show resistance to PBNV and less colonization by vector thrips (Dwivedi et al., 1995). Cultivars such as ICGS 11, Kadiri 3, and ICGS 44 are field resistant to PBND (Reddy et al., 1995). Techniques of plant transformation and regeneration have been used as alternative approaches to conventional plant breeding to develop virus resistant cultivars. The development of transformation and regeneration systems has allowed the introduction of viral and other useful genes into peanut germplasm (Yang et al., 1998; Rohini and Rao, 2001; Higgins et al., 2004 and Tiwari et al., 2008). Genetic transformation of groundnut is an alternative for the improvement of the crop, allowing the transfer of individual genes which confer agronomic traits such as pest and virus resistance or enhancement of protein quality of the seeds (Mansur et al., 1995).

Genetically engineered resistance to PBNV, an enveloped virus with a negative strand RNA genome can be obtained, by transforming groundnut with the gene encoding the viral nucleocapsid protein. This approach may prove to be useful in producing plants resistant to infection by other negative strand viruses as well.

In view of the above considerations, the genetic transformation studies on groundnut using nucleocapsid protein gene of PBNV were undertaken with the following objectives:

- To develop transgenic groundnut plants resistant to PBNV.
- To characterize the putative transformants for integration, expression and inheritance of the introduced gene.
- To carry out evaluation of the transgenic plants for resistance to PBNV under glasshouse conditions.