Introduction

Peanut, (Arachis hypogaea L.) is grown for its oil and edible nuts and it is an annual plant of the family Fabaceae. Peanut plants are little, typically erect, and thin stemmed having feather-like leaves. The leaves arranged like alternate pairs with leaf-like attachments near the stalk. The flowers of peanut plant are yellow, orange, and cream or white. Flowers produce 'pegs', characteristic floral structures which go under into the soil to grow the pod. The pods could reach up to 10 cm (4 in) in length and having 1 and 5 seeds. Height of the peanut plant is about 0.6 m (2 ft) or depending on the variety (Thiessen and Woodward, 2012). Peanut may also be known as groundnut, monkeynut or earth nut.

Groundnut is an important legume crop originated in Argentina region of South America and cultivated in tropical, subtropical and warm temperate regions of the world. It is cultivated in 100 countries located between 40 °N to 40 °S. Groundnut seeds are rich source of edible oil (43-55%) and protein (25-28%). Two third of its production is used for oil extraction and remaining portion for consumption. After extraction of oil from seeds, remaining cake is used as high protein animal feed. It is a valuable source of vitamins B, E and K. It is the richest plant source of thiamine and is also rich in niacin, which is low in cereals (Settaluri et al. 2012). 25.2 million hectare area is used for the production of groundnut around the world with production of 3.59 million tonnes and 1.42 tonnes per hectare (Anon. 2005).

Production of peanut

India is second largest production of groundnut after China (fig. 1). Groundnut is the most important Indian oilseed. It accounts up to approximately 25% of the total production of oilseed in the the country. In India, it occupied an area of 4.20 million ha with a production of 6.9 million tons in 2011, which accounted for a yield of 1655 kg ha⁻¹ (FAOSTAT, 2012). Peanuts production is highly susceptible to rainfall deviations and show huge variation between years. Principal groundnut growing states are Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra which account for more than 80 per cent of Indian production as well as region. Regional estimates of groundnut production are Karnataka (0.5 million tons), Maharashtra (0.5 million tons), Tamil Nadu (1million tons), Andhra Pradesh (1-2 million tons) and Gujarat (1-3.5 million tons) are the main grower states of Peanuts. In khariff (June -
September) around 75% of the crop is produced and in rabi (November - March) remaining 25% is produced (http://www.agrocrops.com/groundnut-division.php).

![Figure 1: Peanut global production in million tons](image)

Plants provide basic needs to other living organisms and they are most prone to different diseases. Plant disease may be caused by fungi, bacteria, viruses, viroids and mycoplasmas and cause damage that reduces yield.

**Diseases of peanut**

More than 55 pathogens of groundnut crop were listed by Grover (1981). Among these 55 only few disease like early leaf spot late leaf spot (*Phaeoisariopsis personata*), *(Phaeoisariopsis arichidicola)*, rust (*Puccinia arachidis*), collar rot (*Aspergillus niger van Tieghem*), afla root (*Aspergillus flavus*), root rot (*Macrophomina phaseolina*), and stem rot (*Sclerotium rolfsii*) are gain economically important in India. In addition to these diseases, nematode disease such as root knot and viral disease peanut mottle, and clump; groundnut bud and stem necrosis are key diseases that limit groundnut production as well as productivity (Ghewande and Reddy, 1986). Pre- and post-harvest aflatoxin contamination in the kernels and meal also reduces groundnut quality as well as export value.
Introduction

Elicitation and characterization of ISR in peanut by PGPR against A. niger

Collar rot disease

*A. niger* causes collar rot / crown rot in groundnut. This disease occurs more in temperate regions (Phipps, 2000; Magnoli et al., 2006). Collar rot disease is seed-borne and the fungus survives on peanut seeds (Magnoli et al., 2006). Seed-borne fungi have been reported to generate abortion of seed, shrunken seeds, reduce seed size, seed rot, seed necrosis, discolorration of seed, physiological alternation of seed and reduction of germination capacity (Elwakil, 2003).

Jochem (1926) first reported that collar rot disease of groundnut is caused by *A. niger*. In India collar rot disease caused by *A. niger* was reported by Jain and Nema (1952). Collar rot is widespread in almost all groundnut growing states of India viz. Punjab, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujarat, Maharashtra, Rajasthan, Karnataka and Orissa. Collar rot disease is causing more damage in sandy loam and medium black soil. Most of the groundnut cultivars are at risk to this disease. Collar rot disease becomes visible in two different phases of germination. In the pre-emergence phase, rot of seeds may occur in soil or sooty black masses of spore covered at place of germination; the emerging hypocotyls are eventually killed by these spores. In the post-emergence phase, on the cotyledons; circular light brown lesions appear initially and as they proceed to the hypocotyl tissue or stem lesion.

Fungal Diseases in Groundnut plants

<table>
<thead>
<tr>
<th>Disease Name</th>
<th>Causative Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflaroot or yellow mold</td>
<td><em>Aspergillus flavus</em></td>
</tr>
<tr>
<td>Crown rot or collar rot</td>
<td><em>Aspergillus niger</em></td>
</tr>
<tr>
<td>Wilt</td>
<td><em>Fusarium soalni</em> and <em>Fusarium oxysporum</em></td>
</tr>
<tr>
<td>Seed and Seedling rot</td>
<td><em>Rhizopus archisus</em> and <em>Sclerotium rolfsii</em></td>
</tr>
<tr>
<td>Stem rot</td>
<td><em>Rhizopus archisus</em> and <em>Sclerotium rolfsii</em></td>
</tr>
<tr>
<td>Root rot, pod breakdown, wilt</td>
<td><em>Rhizoctonia solani</em></td>
</tr>
<tr>
<td>Damping off</td>
<td><em>Pythium ultimum</em> and <em>Pythium myriotylum</em></td>
</tr>
<tr>
<td>Vascular wilt</td>
<td><em>Verticillium alboatrum</em></td>
</tr>
<tr>
<td>Charcoal rot</td>
<td><em>Marcophomina phaseolina</em></td>
</tr>
<tr>
<td>Early and late leaf spot</td>
<td><em>Cercospora arachidicola</em> and <em>Cercosporidium personium</em></td>
</tr>
</tbody>
</table>

Table 1 List of fungal disease of peanut
Introduction

Elicitation and characterization of ISR in peanut by PGPR against A. niger

becomes water-soaked and light brown discoloration appear. The seedlings then collapse and die due to the rotting of the succulent hypocotyls. Due to *A. niger* average 5 per cent yield loss was reported but in some places it may cause as high as a 40 per cent loss. Joshi (1969) surveyed groundnut farming region in the Gujarat state (India) and illustrate as high as 50 per cent seedling blight in some region. Similarly, Ghewande et al. (2002) reported that losses in terms of mortality of plants due to collar rot ranged from 28 to 50 per cent.

**Figure 2** peanut infected with collar rot disease. a) The pre-emergence phase and b) The post-emergence phase.

To overcome the problem of seed born pathogens, agronomy techniques like crops rotation, use of resistant varieties and fungicides treatment to seeds and/or soil with, are followed. However all these methods become unsuitable or not effective, due to the variability in gene presented by the pathogen to the host variety, its capacity to survive in the soil and physiologic flexibility to infect different hosts (Leach and Garber, 1970). Biocontrol agents like elicitor and plant growth promoting rhizobacteria (PGPR) are economically feasible and ecologically sound alternative to induce natural resistance in plants. These beneficial attributes are environmental safety, long lasting effects and efficacy (Hammerschmidt and Kue, 1995).

**Plant growth promoting rhizobacteria**

Plant root exudates and lysate are utilized by soil bacteria for their growth and development around the rhizosphere. Therefore 100 fold higher numbers of bacteria are found in the rhizosphere compared to bulk soil and more than 15 % of root surface is occupied by varieties of bacterial strains (Van Loon, 2007). The microbe-plant interaction in the rhizosphere can be beneficial, neutral and deleterious for plant growth. Kloeper and Schroth (1978) introduced the term ‘rhizobacteria’ to the soil
bacterial community that competitively colonized plant roots and encouraged growth and thereby reducing the incidence of plant diseases. Rhizobacteria that provide beneficial effects on plant development and growth are termed as Plant Growth Promoting Rhizobacteria (PGPR). The term PGPR was first coined by Kloeper and Schroth (1981) but by which mechanism PGPR promote plant growth was not fully understood (Ravisankar and Nithya 2012).

**PGPR implies direct and indirect mechanisms as plant growth promoters and control biological agents**

Direct mechanisms by PGPR include solubilization of phosphorus, zinc and potassium in soil as well as nitrogen fixation for plant use, siderophore production for sequestration of iron, production of plant hormones like auxins, cytokinins, jasmonic acid and gibberellins; production of ACC deaminase that lower plant ethylene levels, etc. (Glick, 1995; Glick et al. 1999; Mayak et al. 2004).

Indirect mechanisms of PGPR comprise production of antibiotics against pathogenic fungi and bacteria, decrease of iron accessible to phytopathogens in the rhizosphere, production of fungal cell wall and insect-gut membrane lysing enzymes, and induction of systemic resistance against various pathogens and pests in plants (Ramamoorthy et al. 2001).

In addition, effects of PGPR on plant growth and yield of groundnut under agricultural settings is one of the ultimate criteria to understand how PGPR influences growth of plants. With this background information and keeping these points in view, the present investigations on seed borne pathogens of peanut was undertaken with following objectives.
Introduction

Objectives

- To isolate PGP rhizobacteria and to study their in vitro and in vivo efficacy to control collar rot disease in germinating groundnut seedlings.

- To identify selected potential bacteria by 16S rRNA gene sequencing.

- To study induction of resistance in peanut seedlings as well as in adult plants against *Aspergillus niger*.

- To study mechanism of induction of resistance by proteome analysis of PGPR treated and untreated seedlings.