Chapter 1

Literature Review

1.1. Introduction

Human health is closely associated with plant growth, health and productivity. Species and ecosystems have certain capabilities to tolerate changes in the environment which is known as resistance. But there are certain limitations to this attribute that represents the threshold of tolerance. When these thresholds are exceeded by further increase in the intensity of environmental stress, substantial ecological changes are caused.

Exogenous stress factors that affect the growth of plants have been divided into 2 categories i.e., Abiotic and Biotic stress factors. Plants are exposed to a diversity of biotic and abiotic stresses throughout their life cycle (Figure 1.1).

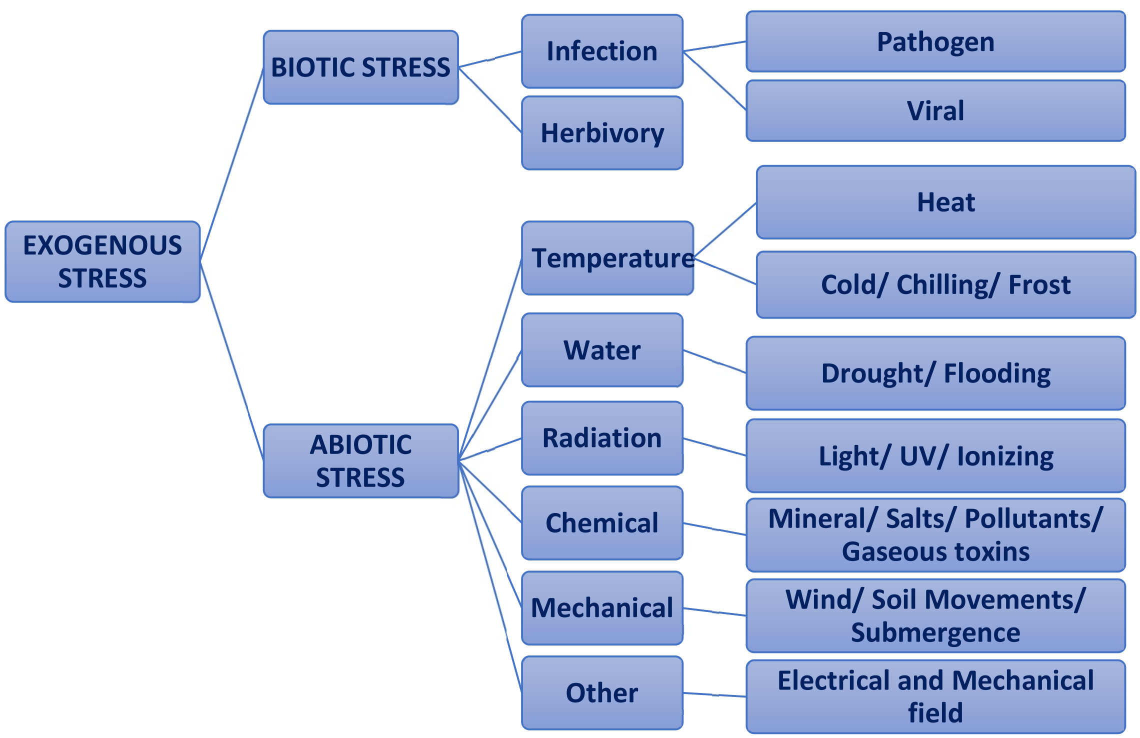

Figure 1.1: Types of environmental stress factors affecting the growth and production of plants.

1.2. Soil salinity and land degradation

Studies show that the changing regional patterns in association with rising temperature, erratic winds and rainfall etc., are due to the effect of global warming phenomenon, and would lead to further increase in aridity. Increase in aridity along with extensive land use practices are causing severe land degradation and increase in climate changes lead to further desertification (P. Singh and Nair, 2012) and increased salinization of the agricultural land area.

Some amount of salt is present in all the soils and is considered healthy for the plant growth up to certain limits. The term 'salt affected soils' (SAS) is used if the salt concentration in the soil is high enough to interfere with normal plant growth of most crop species. Saline soils usually have an electrical conductivity (EC) >4dSm^{-1} (~36mM NaCl) and can sometimes be as high as 68dSm^{-1}. SAS are divided into two categories depending on the amount of salt, type of salts, sodium content and soil alkalinity i.e., Saline and Sodic soils.

Those with a high percentage of soluble salts and exchangeable sodium ions are called saline soils (Figure 1.2a). The exchangeable salts mainly include sodium chloride and sodium sulphate, but also contain sulphates and chlorides of calcium (Ca^{2+}) and magnesium (Mg^{2+}). Salinity is mainly caused as a symptom of change in water balance. It affects vegetation and water quality due to free salts. It can be clearly identified as barren patches among the agricultural or vegetational fields.

Figure 1.2: (a) Saline soil and (b) Sodic soil. The saline soils contain higher amount of soluble free salts while sodic soils are alkaline in nature salt particles tightly bound to clay particles causing low water permeability.

Sodicity affects salt behaviour, high amount of sodium ions is bound to clay particles which cause instability and breakdown of land structures under wet condition. It causes landscape issues such as soil erosion, restricted water movement and high clay dispersion. These are mostly formed in the sub soil regions (Figure 1.2b; indicated by red arrow) and are more unreliable for supporting plant growth. Sodic soils are alkaline in nature (Alkali soils), with the major components being sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) as soluble salts, with chloride (Cl^-), sulphate ($SO_4{}^{2-}$), carbonates ($CO_3{}^{2-}$) and bicarbonates (HCO^{3-}) as the anionic constituents (P. Kumar and Sharma, 2020). The pH >8.2, while EC of the soil solution varies. Cultivation is difficult in sodic soils due to high pH, poor drainage and toxic effects of sodium (Biswas and Biswas, 2014; Essington, 2020).

Soil salinity has affected nearly 20% of arable land worldwide and has been predicted to increase up to 50% by the year 2050 (Etesami and Adl, 2020). While continued development and spread of SAS is seen as a threat to agricultural sustainability, these degraded ecosystems offer immense opportunities to harness the productivity potential through appropriate technological interventions. Even marginal to modest gains in crop yields in such soils would mean dramatic improvements in the lives of thousands of poor farmers in salinity affected regions of the world. Some of these include sodic soil reclamation, essentially aiming at replacement of the soluble Na^+ by soluble Ca^{2+}such as gypsum and calcium chloride. Other practices include treatment with acids and acid forming substances such as sulphuric acid and pyrite, and calcium salts of low solubility such as ground limestone (Sharma and Singh, 2015).

1.2.1. Salinity distribution around the world – India – Gujarat

Soil salinity is spreading dynamically world-wide with no continent spared (Figure 1.3). It is a major challenge to accurately identify and quantify the irrigation-induced salinity hotspots because of the high geographic variability and constant changes in the net salinity levels, however, certain areas in the arid and semi-arid regions are known to be permanently affected by salinity (Hassani et al., 2020).

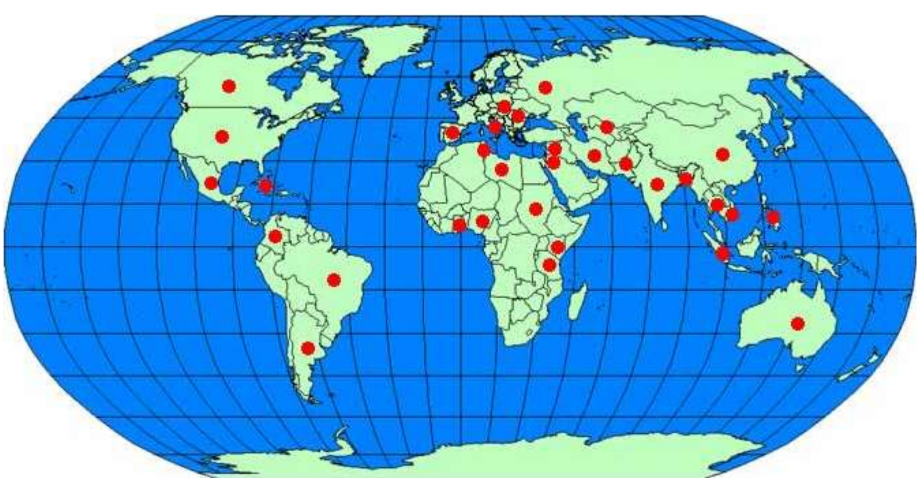

Figure 1.3: World map depicting countries affected by salinity stress. (Source: Pavuluri et al., 2014)

Arid regions are those which receive little precipitation, less than 10 inches or 25cm of rainfall, annually and are severely affected by soil salinity. These areas have a distinct landscape with little vegetation and often loose surface material. Erosion is the main factor in shaping the land surface. Of the total area globally, 18.8% of the land area is arid zone, of which 46.1% is accounted for in Africa followed by 35.5% in Asia.

Area-wise break up of arid regions of the states affected in India are as listed in the Table 1.1 below, which shows that 31.7 million Hectares of land area in India is constituted of arid regions, Figure 1.4a shows land area distribution of arid and semi-arid regions in India (Tewari et al., 2014).

Table 1.1: Distribution of arid area in different states of India. (Source: Tewari et al., 2014)

State (s)	**Area (mHa)**	**Percent of total land area**
Rajasthan	19.61	61.0
Gujarat	**6.22**	**19.6**
Punjab and Haryana	2.73	9.0
Andhra Pradesh	2.15	7.0
Karnataka	0.86	3.0
Maharashtra	0.13	0.4
Total	31.7	**11.8% (Indian soil cover)**

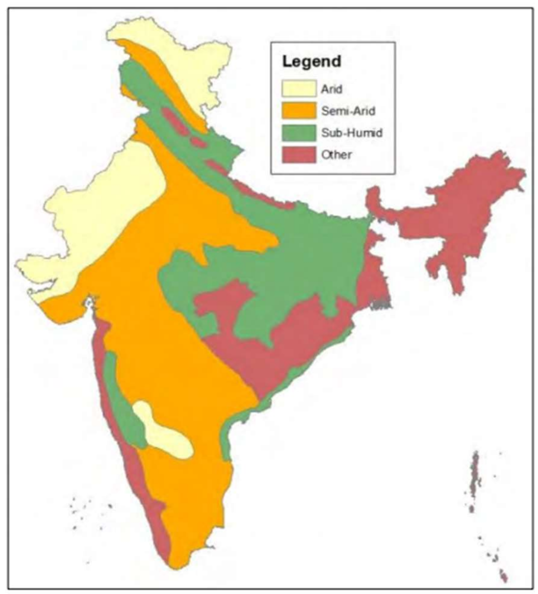

Figure 1.4a: Land areas affected by aridity in India. (Source: Tewari et al., 2014)

About 20% of India's arable land is affected by salinity (Figure 1.4b). Although Rajasthan has the maximum area affected by aridity, coastal Gujarat has the maximum area covered by SAS in the country, followed by UP, etc. (Table 1.2) (Biswas and Biswas, 2014; Essington, 2020).

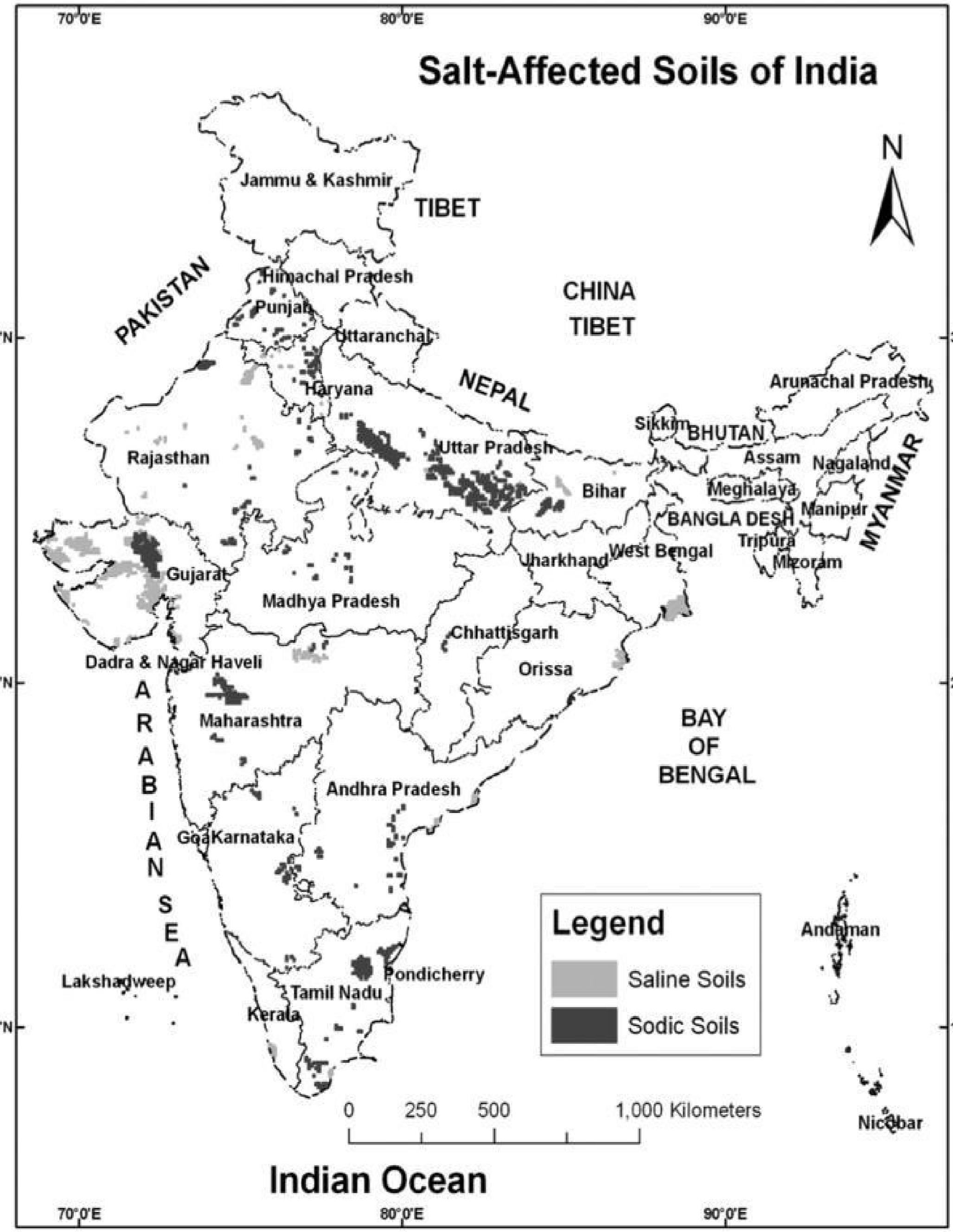

Figure 1.4b: Salt affected regions (SAS) in India. (Source: Essington, 2020)

Table 1.2: State-wise distribution of salinity affected regions in India, from maximum to lowest.

States affected by Salinity	Saline soil (ha)	Sodic soil (ha)	Coastal saline region (ha)	Total (ha)
Gujarat	**1218255 (71%)**	**541430**	**462315 (37%)**	**2222000 (33%)**
Uttar Pradesh	21989	1346971	0	1368960
Maharashtra	177093	422670	6996	606759
West Bengal	0	0	441272	441272
Rajasthan	195571	179371	0	374942
Tamil Nadu	0	354784	13231	368015
Andhra Pradesh	0	196609	77598	274207
Haryana	49157	183399	0	232556
Bihar	47301	105852	0	153153
Punjab	0	151717	0	151717
Karnataka	1307	148136	586	150029
Orissa	0	0	147138	147138
Madhya Pradesh	0	139720	0	139720
A and N islands	0	0	77000	77000
Kerala	0	0	20000	20000
Jammu and Kashmir	0	17500	0	17500
Total	**1710673**	**3788159**	**1246136**	**6744968**

Source: (Biswas and Biswas, 2014; Essington, 2020; P. Kumar and Sharma, 2020)

The saline soils in the state can be divided into four categories ie., saline, dry saline, saline sodic and saline marshy, depending on the salt constituents, pH and also the proximity to sea water. The regions of Kutch, followed by Saurashtra are the most affected by SAS (Table 1.3) having arid to dry sub-humid climate in the area.

Table 1.3: Percentage of area affected by Salinity and climatic extent in Gujarat region.

Region	Saline	Dry saline	Saline sodic	Saline marshy	Climate
Kutch	2.4	13.7	3.1	12.9	Arid to semi-arid
North Gujarat	0.5	8.1	7.6	1.2	Arid to semi-arid
Central Gujarat	2.2	0	0	0.7	Semi-arid
North Saurashtra	4.8	3.5	12.8	2.1	Dry sub-humid
South Saurashtra	11.6	0	5.8	0.9	Dry sub-humid
South Gujarat	2.2	0	0	16.3	Semi-arid to dry sub-humid
Southern hills	**0**	**0**	**0**	**7.5**	Semi-arid to dry sub-humid

Source: (Patel, 2019; Singh and Nair, 2012)

1.2.2. Soil Salinity classification and effect on plant growth

Electrical conductivity of the soil saturation extract (ECe) is the standard measure of salinity. USSL Staff (1954) has described general relationship of ECe and plant growth (Zaman et al., 2018), as below:

- Non-saline (ECe $\leq$ 2dSm^{-1}): salinity effects mostly negligible
- Very slightly saline (ECe 2–4 dSm^{-1}): yields of very sensitive crops may be restricted
- Slightly saline (ECe 4–8 dSm^{-1}): yields of many crops are restricted
- Moderately saline (ECe 8–16 dSm^{-1}): only salt tolerant crops exhibit satisfactory yields
- Strongly saline (ECe >16 dSm^{-1}): only a few very salt tolerant crops show satisfactory yields

1.2.3. Agricultural concerns of soil salinization

Based on the 2012-14 estimates, India suffered an annual loss of 16.84 million tons of farm produce (including cereals, pulses and cash crops) due to soil salinization, worth Rs. 230 billion. Gujarat was among the highest in the list with Rs. 100.63 billion loss, followed by Uttar Pradesh (Rs. 82.29 billion loss), both of which have the largest area affected by salinity (>50% of arable land) in the country (P. Kumar and Sharma, 2020).

1.3. Effect of salt stress on plant growth

Salinity devastatingly affects the plant growth and development. It attacks the vital physiological functions, causes imbalances in nutritional and hormonal regulation (Deepika and Dhingra, 2014). It affects the plant growth because of osmotic stress due to accumulation of excessive Na^+ ions and generation of ROS in the plant tissues. The ion channels for few ions such as Na^+ and K^+ are of similar size, therefore under high Na^+ concentration in the vicinity, Na^+ accumulation gets higher instead of K^+. High Na^+ accumulation prevents K^+ absorption which is involved several key enzymatic reactions responsible for photosynthesis and protein synthesis. Thus, accumulation of salt ions is also responsible for causing metabolic toxicity hindering plant growth (Arora et al., 2021; Egamberdieva et al., 2019; Sharma & Singh, 2015). The major symptoms observed in the plants due to salt stress have been discussed below:

Physiological symptoms – Salinity affects root growth more than shoot development, decreased rate of leaf development and carbon assimilation due to smaller leaf area are commonly observed (Egamberdieva et al., 2017; Neera Garg and Bhandari, 2016; Vives et al., 2018). Seed germination - Increased osmotic pressure restricts the absorption and entry of water into the seeds, certain salt constituents are toxic to the embryo and seedlings that hampers the metabolism of stored materials and may even reduce the germination rate by 50% (Farooq et al., 2017; Q. Ma et al., 2017; Nadeem et al., 2019; Shu et al., 2017). Vegetative growth – Osmotic stress causes stomatal closure leading to reduced carbon dioxide (CO_2) assimilation and transpiration, reduced turgor potential affects the leaf expansion, photosynthetic rate which coupled with spurt in respiration and results into reduced biomass accumulation (Bano and Fatima, 2009; Deepika and Dhingra, 2014; Egamberdieva et al., 2017). Photosynthesis - accumulation of salts in chloroplast inhibits photosynthesis and destroys the organ ultrastructure (Neera Garg and Bhandari, 2016; Torabian et al., 2018). Mineral imbalance – Decreased uptake of mineral ions such as potassium, phosphorus, calcium, iron etc has also been observed (Bano and Fatima, 2009; Garg and Manchanda, 2009; Garg and Bhandari, 2016; Torabian et al., 2018). Reproductive growth and yield – The onset of flowering is delayed, the quantum of reproductive structure such as number of flowers are reduced. Nutritional imbalance and hormonal synthesis is hampered leading to reduction in quantity as well as quality of crop produced (Deepika and Dhingra, 2014). Oxidative stress – decrease in photosynthetic rate increases reactive oxygen species (ROS) formation and activity of enzymes that detoxify their activity (Prakash et al., 2017; Torabian et al., 2018). Root nodulation - salinity affects photosynthesis which limits the availability of enzymes and assimilates necessary for nodulation, causing premature nodule senescence and reduced biological nitrogen fixation (Farooq et al., 2017).

1.3.1. Strategies adapted by plants against salt stress

Adaptation to environmental stress in plants are well known to be governed by genetic traits, plants can sense osmotic changes much earlier, while the sodium specific responses occur later due to the toxic effects of the sodium (Van Zelm et al., 2020). Mechanisms of salinity tolerance falls into three categories:

i) Diminution - Ion toxicity causes reduced water uptake due to which plants respond by reduction in the following aspects: seed germination, photosynthesis (due to stomatal closure and/or structural damage of cell components), decreased root length, uptake and accumulation of essential minerals such as nitrogen (N), phosphorus (P), potassium (K), iron (Fe), calcium (Ca) etc., to avoid competitive uptake of Na^+ and Cl^- ions (Torabian et al., 2018), reduction in phytohormones, reduced water uptake, leaf area, biomass (Ahmad et al., 2013; Farooq et al., 2017; Garg and Manchanda, 2009; Nadeem et al., 2019; Shu et al., 2017).

ii) Avoidance – Strategies are appointed for avoiding the interference of salt ions and away from the parts of the plant where they are harmful. *Salt exclusion* - through filtration at the root surface (eg., Red mangrove), *Salt excreters* remove salt through glands or bladders or cuticle located on each leaf (eg., *Mesembryanthemum crystallinum*). *Secretion through cuticle* (e.g., Tamarix), *salt glands* are the dump sites for removing excess salt absorbed in water from the soil to help plants adapt to life in saline environments (Galvan-Ampudia and Testerink, 2011; Jiang et al., 2019).

iii) Ion homeostasis - maintaining lower Na^+ content and high K^+ in cells (regulating genes for Na^+/H^+ antiporter and stimulation of high–affinity K^+ transporter1 - HKT1 (Y. Liang et al., 2006; Sandhu et al., 2017); osmotic adjustment via accumulation of *compatible solutes* (i.e., uptake of solutes such as quaternary ammonium compounds, proteins, sugars, amino acids etc) (W. Liang et al., 2017; Shi, 2013); *antioxidant enzyme regulation* by breakdown of unwanted ROS and their by-products into non-toxic substances (Etesami and Adl, 2020; Q. Ma et al., 2017).

iv) Salt Dilution By dilution of ions in the tissue of the plant by maintaining succulence. Plants achieve this by increasing their storage volume by developing thick, fleshy, succulent structures Succulence is mainly a result of vacuoles of mesophyll cells filling with water and increasing in size. This mechanism is limited by the dilution capacity of plant tissues. Compartmentation of ions at Organ level - high salts only in roots compared to shoots especially leaves (Abobatta, 2020).

Based on the responses to high concentration of salts, plants are divided into the following groups. 1) Halophytes (salt tolerant crops) - They are native to saline soils and need saline soils for growth (eg., Atriplex or salt bushes); 2) Glycophytes - Literally 'sweet plants', they can tolerate moderate levels of salinity; 3) Non-halophytes - They are sensitive plants and unable to grow under saline conditions. Most of the cultivated crop species belong to glycophytes.

1.4. Importance of legume crops in arid and semi-arid regions

In the current situation, leguminous plants are the only hope for global food security. Due to their low-cost high-nutritional value, being the source of amino acids, carbohydrates, vitamins, minerals and fibre in human diet, fulfilling 33% of the dietary protein requirements and as well as livestock feed. These crops constitute 27% of the major crops throughout the world, occupying 12 – 15% of arable lands (Nadeem et al., 2019). Legume farming helps enhancing soil fertility by crop rotation, these contribute by fixing nearly 40 million tons of nitrogen in the soil each year, thus diminishing the dependence on N-fertilizers for healthy crop output. Because of their symbiotic nitrogen fixing relationships, legumes are highly important in low fertility environments such as arid and semi-arid regions (Etesami and Adl, 2020).

Legume crops are highly sensitive to salt stress. Neera Garg and Bhandari, (2016) reported chickpea unable to survive beyond 100mM NaCl, Kurdali et al., (2019) reported a 46% decrease in the N_2 uptake by *Sesbania aculeate* plants at 8dSm^{-1} (76.8mM NaCl), (Mahmood et al., 2017) reported a 50% decrease in the total biomass in mung bean at 7.81 dSm^{-1} (74.88mM NaCl) and Torabian et al., (2018) reported ~70% reduction in the micronutrient uptake in mung bean plants at 10dSm^{-1} (96mM NaCl).

Pigeon pea (*Cajanus cajan*) is a perennial legume crop which was used as the host plant for present study. It is cultivated world-wide in tropical and sub-tropical regions. It is an important legume crop grown in semi-arid regions with an annual rainfall less than 650mm as it abhors water-logging and is highly salt sensitive. It is major legume crop with 6th most important crop in area and production globally and has multipurpose applications including a major protein source in human diet as green vegetable and daal, while the dried seeds and grass are used as animal fodder (Nix et al., 2015), it

Pigeonpea Plant

also improves soil fertility by nitrogen fixing ability. It is predominantly grown in India, 2nd most important crop after chick pea (Narayanan et al., 2018), but its productivity has stagnated over the past few years under the scenario of changing climatic conditions. Hence the major challenge to increasing pigeon pea productivity, aside from crop improvement, would be abiotic and biotic stress management (Pazhamala et al., 2015; Rathinam et al., 2019).

Scientific classification

Kingdom: Plantae

Order: Fabales

Family: Fabaceae

Genus: ***Cajanus***

Species: ***cajan***

Fungal species known to affect *C. cajan* severely are *Fusarium udum* and *Fusarium oxysporum* (Fusarium wilt), *Rhizoctonia solani* (Web blight), *Aspergillus niger and Aspergillus flavus,* (seedling rot) etc. Wilt infection caused by the *Fusarium udum* is a major limitation to world-wide pigeon pea crop production (Choure et al., 2012; Dukare and Paul, 2021; U. C. Jha et al., 2020).

1.5. Importance of PGPR for stress tolerance in plants

Rhizosphere comprises a variety of soil microbes, around 1-2% of which are highly beneficial to plant growth and are classified as plant growth promoting rhizobacteria (PGPR). These help the plants to protect them from the environmental harshness by developing mutually beneficial symbiotic relationships, colonizing their root surface, internal tissues or the rhizosphere (Backer et al., 2018). Such microbes are mostly found among the phyla Cyanobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Rhizospheric communities play a crucial role in the host’s development, also constituting the first line of defence against soil-borne pathogens and this reservoir of microbes are yet to explored sufficiently. PGPR suppress phytopathogens by producing antagonizing molecules, volatile gases like HCN and antibiotics etc., as well as triggering plant immune responses. A number of PGPR have been identified for dual purposes of biofertilizer as well as biocontrol effects (Jiao et al., 2021). PGPRs have several applications such as boosting key physiological processes, increasing root volume to improve nutrient absorption, stimulate plant growth, protection against phyto-pathogens, bioremediation of soil pollutants and last but not the least stimulating the stress response in plants by boosting antioxidant enzymes under stressed conditions (Figure 1.5).

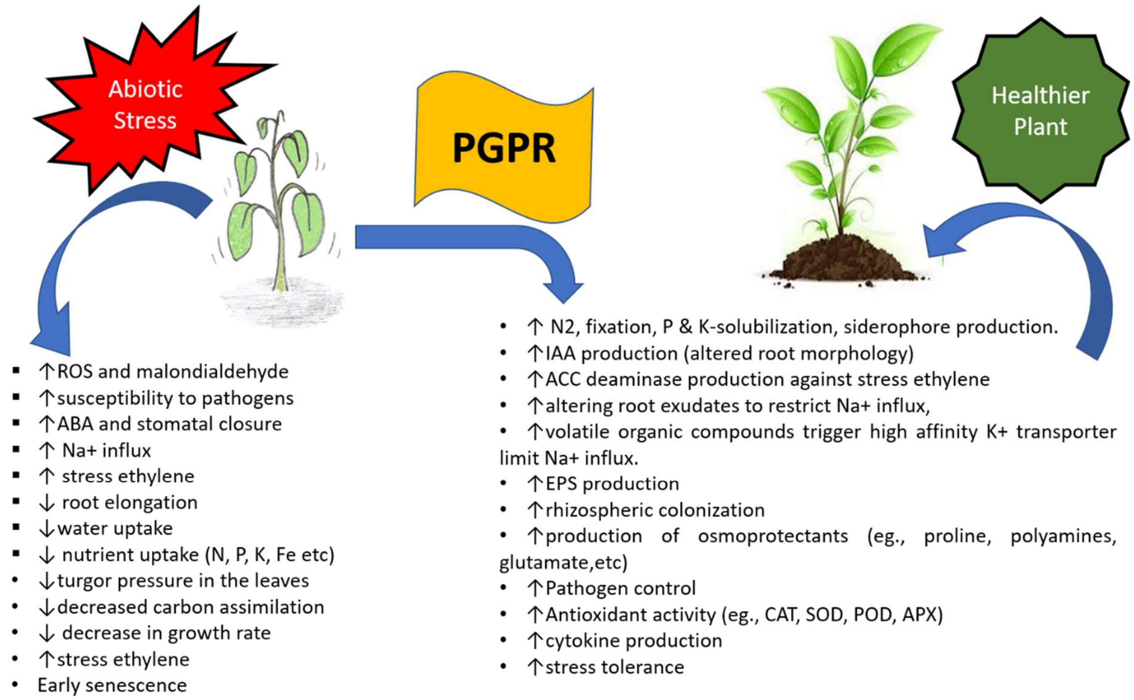

Figure 1.5: Effect of abiotic stress in plants, its bioremediation using PGPR and the traits involved.

In addition, many PGPR also bring about amelioration of different abiotic stresses such as heavy metals, drought, and salinity (Etesami and Maheshwari, 2018). PGPR strains that show mitigation of salt stress to plants often exhibit traits such as indole acetic acid production, siderophore production, phosphate solubilisation, 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase production and also biofilm formation (Mokrani et al., 2020; Shilev, 2020) (Figure 1.6). PGPR inhabiting the rhizosphere belong to genera *Achromobacter, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Klebsiella, Microbacterium, Paenibacillus, Pantoea, Pseudomonas, Serratia, Streptomyces* etc. groups. Various salt-tolerant PGPR belonging to genera *Rhizobium*, *Pseudomonas, Acetobacter, Bacillus, Serratia* and *Azospirillum* have been studied for PGP activity under salinity (Choudhary et al., 2015).

Biofilm formation plays a vital role in the effectiveness of PGP demonstration under salt stress (Figure 1.6) such as increase in soil aggregation which enhances the root soil association, nutrient assimilation by entrapping them within the matrix thus increasing their bioavailability, increased water uptake, nodulation which is a highly sensitive process gets a protective anaerobic casing due to which it is not affected by external stress factors, quorum sensing among the encased microbes with increased sensitivity, production of secondary metabolites such as siderophores which can be exclusively utilized by the enclosed microbes and most importantly exclusion of salt ions for protection against the osmotic shock to the plants (Fatima et al., 2020).

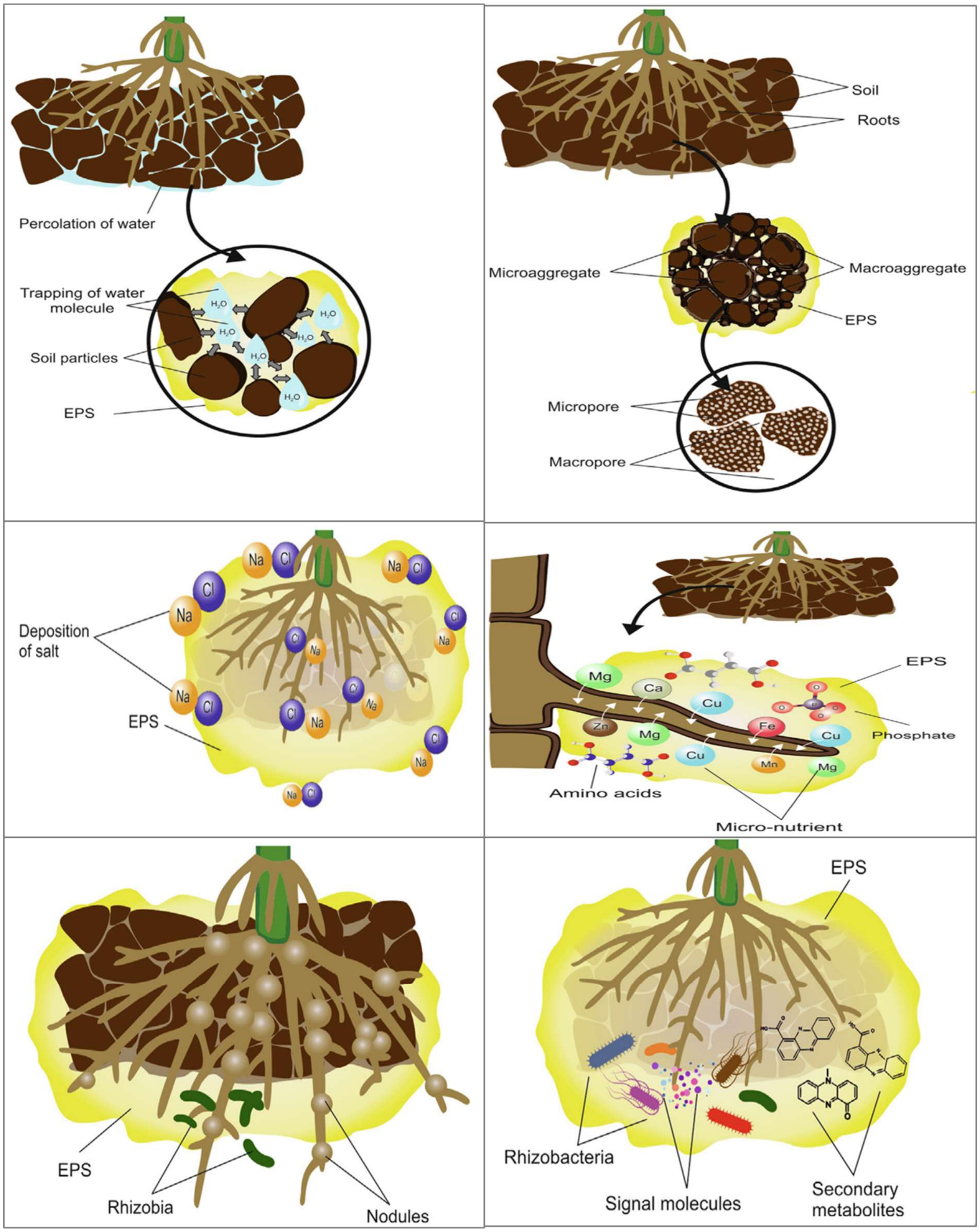

Figure 1.6: Soil aggregation due to EPS formation, increased water uptake, nutrient assimilation to increase bioavailability, salt exclusion, enhanced nitrogen fixation due to protective and optimal environment, quorum sensing and secondary metabolite production.
(Source: Arora et al., 2020)

1.6. Biotic stress

Most of the cultivable and atleast half of irrigated lands around the world are severely affected by environmental stresses. Food and Agriculture Organization (FAO) estimates state 25% crop losses globally are caused by pests and diseases (FAO, 2015). Out of different types of pathogenic infection of plants, two-thirds are caused by fungal pathogens (Goswami et al., 2016). With the traditional practices of using chemical pesticides and fungicides many phytopathogens have developed resistance due to long term and extensive usage has led to bioaccumulation of toxic substances in food chain (Jiao et al., 2021). The side effects of chemical pesticides also include harmful impact on plant beneficial microbes. The need for adoption of eco-friendly alternatives for sustainable agriculture has led to identification of many biofertilizers with the ability of biopesticide activity. Biological control of plant pathogens by biocontrol PGPR, is a rapidly increasing, highly potential means to avoid chemical agents and is known to be a cheap, effective and eco-friendly method for the management of crop diseases. Studies have also reported additive effects of combined biotic and abiotic stress factors on plant growth due to shared signals and response genes as a possible scenario (Chojak-Koźniewska et al., 2018) which also needs to be given serious attention.

1.7. Biocontrol effect of the PGPR

Members of the genus *Pseudomonas,* especially the fluorescent pseudomonads, have long been known for their potential to reduce the plant diseases, along with plant growth promotion, has been gaining considerable importance as potential antagonistic but eco-friendly microorganisms. They possess some remarkable traits which make them suitable as biocontrol agents such as i) robust mass production, ii) thrive on plant exudates iii) rigorously colonize the plant rhizosphere and endosphere iv) production of broad-spectrum bioactive metabolites (such as - antibiotic molecules, siderophores, hydrogen cyanide gas) v) phytohormone production (IAA, jasmonic acid), vi) aggressively compete against soil microbes for survival and vii) adaptability under stressed conditions (Weller, 2007). Prominent secondary metabolites produced by fluorescent pseudomonads that act as bioactive molecules include 2,4-diacetylphloroglucinol (DAPG), phenazines (PHZ), pyrrolnitrin (PRN), pyoluteorin (PLT), cyclic lipopeptides (CLPs) and volatile organic compounds such as hydrogen cyanide (HCN) (Mishra and Arora, 2017). The *Pseudomonas* sp. are well known for biocontrol activity against the fungal pathogen *Fusarium* sp. which causes wilt disease in the legumes especially Pigeon pea crop (Duffy and Défago, 1999; Molina-Romero et al., 2021; Akanksha Singh et al., 2013).

The relatively narrow spectrum activity of individual biocontrol agents against plant pathogens is a major constraint limiting their commercial exploitation. The approach to transcend this difficulty is to develop strain mixtures with superior biocontrol activity and plant growth promoting ability. Mixed populations can perform functions that are difficult or even impossible for individual strains or species. Balancing two or more tasks so that they are efficiently completed within one organism can pose insuperable challenges in some situations (Alizadeh et al., 2013).

1.8. Importance of developing PGPR consortia

In recent times, PGPR research is directed towards developing mixtures of strains, or consortia, with different PGP traits, contributing to plant growth stimulation by various mechanisms collectively, normally not possible by single strain (Santoyo et al., 2021). Mixed inocula are considered superior to individual strains due to their concerted benefits to the plants (Parihar et al., 2020; Samaddar et al., 2019), their ability to interact synergistically (Fuentes et al., 2016) as well as their better adaptability to field conditions (Bradáčová et al., 2019). Another important feature of a consortia is the ability to successfully complete tasks wherein multiple functions need to be accomplished by different cell types (Brenner et al., 2008), such a task can only be fulfilled by using a mixed consortium (Table 1.4). It has also been suggested that developing a consortium could be a better strategy for longer survival and activity of PGPRs, when applied as inocula they should not only compete with others for survival but also complement each other for plant growth promotion (Jha and Saraf, 2012).

Table 1.4: Studies showing application of mixed consortia and their synergistic effect on plant growth promotion.

Microbial consortia	Plant	PGPR effect	Reference
12 bacterial strains + 5 fungi + 2 algal species	Tomato (*Lycopersicum esculentum*)	Increased vegetative growth, fruit yield	Bradáčová et al., 2019
Azospirillum sp. + *P. putida* + *Acinetobacter* sp. + *Sphingomonas* sp.	Maize (*Zea mays*)	Increased rhizospheric colonization, plant growth	Molina-Romero et al., 2021

Azospirillum sp. + *Pseudomonas* sp. + *Glomus* AMF	Maize (*Zea mays*)	Useful in early stimulation of crop growth	Couillerot et al., 2013
Bacillus brevis + *Bacillus licheniformis* + *Acinetobacter calcoaceticus*	Jatropha (*Jatropha curcas*)	Increased plant germinability and plant growth along with high IAA, biofilm, phosphate and siderophore production.	Jha and Saraf, 2012
Trichoderma + *Pseudomonas* sp.	Cucumber (*Cucumes sativus*)	Enhanced phytoprotection due to activation of different signalling pathways.	Alizadeh et al., 2013

The premise that plant growth promoting traits do not work independently of each other but additively as suggested in the "additive hypothesis" (Bashan and Holguin, 1997), can be exploited beneficially by developing consortia which together present multiple mechanisms, such as phosphate solubilization, di-nitrogen fixation, ACC deaminase and antifungal activity, IAA and siderophore biosynthesis etc. are responsible for the plant growth promotion and increased yield.

1.8.1. Importance of consortia in stress alleviation

Besides use of consortia to supplement plant nutrition and reduce fertilizer application rates (Molina-Romero et al., 2021), consortia are also effective at management of plant abiotic stressors such as heavy metals, drought as well as salt (Shilev, 2020) (Table 1.5). An important aspect of developing consortia is to understand the dynamics of cooperative behaviour and to be able to design and screen consortia that show resilience and redundancy to perform as a community under variable field conditions. In addition to PGPR traits, the colonization and persistence also needs to be evaluated (Amaya-Gómez et al., 2020).

Table 1.5: Studies indicating effect of microbial PGPR consortia for alleviation of Biotic and Abiotic stress alleviation in plants.

Microbial consortia	**Stress alleviation**	**Plant**	**PGPR effect causing stress alleviation**	**Reference**
Paenibacillus polymyxa + *Rhizobium* sp.	Drought	Common bean (*Phaseolus vulgaris*)	Increased plant growth, nitrogen, nodulation, leaf ABA content while decrease in leaf zeatin	Figueiredo et al., 2008
Bradyrhizobium sp. + *Bacillus* sp.	Salinity	Cow pea (*Vigna unguiculate*)	Increased nitrogen fixation, carbon metabolism	Santos et al., 2018
Bacillus cereus + *Bacillus subtilis* + *Serratia* sp.	Drought	Cucumber (*Cucumes sativus*)	Increased leaf proline content, leaf SOD activity	Wang et al., 2012
Trichoderma + *Pseudomonas* sp.	Biocontrol (*Fusarium*)	Cucumber (*Cucumes sativus*)	Induced systemic resistance by enhanced priming of defence related genes	Alizadeh et al., 2013
Pseudomonas sp. + *Rhizobium* sp. + *Trichoderma*	*S. rolfsii* (collar rot)	Chick pea (*Cicer arietinum*)	Increased phenylpropanoid activity with high SOD, POX. Increased host antioxidant mechanisms	Singh et al., 2013a; Singh et al., 2013b
P. putida + *P. fluorescence* + *B. thuringiensis*, *P. synxantha*	Heavy metal (Zn, Cd)	Spinach (*Spinacea oleracea*)	Decreased accumulation of the heavy metal ions led to stress alleviation	Shilev and Babrikov, 2019
Rhizophagus fasciculatus + *Gigaspora* sp.	Salinity	Pea (*Pisum sativum*)	Enhanced antioxidant enzyme system, ionic balance, MDA and phenolic compounds, crop growth and yield	Parihar et al., 2020
Pseudomonas fluorescence + *Sinorhizobium fredii* + *Azotobcter chroococcum*	*Fusarium udum* (wilt)	Pigeon pea (*Cajanus cajan*)	Enhanced disease suppression, plant growth promotion	Choure et al., 2012
Pseudomonas sp. + *Bacillus* sp.	Nutrient deficiency (Phophate)	Wheat (*Triticum aestivum*)	Enhanced plant growth and yield and increased P-uptake	Emami et al., 2020

Enterobacter sp. + *Serratia* sp. + *Achromobacter* sp.	Salinity	Wheat (*Triticum aestivum*)	ACCd activity, IAA production to mitigate salinity stress.	Barra et al., 2016
B. licheniformis + *Bacillus* sp. + *P. aeruginosa* + *Streptomyces sp.*	Biocontrol (Sunflower necrosis virus)	Sunflower	Disease suppression by reducing the virus titre with concomitant increase in plant growth.	Srinivasan and Mathivanan, 2011
Pseudomonas sp. + *Paenibacillus* sp. + *Pantoea* sp. + *Bradyrhizobium* + *Stenotrophomonas* sp.	Heavy metal (Cd)	Soybean (*Glycine max*)	Reduced metal bioavailability, enhanced antioxidant system, increased phenolics and GST.	Zaets et al., 2010
Bacillus haynesii + *Bacillus licheniformis* + *Bacillus paralicheniformis*	Drought	Rice (Oryza sativa	ISR, increased plant growth and crop yield, EPS, IAA production, mineral solubilization, ACCd activity.	Joshi et al., 2020

1.9. Scope of the thesis

Three different groups of PGPR's, each with specific type of PGPR properties, were chosen for the present study.

Members of PGPR Consortia:

- ***Enterobacter* sp. C1D (*E.*C1D):** It is a lab isolate (collected from a sediment sample, at an Industrial Waste Effluent settling lagoon situation near Sarod, Mahi river estuarine area, Gujarat, Western India) is known to alleviate heavy metal stress in plants as a PGPR. It is an efficient Phosphate solubilizer (Subrahmanyam et al., 2018).
- ***Pseudomonas* spp.:** Lab isolates *Pseudomonas* sp. G16, P.G19, P.G22, P.G38 (Chaubey, 2012) and 2 standard strains *P. protegens* CHA0 (Haas and Keel, 2003) and *P. protegens Pf-5* (Patel and Archana, 2018), were used in this study. These are known to have good PGPR traits along with the biocontrol properties.
- **Rhizobia: *Rhizobium*** sp. IC3109 has been obtained from Indian Agricultural Research Institute, Delhi, India and is known to be a good nitrogen fixer strain. While, ***Rhizobium*** sp. ST1 is a lab isolate (Geetha et al., 2009) also known to show siderophore production and is a good nitrogen fixer strain.

***Sinorhizobium fredii* NGR234:** A unique alpha-proteobacterium (order Rhizobiales) that forms nitrogen fixing nodules with more legumes than any other microsymbiont. It is known to be capable of forming nodules more than 120 genera of legumes and the nonlegume *Parasponia andersonii.* (Saldaña et al., 2003)

As discussed before, salinity stress not only affects the plant growth drastically, but it is also harmful for the growth of PGPRs which are present in the rhizosphere. Competition for nutrient acquisition, such as iron or phosphate deficiency, only adds up to the stress under already present saline conditions. Thus, it is rather necessary to understand the bacterial interactions at cellular levels under such multiple stressed conditions, to understand the effect of consortia development under stressed conditions as well as the bacterial interdependency for alleviation of the stress. Molecular or genotyping techniques have been used earlier for determining the cell count estimates for the samples released into environment (Reddypriya et al., 2018; Von Felten et al., 2010). Similar techniques can also be applied for estimation of cell count in consortia samples for *in vitro* studies as well, which can help us to understand the inter-relation between consortia members under multiple stresses, and to obtain the consortia which can help to alleviate the maximum stress alleviation in plants.

Most of the studies on stress alleviation have been focussed on application of PGPR and their consortia directly to the plants in stressed condition. But the physiological consequences of individual strains present in the consortia have rarely been studied. The present study was aimed at evaluating the effects of salt stress on the phenotypic demonstration of PGPR traits in bacterial strains belonging to different genera of *Pseudomonas*, rhizobia and *Enterobacter* and their consortia. In addition, it was of interest to study consortia developed using these bacteria of diverse genera to check their resultant PGPR traits in a consortium under increasing concentrations of saline stress. The interaction among bacteria in a consortium under salt stress was assessed using molecular techniques to understand the survival of consortial members under nutrient deficiency and salt stressed conditions.

Further work was emphasised on the application of such consortia for microcosm studies, and the plants were subjected to abiotic (salinity) and biotic (fungal) stresses while seedlings treated with bacterial consortia were grown to observe which treatment alleviated the stress to maximum limit while helping the growth of healthiest plants for the longest period. For this purpose, the leguminous plant *Cajanus cajan* (Pigeon pea) was selected as the host plant.

Objectives under study

- Effect of salt stress on PGPR traits of PGPR strains individually and in consortium
- Functioning and compatibility of the PGPR consortia in presence of multiple abiotic stresses using PCR based approach.
- Plant inoculation studies with PGPR consortia for salinity and biotic stress with *Cajanus cajan.*