CHAPTER 2

REVIEW OF LITERATURE

2.1 Systematic position of *Oryza Sativa*

Kingdom  
Plantae

Division  
Angiospermeae

Class  
Monocotyledoneae

Order  
Poales

Family  
Poaceae

Genus  
*Oryza*

Species  
*sativa*

2.2 General description of *Oryza sativa*

Rice (*Oryza sativa* L) is a member of Poaceae (Gramineae) family, which is an annual herb, the height of plant ranges from 1-1.5 meter. Roots of rice emerge through coleorhizae and coleoptile and finally develop into secondary and adventitious roots. The hollow stem or culm contains nodes and internodes. The leaves are sessile, panicle type inflorescence which produces spikelets at the top of the culm. Each spikelet develops into flower, flower which is enclosed in the lemma and palea. Flowers are bisexual consist of six stamens and one pistil. Rice is a self-pollinating crop. Rice grain is enclosed in lemma and palea. The dehulled rice grain is called as brown rice since it is covered with brown pericarp.

The genus *Oryza* possess twenty-four species, out of which only two species are cultivated viz *O. sativa* and *O. glaberrima*, others are wild type. Most of the cultivars grown in Asia, America and Europe are from *O. sativa* and *O. glaberrima* is cultivated in West Africa *O. sativa* cultivars commonly grouped into three subspecies such as Indica (grown in India), Japonica (cultivated in Japan) and Javanica (cultivated in Indonesia)
Rice occupies 23.3 percent of the cultivated area of the Indian sub-continent. The contribution of rice for total food grain production is 43 percent and total cereal production is 46 percent in India and contributes a major portion in national food grain production. Worldwide rice production is ranked third after wheat and maize. India holds the second rank in rice production (about 45 million ha.).

Due to urbanization and industrialization agriculture area are reduced and there is no extra land is available for rice cultivation. In fact, the area for rice cultivation is going to be limited in several countries due to urbanization [17]. To face the challenges in rice production high yielding and resistance (biotic and abiotic stress) cultivars of rice are needed.

Plants are sessile and face diverse environmental stresses including biotic and abiotic factors during their lifespan. These factors either individually or in combination alter the plant growth, development, and metabolism [18]. In an average, saline stress, water limitation, chilling or freezing and extreme temperature stress reduced the survival, growth, and yield of crops approximately 70% [19]. These different climatic factors are considered as inhibiting factors for agriculture productivity.

2.3 Mechanism of abiotic stress tolerance

Plants are sessile and to cope up with adverse environmental conditions, develop unique and sophisticated adaptation mechanisms. To survive under unfavourable environmental conditions, plants develop the mechanisms of tolerance, resistance, or avoidance. The secondary messengers including cAMP, cGMP, protein kinase C, Ca^{2+}-dependent kinases and calmodulin-dependent kinases are involved in stress signaling in plants [20].

Stress can also enhances the concentration of signaling molecules such as Jasmonic Acid (JA), Salicylic Acid (SA) Abscisic Acid (ABA) and ethylene in plants. To regulate the signaling and to cope up with stress these
molecules may interact with each other [21]. There may be multiple pathways for signal transduction and regulation of gene expression. According to [22] a network of signal transduction during abiotic stress divided as (A) Osmotic or oxidative signaling (B) Ca^{2+} dependent signaling (C) Ca^{2+} dependent SOS signaling for ion homeostasis. The second round of signaling depends on regulatory molecules such as ABA, salicylic acid, and ethylene. However, abiotic stress signaling requires all the signaling molecules, adaptors and scaffolds [23].

2.4 Role of salicylic acid under abiotic stress condition

Salicylic acid (SA) chemically is a phenolic compound and plays a critical role in plant defense from pathogens. SA also plays important role during drought stress, temperature, salt stress, UV radiation, and heavy metals. The accumulation of endogenous SA reported during various abiotic stresses. Two major pathways such as Phenyl Ammonium Lyase (PAL) and Isochorismate Synthase (ICS) from chorismate synthesize the SA. The genes involved in SA synthesis (ICS1 and ICS2) are localized in the chloroplast and SA is synthesized in plastids [24].

In *Phillyrea angustifolia* endogenous SA level increased up to five-fold during drought stress [25]. Previous study [26] reported that 100 mM NaCl treatment made less harm to the *Arabidopsis* (NahG) mutant, which do not accumulate SA compared to wild-type plants. SA-treated maize enhanced growth and reduced lipid peroxidation and membrane permeability when grown under salt stress [27]. SA regulates the activity of antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase. In sunflower plants, foliar application of SA enhanced the salt tolerance as well as yield and oil production [28].

During drought stress, SA accumulation was reported in different species which also induces PR1 (Pathogenesis-Related Protein 1) and PR2 (Pathogenesis-Related Protein 2) genes. SA plays a significant role in seed
germination, the lower concentration of SA (0.5 mM) affects ascorbate-glutathione cycle to ameliorate the drought stress [29] in wheat.

2.5 Role of jasmonic acid in abiotic stress

The role of Jasmonic acid (JA) is critical during abiotic stress conditions. Jasmonate increased the tolerance in plants during water limitation. The application of JA enhanced the activity of antioxidant enzymes during water stress. Previous studies revealed the role of JA in signaling of drought-induced antioxidant response including ascorbic acid metabolism. Reported the exogenous JA application protects plants from oxidative damage induced by drought [30].

Transcriptome analysis of JA biosynthesis genes such as AOC1, AOC2, AOS, LOX3 and OPR3 upregulated during saline stress [31]. The JA-biosynthesis gene OPR1 overexpressing in Arabidopsis resulted in inhibition of formation of roots. In rice, salt-sensitive 3 (RSS3), interacts with the OsbHLH089 and OsbHLH094 transcription factors and mediates root elongation during salinity [32].

OsCYP94C1-related protein that is encoded by OsCYP94C2b gene when over expressed enhances the salt tolerance in rice [33]. These investigations proposed the part of JA as a positive and negative controller of saline stress [34]. However, modulation of JA pathway during saline stress is not fully explored. Above findings suggested that signaling route of JA induced during saline stress and alters physiological mechanism in plants.

2.6 Saline stress

Saline stress is one of the most challenging factor that affect crop productivity. Saline stress inhibits yield and converts the fertile area into a barren land. Rice is a salt-sensitive crop and about 30% of the rice producing area is affected by salinity worldwide [35].
A Higher level of salt in soil affects plants in various ways such as ion toxicity, water stress, nutrient deficiency, generation of reactive oxygen species, oxidative burst, membrane dysfunction and inhibition of growth, differentiation and genotoxicity [36]. Salinity reduces the leaf expansion and leads to ionic stress which ultimately causes premature leaf senescence or cell death. During saline stress, a number of agronomic traits such as panicle weight, tiller numbers per plant, grain yield and harvest index relative water content [37], chlorophyll content [38] are significantly reduced. Accumulation of salts in soil such as sodium chloride, sodium sulphate, sodium carbonate or magnesium causes the saline stress. Saline stress alters various physiological and biochemical processes of plants such as higher rate of respiration and toxicity due to the accumulation of ions, modulates mineral distribution, membrane permeability and reduced photosynthetic efficiency [39]. Susceptibility and tolerance to saline stress is an orchestrated mechanism of multiple genes responsible for stress cross talk with each other signal transduction pathways.

The stress-responsive genes are classified into two main groups, the first is involved in signal transduction cascade, transcription and post-transcriptional modifications. While the second group of genes regulates membrane homeostasis, osmoprotection and ROS scavenging [40]. In different plant species salinity induced the up-regulation of genes and transcription factors. These genes are classified based on their functions such as ion transport gene (AtNHX, H⁺-ATPase, SOS), ROS scavenging (CAT, SOD, POX), molecular chaperones or heat shock proteins (HSPs) and transcription factors (MAPKs, WRKY, DREB).

Salt stress affected various functions of proteins including the proteins involved in ion transport, signaling, energy, lipid, osmolyte and phytohormone metabolism and stress-related proteins. Comparative study of salt sensitive and tolerant plant species showed the differential protein abundance under saline stress. Hence to get a clear picture, comparative proteomics will be helpful. Hence, there is an urgent need to develop the salt-resistant cultivars
with increased crop production to fulfill the food demand all over the world to satisfy the demand of growing population.

2.7 Autophagy

Autophagy is a tightly regulated catabolic process during which cytoplasmic content undergoes the degradation either by lysosome (animal cells) or by vacuole (yeast and plants). It is a turn over process of organelles and macromolecules, allowing cells to survive under nutrient starvation. The main feature of autophagy is the formation of the autophagosome [41]. Autophagosome is a double membranous vesicle, engulfs the cytoplasmic material and degrades it. After the degradation, new macromolecules are synthesized via the recycling of degraded products. Autophagy divided into three major cateogaries viz macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [42]. Autophagy has been well studied in Saccharomyces cerevisiae and at least, 36 Autophagy Related Genes (ATG) have been identified out of which 18 ATG genes are involved in the formation of autophagosome [43]. Autophagy is considered as an evolutionarily conserved pathway from lower eukaryotic yeast, mammals and plant kingdoms [44].

2.7.1 Molecular mechanism of autophagy

Three major systems are involved in autophagy mechanism are mentioned below:

2.7.2 ATG 9 cycling system

ATG9 is the only identified integral membrane protein required for autophagosome formation, and it is thought to cycle between the membrane sources and the phagophore assembly site (PAS). Thus, ATG9 plays an important role as a membrane carrier. A binding partner of ATG9 is ATG11 which has been identified in the yeast two-hybrid screening system and showed that ATG11 mediates the direct transport of ATG9 to the PAS along
with the actin cytoskeleton [45]. Most of the ATG proteins, primarily display single punctate localization at the PAS while ATG9 localized in multiple punctate structures, including PAS. The cycling of ATG9 between PAS and non-PAS punctate structures are essential for autophagosome formation. In the absence of ATG11, the transport of ATG9 to PAS is blocked. Direct movement of ATG9 to the PAS also involves ATG23 a peripheral membrane protein and ATG27 a type-I transmembrane protein, which forms a cycling unit with ATG9. Both of these proteins are required for the cytoplasm to vacuole (CVT) pathway and for efficient autophagy [46].

Figure 2.1 Mechanism of Autophagy. Autophagy mechanism includes a series of steps including Induction, Cargo Identification, Packaging, Vesicle Nucleation, Vesicle Completion and Vesicle Fusion with Vacuole/Lysosome. Different classes of ATG proteins are governing the functions of various steps in the autophagic pathway

2.7.3 Phosphatidylinositol 3-Kinase complex

The class III Phosphatidylinositol 3-kinase (PtdIns3K) also known as Vacuolar Protein Sorting 34 (VPS34) protein is first identified in yeast, is responsible for the synthesis of phosphatidylinositol 3-phosphate. It forms
two complexes, complex I and complex II in yeast. Each complex consists of VPS34, VPS15, and VPS30/Atg6 proteins. VPS15 is essential for association with VPS34 [47]. The role of VPS30/ATG6 in PtdIns3K complex is not well understood. These proteins are reported as an important regulator of autophagy, vesicular trafficking, G-protein signaling and nutrient sensing mechanism.

In plants, PtdIns3k has been identified which showed higher homology with yeast PtdIns3k (VPS34) when compared to the mammalian P110 PI3 kinase. It has also been reported that AtVPS34 is essential for pollen development and vacuole reorganization in plants [48], [49]. It has been reported that plants deficient in ATG6 have shorter roots, early leaf senescence, increased anthocyanin production, developed dwarfism, with fewer flowers and sterile. Furthermore, antisense plants of AtATG6 are failed to limit the pathogen associated cell death response [50].

2.7.4 Ubiquitin-Like Protein conjugation system

In eukaryotes, Ubiquitin is a small 8.5 kDa regulatory protein ubiquitous in all the tissues. These proteins are involved in post-transcriptional modification and bind with the substrate protein and modify the proteins in three steps such as activation, conjugation, and ligation [51]. The modification can be achieved either by single ubiquitin or by the chain of ubiquitin. In selective and non-selective autophagy, two types of conjugation systems are involved including ubiquitin-like proteins ATG12 and ATG8 [52].

In autophagy, ATG12 has covalent conjugation with ATG5. To initiate this conjugation, additional proteins are required such as ATG10, and ATG7, where ATG7 act as E1 ubiquitin-activating enzyme and ATG10 function as an E2 ubiquitin-conjugating enzyme. Furthermore, ATG12-ATG5 conjugate, ATG5 noncovalently binds with a coiled protein ATG16 and forms a trimeric complex ATG12-ATG5-ATG16, which is essential for autophagy [53]. ATG8 ubiquitin-like protein binds to phosphatidylethanolamine (PE) an integral membrane protein. However, it is reversible conjugation of ATG12-ATG5
conjugate where ATG4 release ATG8 from lipid. This free Atg8 allows processing of CVT pathway [54]. The two yeast conjugation systems are also well conserved in Arabidopsis. Arabidopsis has nine members of the AtATG8 family (AtATG8a - AtATG8i) two members of the AtATG4 family (AtATG4a, AtAATG4b), and two members of the AtATG12 family (AtATG12a, AtATG12b [55].

2.7.5 Role of autophagy during abiotic stress

Two most common environmental stresses including excess salt and water limitation affect plant growth and productivity of agricultural crops. These stresses cause the oxidative damage, higher accumulation of ROS and degraded proteins. Autophagy has been considered as a scavenger of these oxidized proteins and regulates the ROS accumulation. Thus it exhibits a crucial role in during saline and drought stress. The transcriptome profiling of Arabidopsis suspension culture cells grown under sucrose starvation showed the induction of vacuolar autophagy with enhanced vacuolar proteases expression within 24 hours. Vacuolar proteases are essential for degradation of delivered cytoplasmic components and nutrient remobilization [56]. Furthermore, the viability of cells was decreased after 72 hours of starvation.

The role of Arabidopsis AtATG18 gene family is essential for autophagosome formation during nutrient deficiency and senescence. The role of starvation-induced ATG genes in Arabidopsis. ATG genes showed transient up-regulation in a coordinated fashion during starvation and served as the marker for autophagy in plants. Earlier autophagy proteins such as ATG8 in Arabidopsis and ATG10b in rice expressed under salt and osmotic stress [57]. Application of hydrogen peroxide in Arabidopsis showed oxidation of proteins and induced autophagy, however defective AtATG18a-RNAi transgenic plants were sensitive to H\textsubscript{2}O\textsubscript{2} and accumulate more oxidized proteins than wild-type plants due to decreased rate of degradation by autophagy. This suggested that oxidized protein downgraded by autophagy under oxidative stress conditions [58].
The \textit{atg10-1} mutant plants are highly sensitive to N and C limitation than wild-type and showed accelerated senescence of leaves and PCD and failed to accumulate autophagosome in the vacuole \cite{59}. Under normal conditions NADPH oxidase dependent pathway controls the autophagy while during drought stress NADPH oxidase-independent pathway. The autophagy imparts a critical role in chloroplast degeneration during aging \cite{60}. Similarly, accumulation of ROS in \textit{atg2} and \textit{atg5} mutants observed in WT plants. The cadmium stress induces ROS that may function as a secondary messenger to initiate the formation of autophagosome \cite{61}. Previous study showed the expression of autophagy homologs in rice and found 33 ATG homologs in rice and classified them in 13 ATG subfamilies \cite{62}. They also analyzed the role of various ATG genes during different abiotic stresses (salinity, drought, cold and dark) hormones and nutrient deficiency condition. The results showed that most of the OsATG homologs expression was regulated by stress condition. The study suggested that identification of OsATG homologs whether constitutively expressed or induced by an environmental stimulus which provides a novel insight into extensive analysis and characterization of important selected genes in \textit{Oryza sativa}.

The role of autophagy during oxidative stress was assessed and showed that oxidative stress induces autophagy in animals as well as in plants \cite{63}. During aluminum stress, ATG mutants exhibited more accumulation of ROS and oxidative damage than the wild-type plants. It was observed that during nitrogen starvation condition autophagy-defective mutants showed accumulation of higher level of reactive oxygen species which caused the dysfunction of respiratory function resulted in the loss of mitochondrial DNA \cite{64}. The study suggested that autophagy plays a significant role to scavenge the ROS and maintains the function of mitochondria during nutrient deprivation. \textit{Physcomitrella atg5} mutant exhibited senescence phenotype under nitrogen and carbon starvation medium when compared to wild type. This occurred due to the absence of cytoplasmic degradation by autophagy which leads to amino acid imbalance.
These studies suggested that autophagy plays a critical role during nutrient starvation [65].

2.8 Programmed Cell Death (PCD)

The growth and development of multicellular organisms depend upon removal harmful cells through a complex molecular mechanism known as Programmed Cell Death (PCD). PCD is complex, genetically controlled process. Cell death controls the developmental processes as well as maintains homeostasis of cells, defense against various environmental stresses etc. [66]. It has been reported that animal antiapoptotic genes including CED-9 and Bcl-2 are overexpressed in plants and showed increased tolerance to various abiotic stresses [67]. PCD in plants represents the similar features of animal apoptosis such as DNA fragmentation, laddering, and activation of caspases [68]. The key difference between mammalian and plant cells is that mammalian cells involve the macrophages and phagocytosis to eliminate the apoptotic cells. However, cells showed an alternative vacuole mediated autophagy to engulf the damaged and degraded cellular components [69].

2.8.1 Role of PCD during saline stress

The phenomenon of Programmed cell death (PCD) is genetically controlled and conserved throughout all eukaryotes [70]. The hallmarks feature of PCD includes the fragmentation of DNA by nuclease (DNA laddering), condensation and shrinkage of the cytoplasm, release of cytochrome c from mitochondria, elevation of the cytosolic calcium concentration ([Ca^{2+}]_{cyt}), generation of reactive oxygen species (ROS), and activation of caspase-like proteases [71].

The various abiotic stresses including salinity, drought, cold, UV radiation, extreme temperature, metals, herbicides, and gaseous pollutants including reactive oxygen species (ROS) are induced PCD and affects plant growth and yield [72]. Plants try to adapt to the stress condition by activating
various mechanisms including PCD. Abiotic stress reduces the growth and development of different plant parts [73]. Saline stress has an adverse negative effect on plant growth and development [74]. The higher level of salinity induces the osmotic, ionic imbalances, cell leakage, accumulation of ROS and rapid oxidative burst in plants that may leads to various signaling pathways and lead to programmed cell death in higher plants [75]. Such PCD could be considered as a salt adaptation mechanism [76]. Oxidative damage occurs mainly due to higher accumulation of ROS during saline stress [77]. To cope up with adverse condition of stress plants have potential ROS scavenging with nonenzymatic (ascorbate, glutathione, carotenoids, tocopherols) and enzymatic antioxidant system [78]. In plants generation of ROS can occur via different processes in different compartments like including peroxisome, chloroplast and mitochondria [79].

Among these, mitochondria are gaining attention due since it is present in both plants and animals as a common organelle to study the ROS mediated PCD [80]. Mitochondrial electron transport chain is the main site for ROS generation that directly affects the mitochondrial proteins, lipids and leads to dysfunction by creating the permeable transition pores (PTP) and release caspase like proteases [81]. The formation of PTP and release of cytochrome c, caspases proteins including caspase 9 or caspase-independent apoptosis-inducing factor (AIF) that results in the cascade of downstream events leading to PCD. In contrast to animals, plants possess plastid that induce the ROS during stress-induced cell death in Arabidopsis suspension culture or leaf peels [81]. Chloroplasts have been considered as a source and as well as the primary target for plant specific PCD signalling pathway [82]. Salt stress induces the early senescence and death in plants [83]. The characteristic feature of senescence is yellowing of leaves due to rapid degradation of chlorophylls and distortion of chloroplast by affecting the photosynthesis and cell death.. Although still, it is not clear whether stress-induced plastid disorganization leads to PCD.

Chloroplast and vacuoles are the two important organelles in plants that exhibit characteristic features of PCD [84]. Vacuoles are the main
organelles in plants for storage of different hydrolytic enzymes and defense proteins. It plays various roles in stress response, development and defense [85]. The vacuoles present in plants can be categorized into two types i.e. Protein Storage Vacuoles (PSVs) and Lytic vacuoles (LVs). PSV mainly store all types of protein including defense, storage proteins, and carbohydrates. In contrast, LV posses different hydrolytic enzymes and functions as similar to lysosomes and maintains acidic condition [86]. It has been reported that Vacuole induced cell death is a response to various stresses [87].

The VPEs are discovered as cysteine protease with aspartic acid residue and present in plant vacuoles [88]. VPEs can hydrolyze the vacuolar proteins and causes the rupture of vacuole same as animal caspase in regulating PCD [89]. The rice genome contains four VPE homologous qualities as takes after OsVPE1 (Os04g45470), OsVPE2 (Os01g37910), OsVPE3 (Os02g43010) and OsVPE4 (Os05g51570) [90]. Phylogenetically, OsVPE1 and OsVPE3 are more like Arabidopsis AtVPEβ, though OsVPE2 and OsVPE4 are more like AtVPEα and AtVPEγ. OsVPE1, a homolog of AtVPEβ, assumes a vital part in the development of glutelins in seeds [91]. Saline stress instigated the PCD in rice root tips. Saline stress induced the PCD hallmarks in root tips, such as DNA laddering, nuclear condensation and fragmentation. Cytochrome c discharge from mitochondria into cytosol was also observed [92].

Autophagy and programmed cell death are two important pathways and involved in various developmental as well as stress responses. Autophagy regulates the PCD during plant innate immune response [93]. However, mechanism regulating autophagy and PCD are not completely understood. Interaction of Bax- Inhibitor-1 (BI-1) a conserved cell death protein interacts with ATG6 a core autophagy protein. Gene silencing of BI-1 reduced the ATG6 activity and enhanced cell death during Tobacco mosaic Virus and Methyl viologen treatment. In contrast overexpression of BI-1 increased autophagic activity and causes autophagy dependent cell death. The results of the study concluded that BI-1 has both prosurvival and prodeath effects in different physiological contexts and both depend on
autophagic activity. Autophagy plays a basic part in plant protection from necrotrophic pathogens. ATG18a, protein in *Arabidopsis*, communicates with WRKY33, a translation factor that is required for protection from necrotrophic pathogens. In *Micrasterias denticulata* (unicellular green alga) during saline stress formation of autophagosomes enhanced ROS accumulation and DNA laddering were observed [94]. Cell death in plants is mainly of two types vacuolar cell death and necrotic cell death. This category includes HR cell death with autophagic features. Further studies using tools of genetics, biochemistry and cell biology are required to understand molecular mechanisms underlying cell death mechanism.

**2.9 Panchagavya (PG) an important biostimulant**

The soil is a reservoir of nutrients however most of the nutrients cannot be assimilated by plants and very small quantity of nutrients is absorbed by plants through biological processes which are not sufficient enough for the larger agricultural production [95]. The growth and development of plants depend on the availability of nutrients. Plants also require macro, micro and trace elements for their growth and development. Plants receive the macronutrients (Carbon (C), hydrogen (H), and oxygen (O2)) from atmosphere and water while remaining 13 essential elements nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (mb), and chlorine (Cl) are absorbed either from soil minerals organic matter or supplemented in the form of organic or inorganic fertilizers [96].

Plants absorb the nutrients through roots from soil but the amount of nutrients in the soil is unpredictable and unable to reach the requirement for plant growth. Therefore, now a days nutrients are supplied in the form of NPK fertilizers in the blended form [97]. Fertilizers are classified into three types; Chemical, Organic and Bio-Fertilizers. In the context of nutrient supply, environmental impact and crop growth and yield, chemical and organic fertilizers have both advantages and disadvantages.
Synthetic fertilizers are toxic to the soil, mixed with running water, decrease the oxygen content and pollute the water. A large number of toxic substances produced in the soil by the use of chemical fertilizers or leached the nutrients from the soil that makes the land unfit for the cultivation of crops. Chemical fertilizers are also expensive and need to apply in a larger quantity which is expensive to the farmers. Excessive use of chemical fertilizers increases the acidity of the soil, which destroys the microbial flora and deteriorates the soil texture. Continuous accumulation of heavy metals in soil leads to heavy metal toxicity in soil, recycled in the ecosystem and causes severe health issues in animals and plants [98]. These risks can be overcome by the use of biofertilizers or biostimulant for the production of agricultural crops.

Nowadays, the use of biofertilizers has tremendously increasing to improve the nutrient supply and reduced the utilization of chemical fertilizers for the eco-friendly and sustainable agriculture [99]. Biofertilizers have rich microbial flora which enhanced the plant defense to biotic and abiotic factors and reduced the environmental degradation and heavy metal contamination. Biofertilizers are enriched with microorganisms including N-fixing bacteria, phosphate soluble and growth enhancers. Rhizobium is useful to fulfill nutrient requirements of crops to increase yield. The Nitrogen-fixing bacteria. Azospirillum is used as biofertilizer which could enhance the productivity of rice. Nitrogen-fixing bacteria have the ability to fix atmospheric nitrogen and P solubilizing microbes to solubilize phosphate into a soluble form [100].

In India, organic farming has been well established and used in agriculture. According to Ayurveda, various cow products including cow's urine, dung, milk, ghee, and curd are used to treat various diseases in human [101]. Panchagavya (PG) commonly applied to crop plants is an organic product mixed with five components from the cow (dung, urine, milk curd, ghee) as a growth promoter and immunity booster through the foliar spray, soil application and seed treatment [102]. Panchagavya is used for the protection of plants and enhance crop production and applied exogenously on plants, blended with irrigated soil, and seed priming etc. In PG, the total N supply is enhanced and total ammonia uptake was increased by the
presence of chemolithotrophs and autotropic nitrifiers (ammonifiers and nitrifiers) which colonize in the leaves [103]. Microorganisms in PG act as catalysts to foster the useful microbial environment. Microorganisms in the presence of organic matter produce proteins, organic acids, and antioxidants and produce energy, therefore, the microflora and fauna of PG have the power inhibit pathogens in soil.

Naturally different microorganism present in PG including Lactobacillus, photosynthetic bacteria (Rhodopsuedomonas), yeast, Actinomyces and certain fungal species enhanced the soil quality, growth and production in sweet corn [104]. The combination of poultry manure and PG in Ashwagandha enhanced the root growth. Soil mixed with PG showed enhanced roots, leaves, leaflets and leaf lamina in treated plants when compared to control. PG in microbial medium with different concentration (10 μl, 100 μl, 500 μl and 1000 μl) of PG mixed with 1.5% water [105]. Results of the study revealed that low concentration of PG are having potential for antifungal activity and enhance growth.

PG, being eco-friendly biostimulant is used widely to improve crop production, protection and to replace chemical fertilizers and pesticides [106]. Physico-chemical parameters and preliminary phytochemical analysis provide important information about any formulation and play an important role in analysis, especially for natural products which contain a number of chemical moieties and therapeutically active ingredients. The 3% concentration of PG is sufficient to improve all the physiological and biochemical attributes of black gram [107]. PG also has the properties of fertilizers and biopesticides. The seed priming of Jatropha curcas and Pongamia pinnata using different dilutions (1-5%) of PG showed more germination and seedling vigor index at 2% and 5% concentration when compared to control. PG functions as a potent biostimulant for crop production, plant growth, development, protection from various abiotic and biotic stresses. Most of the earlier studies focused on the application of PG and enhancement of crop yield. Although, it is required to identify the essential components present in PG formulation and identify their targets.
through biochemical and molecular mechanisms. We also investigated the efficacy of PG at the molecular level and in alleviating the saline stress [108] and identified the active constituents of PG by using HPLC and FTIR analysis. Furthermore, the novel compounds were isolated, extracted, characterize and tested their efficacy in the alleviation of saline stress in rice seedlings. The study provides a strong base for future research to identify targets to enhance the tolerance in plants for sustainable agriculture.