GENERAL INTRODUCTION

*"Vasansi jirnani yatha vihaya navani grihnati naro aparnai,
thata sarirani vihaya jirnanyanyani sanyati navani dehi"*

[Swami Prabhupada 1992]

According to Hindu mythological philosophy, The Soul is immortal, but the body is mortal. So The Soul wears a new body as a new dress, when the present body becomes unable to serve the 'The Soul' any more. Now, if we extend this philosophy little bit more with respect to our material outlook, we can easily say that, the earth is divided in two different worlds. One of them is- "World of living" and the other one is- "World of non-living"; and without moving the way towards any spiritual or supernatural discussion; the above terms are more precisely called 'Biotic World' and 'Abiotic World'. Biotic World is the key factor, which makes this planet so special, amongst its relatives. The concept and flourishing beauty of life is the ultimate execution of Biotic World. Still, the integrity of life is completely dependent on the service and supply of the enormously potential members of the Abiotic World. Those members, the matters, molecules and atoms; create building blocks like protein, nucleic acids, lipids etc; connect among different systems through metabolism and care for the benefit and endurance of life. 'The Soul' with its present body is the member of 'Biotic World', but when body becomes 'Jirna' or necrotized, it becomes a part of 'Abiotic World'. So, in a snapshot, life is the most beautiful and delicate manifestations of ever happening interactive relationship between biotic and abiotic world, where non-livings create, connect, care and livings perform.

Now the performance of any living organism is mostly dominated by its communication skill, the art to connect and to be connected. And the execution of this skill/art is mainly dependent on signaling molecules; they connect cell to cell, create unity between them and even unfold the extremes. Biotic world shares lots of signaling molecules in the form of organic compounds, especially bio-molecules like proteins, peptides, hormones, enzymes etc., inorganic compounds and energy. This work is completely dedicated for the behavioral study of a specific group of bio-molecules called 'Peptides'.

Emil Fischer is the founding father of the field of peptide chemistry and originator of the term peptide. In present day scenario, the term 'Peptide' is well established and widely accepted group of biomolecules. Peptides are relatively simple and small biopolymers of different amino acids which resemble proteins except that the latter are higher molecular weight substances. X-Ray diffraction studies reveal that the peptide bond is flat, i.e.- carbonyl carbon, nitrogen and the hydrogen attached to them, lie in a same plane. The C-N Bond distance of peptide came out to be 1.32 A°, compared to usual C-N single bond distance of 1.47 A°, indicating that C-N Bond has resonating 50% double bond character [Morrison and Boyd, 1983].

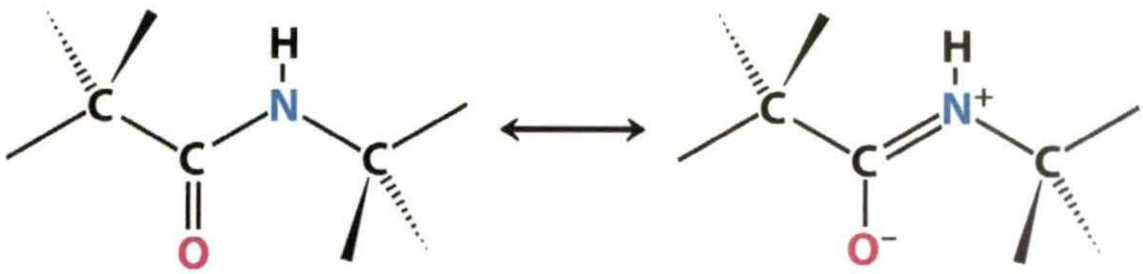

Figure 1 Resonance stabilization of peptide bond (Berg *et al.*, 2012)

Peptides are vastly distributed in nature. They play the roles as hormones, antibiotics, toxins, herbicides, food preservatives, biotic and abiotic stress elicitors along with metabolic and signal intermediates. They are widespread in mammals, amphibians, insects, plants, bacteria and even viruses. Now, instead of their variable roles, the genesis of peptides is highly organized. This can be through central dogma, by regulated catalysis of different large proteins or even some peptides can be of auto-replicating in nature. A large amount of information also has shown that non-ribosomal antimicrobial peptide synthesis is performed according to the multiple carrier thio-template mechanism (Stein *et al.,* 1996). In this template driven assembly, a series of very large multifunctional peptide synthetases with a modular arrangement, perform the peptide synthesis in an ordered fashion. Even antimicrobial ribosomally synthesized cationic peptides have been recognized recently through evolutionary tree.

Apart from all the activities, in recent years, peptides have gained their justified reputation in living kingdom mainly because of their signaling behaviour. They have their widespread impact right from the unicellular bacteria to the multicellular, most evolved angiosperms and mammals. They are the most utilized class of intercellular signals in gram-positive bacteria. Oligopeptide pheromones can be considered as the first class of prokaryotic communication molecule to be detected (in the 1960's) as competence factor in *Streptococcus pneumoni* and *Bacillus subtilis*. The tremendous diversity of oligopeptides makes them especially suitable when a high degree of discrimination is required. This specific group of molecules control fruiting body development, chemotactic auto-aggregation, sporulation, transformation, competence, virulence factor secretion, agglutinating plasmid transfer etc. in bacteria.

One of the most interesting phenomena in bacteria is its quorum sensing behaviour. Quorum sensing bacteria produce and release chemical signal molecules termed auto-inducer, whose external concentration increases as a function of increasing cell population density. Bacteria detect the accumulation of a minimal threshold stimulatory concentration of these auto-inducers and alter gene expression, and therefore behaviour. The major parts of this bacterial secreted auto-inducing chemical are of peptide in nature, known as AIP (Auto-inducing peptides).

These molecules also have an undoubted impact on the animal kingdom. Beginning with the discovery of insulin in 1922, polypeptides have been established as signals that regulate broad spectrum of physiological processes. For example, Oxytocine (nine amino acid residue), which is secreted by the posterior pituitary and stimulate uterine contraction; Vasopressin having anti-diuretic effect; Bradykinin (nine residue) which inhibit the inflammation of tissues and Thyrotropin Releasing Factor, which is formed in the hypothalamus and stimulate the release of another hormone Thyrotropin from anterior pituitary gland are all peptide hormones. There are still lots of examples of peptide hormones in animal world.

Among neuropeptides, several endogenous opioid peptides like endorphin, enkephalin, dynorphin, desmorphin, deltorphin etc. possess interesting morphinomimetic properties. Opoid peptides and their G-Protein coupled receptors (delta, kappa mu) are located in the central nervous system and peripheral tissues. Two types of endogenous opoid peptides exist, one containing Tyr-gly-gly-phe as the message domain (Enkephalin, Endorphin, Dynorphins) and the other containing Trp-pro-phe/trp sequence (Endorphin 1 and 2). In overall approach, peptides play versatile role in the integral survival of the animal kingdom and communicate with the essential metabolic junctions of critical and dynamic form of life. Recently opoid like bioactive soybean peptides are isolated by comparing amino acid sequence databases with the help of bioinformatics tools.

Nearly three decades ago, Green and Ryan (1972) first indicated the presence of a wound induced proteinase inhibitor in plant leaves as a possible defense mechanism against insects. The idea ultimately led to the discovery of first peptide hormone in plants, 'Systemin'. Presently at least five peptides are well known for peptide signaling in plants: Systemin, PSK, ENOD 40, CLV 3 and SCR. Tomato systemin is the first identified plant signaling peptide. It is composed of 18 amino acids and is able to confer a systemic wounding response in tomato plants attacked by herbivores and insects (Pearce *et al.*, 1991). Although more than 100 diverse plant species respond systematically to herbivores attacks, homologues of systemin could be identified only in some other Solanaceae (Constabel *et al.,* 1998). It was known that tomato systemin could activate a Mitogen Activated Protein Kinase (MAPK) in the medium (Schaller and Ryan, 1994). In tomato plant, tomato systemin is able to induce expression of proteinase inhibitor encoding genes (Pearce *et al.*, 1991). Recent advancement shows that small peptides may play a role in response to phytohormone. Miklashevichs *et al.* (1997) have reported that small peptides (ranging from 12-22 amino acids) may play a role in cell response to phytohormone. Their experiment with isolated protoplast showed induction of cell division by auxin and similar action was mimicked by a peptide of 10 KDa. In the same year van de Sande *et al.* (1996) while working with legume plants observed that the gene ENOD 40 is expressed during early stages of legume nodule development. A homologue was isolated from tobacco, which like ENOD 40 of legumes encodes one oligopeptide of about 10 amino acids. In tobacco protoplasts these peptides change the response of auxin at concentration as low as 10^{-12} to 10^{-16} (M) range. They have suggested that the peptides encoded by ENOD 40 appear to act as plant growth regulator. Ryan *et al.* (2002) proposed that the two ENOD 40 polypeptides may serve as a regulatory role in unloading sucrose from the phloem, either by modulating enzyme activity or by directing the enzyme to specific intracellular sites. Rapid Alkalization Factors (RALF) are another group of peptide hormone, initially identified from expressed sequence tag libraries of various organs from 16 species of plants representing 9 families (Pearce *et al.,* 2001). RALF is proteolytically processed from a larger preprotein. RALF inhibits root-growth, cell division and root hair formation but induce MAP Kinase activity more rapidly than Systemins.

Two small polypeptide signals were reported by Matsubayashi and Sakagami (1996) to regulate plant cell proliferation interspersed *Asparagus* suspension cultured cells. The rates of cell division of suspension-cultured cells have been known for years to be strictly dependent on the initial cell density (Stuart and Street, 1969). At low density, the mitotic activity is very low, but if the cultures are supplemented with media taken from high-density cell cultures (conditioned medium [CM]), the low-density cells rapidly proliferate. Two mitogenic factors were isolated from CM of *Asparagus officinalis* mesophyll cell cultures. Structural analysis of the two components revealed that they were both small polypeptides modified with sulfate groups (Matsubayashi and Sakagami, 1996), and were named Phytosulfokines (PSK-α and PSK-β). The *in vivo* functional role of PSK-α in plant has not been established, but the polypeptide enhances the differentiation of cell cultures of *Zinnia* into tracheary elements and causes somatic embryogenesis in carrot (Kobayashi *et al.*, 1999). CLAVATA3 (CLV3) is another extra cellular signaling polypeptide of 79 amino acids in length that is involved in the determination of cell fate in the shoot apical meristem (SAM) of *Arabidopsis*. The polypeptide synthesized from *clv 3* is hypothesized to be secreted from superficial cell layers in the central region of the SAM, interacting with CLV1, a 105 kD receptor-like kinase (Clark *et al.*, 1997) in underlying cells, coordinating the enlargement of CLV1 domain with a restriction of the stem cell population (Fletcher *et al.*, 1999). The amino acid sequence of CLV1 suggests that it is a trans-membrane protein consisting of a leader sequence, a putative extracellular domain of 21 leucine-rich repeats and 15 putative N-linked glycosylation sites.

A family of intercellular signaling polypeptides of 47 to 60 amino acids in length, collectively called S-locus cysteine rich proteins (SCR) or S-locus protein 11 (SP11) (Schopfer *et al.*, 1999), play a central role in pollen self-incompatibility in the Brassicaceae. Each mature SCR/SP11 polypeptide contains eight cysteine residues. The properties of the SCR polypeptides are consistent with their roles as signals that determine pollen SI specificity when recognized by the appropriate receptor. The polypeptides are secreted from developing microspores in the tapetum and transported to the pollen coat exine layer. SCR translocates into cell walls of the stigma epidermal cells at the pollen-stigma contacts, activating a receptor-mediated cascade leading to SI.

Recent peptide researches have looked for homologues of animal polypeptide hormones in plants. A 4-kD polypeptide called leginsulin (Watanabe *et al.*, 1994) was isolated that caused the autophosphorylation of a 7S Globulin and exhibits some characteristics of an insulin receptor. Gehring *et al.* (1996) suggested that a vertebrate peptide hormone, rat atrial natriuretic peptide that function in animals as regulators of salt and water balance, and is a mixture of 3- to 10-kD polypeptides induce stomatal opening in *Tradescantia* sp., which is mediated by protein synthesis. They proposed a plant analogue of this ANP, which regulates transcription and solute movements in plants. Natriuretic peptides could be immunoprecipitated from *Hedera helix* with anti-animal natriuretic peptide antibodies. Jones *et al.* (1998) suggested that the treatment of wild oat aleurone protoplasts with the peptide Mas 7, which stimulates GDP/GTP exchange by heterotrimeric G proteins, induced the synthesis of α-amylase. Mas 7 were sequence analogue of Mastoparan, originally isolated from wasp. The observed conservation of peptide signaling in plants and animals implies that peptide signaling already was operational in a common ancestor, and that, in time, kingdom specific features have evolved, such as specific proteases and receptors.

Under stress condition, plants and mammals synthesize different kinds of cysteine rich peptide molecules. Glutathione (GSH), present in plants, animal and some bacteria, often at high levels, can be thought of a redox buffer. Glutathione probably helps in maintaining the sulphydryl groups of proteins in the reduced state and the iron in the heam in the ferrous (Fe^{2+}) state. Its redox function is also used to remove toxic peroxides formed in the normal course of growth and metabolism under aerobic conditions (Xiang *et al.*, 2001).

Among a variety of adaptive responses to heavy metal toxicity in plants, induction of the heavy metal binding peptides, Phytochelatins, whose function is to sequester and detoxify excess metal ions, is an important one. Phytochelatins are a family of related peptides with structures $(\gamma\text{-GluCys})_n\text{-Gly}$, where n=2 to 7. Phytochelatin is a component of a shuttle system for transfer of metals from cytoplasm to vacuole. Complexed Cd may be replaced from Phytochelatin at a vacuolar pH of 3.5-6.0. Displacement of Cd by protons may allow association of the metals with other ligands like organic acids or phytic acids (Cobbett, 2000).

Most of the work discussed above mainly focused on the systematic and exclusive behaviour of the specific peptide, more particularly related to multicellular communication and amplification circuits. But as the living system is a dynamic model of multi-factorial bionetwork, so the behaviour of every molecule within its population can be an interesting field of study. This work is solely concentrated on biological function of peptide pool, both temporal and spatial, with special interest on the extraction and purification of low molecular weight group between 3000 Da to 500 Da. Attempts were taken to determine the biological activity of low molecular weight peptides under *in vitro* condition, their possible interactive behaviour with standard hormones and phenolic compounds considering special attention on germination and stomatal aperture control. On the other hand, the possible dynamic changes on the expression of peptides will also be accessed, particularly using 2D Paper Chromatography and Capillary electrophoresis. Beside this, characterization of expressed low molecular weight peptides were also performed through gel and spectrophotometric scanning, HPLC, amino acid composition and sequencing. Investigations were performed for considering the role of peptides in heavy metal tolerance and changing the response to morphological figure and anti-oxidative defense enzymes by the application of peptides, isolated from same stress acclimated plants *in vitro*.