APPENDIX-1

LIST OF PUBLICATIONS FROM THIS THESIS:

1. Ghosh A, **Mandal P** and Sircar PK. (2010). Wheat (*Triticum aestivum*) peptide(s) mimic gibberellin action and regulate stomatal opening. ***Indian journal of Experimental Biology*** 48: 77-82.

2. **Mandal P**, Misra TK, Ghosh A and Sircar PK. (2009). Role of germination induced peptide pool in plant tissue culture. ***NBU Journal of Plant Sciences*** 3: 53-58.

3. **Mandal P**, Misra TK, Sarkar A, Ghosh A and Sircar PK. (2008). Dynamic peptide profiles of germinating mungbean: In relation to their nature and separation pattern. ***Indian Journal of Plant Physiology*** 13 (2): 111-117.

4. **Mandal P**, Misra TK, Ghosh A and Sircar PK. (2008). Germination induced peptide pool regulate water homeostasis in plants. ***Journal of Plant Biology*** 35 (2): 121-130.

5. Kunduchoudhuri R, Ghosh A, **Mandal P**, Chaudhuri RK and Sircar PK. (2007). *Vigna catjang* intermodal peptides (0.5-3.0 KDa) modulate root tip mitosis of *Allium sativum*. ***Journal of Botanical Society of Bengal*** 61(1): 51-54.

6. **Mandal P** and Sircar PK. (2005). High proton mediated phytotoxicity, alteration of peptide profile and sugar partitioning in germinating seedlings of *Vigna radiata* (L.) Wilczek. In: ***Stress Biology***. Eds. U Chakraborty and BN Chakraborty. Narosa Publishing House, New Delhi. pp. 158-163.

7. **Mandal P**, Ghosh A and Sircar PK. (2000). Poster Presentation No. 12: Plant physiological roles of isolated plant peptides (500-3000 Mol. Wt.), Proceedings to the National Seminar on recent Advances in Plant Biology, (CPCRI, National Academy of Agricultural Sciences, Kasaragod, Kerala).

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Wheat (*Triticum aestivum*) peptide (s) mimic gibberellin action and regulate stomatal opening

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Wheat peptides (0.5 to 3 KDa M_r) mimick hormonal activity like that of gibberellins and forced open dark closed stomata. The deionized amphoteric peptides solution after passing through cation and anion exchanger resins was run through Amicon's ultrafilters, 10, 3 and 0.5 kDa (M_r) cut off system. The 3 to 0.5 kDa fraction passed through sephadex LH-20 column and collected in 140 tubes (5 ml in each tube). The two fractions F 9 (91-100 tubes) and F 12 (121-130) were found much active on stomatal opening and α-amylase activity, respectively and were ninhydrin positive. Capillary electrophoresis of F 9 fraction yielded several peptides ranging 1600 to 2200 (M_r) and F 12 fraction showed 1800 – 2800(M_r). Both the fractions were totally hydrolysed for amino acid analysis by HPLC. Most of the amino acids were present except cystein in both the fractions. The F 9 fraction, (peptide present in 10 µg fresh wt tissue per ml) induced the dark grown closed stomata to open upto 70%. In F 12 fraction, (peptide present in 10 µg fresh wt equivalent tissue per ml) showed α-amylase induction which was much higher than GA_3 (10^{-5} *M*). The peptide might be present in membrane and bound with GA that activated α-amylase m-RNA synthesis. The peptide might act directly on α-amylase gene.

Keywords: α-Amylase, Gibberellin, Peptide(s), Plant hormone, Stomata, *Triticum aestivum*

In recent years, a large number of biologically active peptides have been isolated from bacterial, fungal, plant and animal sources. Naturally occurring peptides are found to regulate several physiological processes in plants also. Some of these low molecular weight peptides have been characterized in details. Peptides in plant system play a definite role in amplifying signals[1], nitrogen fixation[2], cell proliferation[3], generation of polarity[4]. Concept of bioactive peptides like systemin, phytosulfokine, ENOD 40, CLAVATA 3 and S- Locus factors trigger challenges over the classical definition of narrow viewpoint of plant hormones[5]. Other physiological roles of plant peptides may not be unusual like induction of alpha amylase activity like that of gibberellins[6]. Relevant informations on short peptides like receptor for GA have been cited[7]. It has been shown that *Vigna catjang* internodal peptides (0.5 to 3.0 kDa) modulate root tip mitosis of *Allium sativum*[8]. The internodal peptide shows promotive effect on root growth of *Allium cepa* which has been supported in maize[9]. In 2008, dynamic peptide profiles of germinating mungbean in relation to their nature and separation pattern and their induction on alpha amylase synthesis has been reported[10]. In the same year, it has also been reported that germination induced peptide pool regulate water homeostasis in plants[17]. With the advancement of analytical instrumentations and advanced methodologies, the heavy molecular weight proteins have been reported, but the role of naturally occurring low molecular weight peptides are yet to be unveiled. The present study suggested that peptides of 0.5 to 3 kDa molecular weight regulate many important plant physiological processes.

Materials and Methods

To obtain aqueous extract of seedlings, the wheat seeds collected from National Seeds Corporation Limited were sown and 7 days old wheat (*Triticum aestivum* cv *sonalika*) seedlings (1kg) (were harvested, washed under running water, then with sodium hypochlorite solution (0.2%) to avoid excessive contamination and finally washed with

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Role of germination induced peptide pool in plant tissue culture

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Abstract
Low molecular weight peptides (ranges from 3.0 KDa to 0.5 KDa) were extracted and purified from rice, wheat, chickpea and mungbean through cryocrushing, cold centrifugation, ether fractionation, cation and anion–exchange column chromatographic separation, lyophilisation and ultrafiltration. The profile of heterogeneous peptides was detected through one dimensional paper chromatography. Peptide fractions isolated from different germination hours of mungbean enhanced mitotic index, more particularly prophase of root tip of *Allium cepa* L. and executed definite control over morphogenesis of excised and cotyledonary embryo culture of chick-pea seeds (*Cicer arietinum* L.). Restricted callogenesis in carrot pith culture was observed by the application of wheat peptides without any other hormones. Our speculation is that the peptides can mimic the action of hormone and behave as novel kind of bioactive molecule through which the physiological responses can be modulated.

Keywords: peptides, bio-activity, amylase induction, embryo culture, pith culture

The growth and development of higher plants can be considered to be characterized by the execution of cell division, expansion and differentiation along two axes: the apical-basal and radial patterning. In evolutionary terms, the apical-basal axis of development can be considered to have a strong selective advantage based upon plant competition for light, water and nutrients. Because plants are sessile organisms, their success in a particular environment will depend on their ability to integrate a complex range of external and internal information that may vary from time to time.

It is now fully recognized that plants exhibit greater morphological and developmental plasticity than animals. This conclusion has emerged as a result of integrating the data from molecular biological and genetic approaches with data gained from whole-plant physiological investigations (Trewavas and Knight, 1994). This organogenic plasticity in plants is coordinated by complex interplay between diverge signaling systems, leading to its immediate translation in the rate and plane of cell division, and cell expansion. The totipotency and plasticity of plant cells and tissues can be vividly exhibited when they are cultured *in vitro* with changing hormonal profiles. Basically plant tissue culture relies on the fact that more plant cells have the ability to regenerate a whole plant. Importantly totipotent cells must be able to differentiate not only into any cell in the organism, but also into extra-embryonic tissue associated with the organism. During development, the activities of plant hormones such as auxins, cytokinins, ethylenes, gibberellins and abscisic acid depend on cellular context and exhibit interactions that can be either synergistic or antagonistic. For example, auxin can suppress cytokinin biosynthesis (Nordstrom *et al.*, 2004), auxin and cytokinin can act synergistically to induce ethylene biosynthesis (Vogel *et al.*, 1998) and ethylene can modify auxin responses and meristem function (Souter *et al.*, 2004; Stepanova *et al.*, 2005).

To date, researchers have identified four major group of peptide-ligand-receptor pairs in plants (Ryan *et al.*, 2002), which are involved in a variety of developmental processes, such as wound responses, cellular dedifferentiation, meristem organization and self-incompatibility. However, these must only be part of the story, because plant genome sequencing has revealed many genes predicted to encode small peptide ligands and receptor-like kinases, whose function remain to be uncovered (Shiu and Bleecker, 2001). Furthermore, induced mutation in prohormone processing proteases has been shown to disrupt plant growth and development.

In this context peptide fractions isolated from different germination hours of *Vigna radiata* cv. Sonali B1 were analyzed for their bioactivity related to morphogenesis, cell division and dedifferentiation. Now, proper embryo development and germination are the learning phase for any plant to face the challenge of its adverse world. These two phenomena set the 'programming' of upcoming metabolic machinery, which will last till the death with minimum 'tuning'. 'Germination' is that crucial period where autotrophic plant also behaves heterotropically, which is an excellent manifestation of its selfishness and dependence, and slowly empowers its machinery for their future 'autotrophic' nature. This 'switch over' is controlled by a number of intrinsic factors, triggered by internal and external signal perceptions. This report is an attempt towards the understanding of changing pattern of peptide bioactivity isolated from different germination periods (i.e. – hrs of

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DYNAMIC PEPTIDE PROFILES OF GERMINATING MUNGBEAN: IN RELATION TO THEIR NATURE AND SEPARATION PATTERN

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SUMMARY

Low molecular weight peptides (3 KDa to 0.5 KDa) from different germinating phases of mung bean [*Vigna radiata* (L) Wilczek. cv. Sonali B1] were purified through ion exchange, ultrafiltration and sephadex gel permeation chromatography. The peptides were subjected to paper chromatographic and capillary electrophoretic separation, for the determination of dynamic and temporal manifestation of their occurrence. Analysis of peptides revealed mainly two groups, one of which is constitutive throughout while the other mainly appeared after 24 hrs and disappeared at 5 days of post germination phases. Amino acid analysis of these peptides indicated adequate similarities with legume storage protein. Lack of cysteine and methionine in peptide pool indicated that they are active in all different shapes suitable for transport and absorption.

Key words: **Amino acid analysis, germinating seeds, mungbean, peptides.**

INTRODUCTION

In recent years, a vast array of bioactive peptides are isolated from different spectrum of life form and only some of these low molecular weight peptides have been characterized in detail. Peptides in plant system possess definite role in amplifying signals (Lindsey *et al.* 2002), nitrogen fixation (Mylona *et al.* 1995), cell proliferation (Matsubayashi and Sakagami 1996), generation of polarity (Souter and Lindsey 2000), differentiation, self incompatibility and mediating biotic and abiotic stress elicitation with metabolic intermediates. Concept of bioactive peptides like Systemin, Phytosulphokine, ENOD 40, CLAVATA 3 and S-Locus Factors (Ryan *et al.* 2002) trigger challenges over the classical definition of narrow viewpoint of plant hormones. The genesis of numerous small peptides in different phases of plant system is not at all random but oriented through specific molecular programs that may not be associated with central dogma of protein synthesis (Lee *et al.* 1996, Fletcher *et al.* 1999).

Now, the basic aim of this work is to explore the separation profile of peptides isolated from germinating mungbean [*Vigna radiata* (L) Wilczek. cv. Sonali B1], which is one of the important cultivated variety of mung bean with excellent aroma, taste and flavour. Till now there are very few reports in plants solely related to peptide profile or fingerprint. This may be due to technical difficulty related to isolation, purification and characterization of peptides. More often peptides are characterized through Capillary zone electrophoresis (Heintz *et al.* 2004, Wetterhall 2004), Mass-spectrometry

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ARTICLE

Germination-Induced Peptide Pool Regulate Water Homeostasis in Plants

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Low molecular peptides from 3.0 kDa to 0.5 kDa were isolated from germinating seedlings (after 64 hours of soaking) of *Vigna radiata* (L) Wilczek cv. sonali BI and purified through ion-exchange, ultrafiltration and gel permeation chromatography. Heterogeneous peptide profile of germination pool was detected through paper chromatography and capillary zone electrophoresis. Isolated peptides were further purified through paper chromatography in accordance with their four distinct R_f zones. Peptide fractions isolated from first and third lower R_f zones appreciably increased the flow rate, absorption, transpiration of solute, root and shoot lengths of seedlings. Dark induced stomatal aperture opening and enhancement of protoplast volume was also observed by the same peptides. These peptides antagonized the inhibitory action of ABA and mimicked the bioactivity of cytokinin. cGMP may be the mediator of this signal as established when LY 83583 decreased the protoplast volume after peptide application. Amino acid analysis of third R_f zone peptide strongly support the immunoreactive atrial natriuretic behaviour of peptides. Peptides representing second lower R_f zone did not display bioactivity and peptides from highest R_f zone decreased the above biological perception. In general, functional diversity of peptide pool is particularly related with the physiological conditioning of water and solute homeostasis of plants.

Keywords: Amino acid analysis, capillary electrophoresis, cGMP, homeostasis, paper chromatography, peptides, *Vigna radiata*.

Introduction

In recent years, a vast array of bioactive peptides is being isolated from different species and only some of the low molecular weight peptides have been characterized in details. Over the last decade it was apparent that plants also contain peptidic signalling molecules that play vital roles in cell-to-cell communication (Lindsey *et al.*, 2002; Matsubayashi *et al.*, 2002). Plant peptides are protein molecules smaller than 10 kDa that can essentially be divided into two categories: bioactive peptides that are produced by selective action of peptidases on longer precursor proteins (Ito *et al.*, 2006), and degraded peptides that result from the activity of proteolytic enzymes during protein turnover (Richer and Lamppa, 1999). Although both groups are products of proteolysis, they differ in how they act within the cell. The first group plays key roles in various aspects of plant growth regulation through signalling, endurance against pests and pathogens by acting as toxins and elicitors and detoxification of heavy metals by sequestration. Often these peptides bear certain sequence patterns or motifs. By contrast, the second group has no such pronounced cellular effects, but may play an important role in nutrient mobilization across cellular membranes (Higgins and Payne, 1982) or in functions that remain to be defined.

Evidence was obtained that suggests that low molecular weight peptides have a function in modulating plant water and solute homeostasis (Gehring, 1999). Water movement in higher plants is treated as a symplastic fluid flow incorporated into a unified hydrodynamic system comprising the apoplast and vessels (Zyalalov, 2004). It is argued that colonization of terrestrial areas by plants became possible due to the appearance and maintenance of a gradient of water chemical potential between the rhizosphere and atmosphere, which drives water flows. As water flows through plants in well-constructed interconnected system of vessel elements, rate of water absorption by the root is dependent on stomatal aperture movement, dynamics of changing water potential

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Vigna catjang internodal peptides (0.5–3.0 KDa) modulate root tip mitosis of *Allium sativum*

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Crude aqueous extracts of peptides, from internodes of 28 days grown *Vigna catjang* var. K851 were semi-purified by Ultrafiltration (0.5 - 3 kDa). The extracts showed high mitotic indices (MI) in *Allium sativum* -root tip cells along with a significant increase in prophase percentage over other divisional phases. Later, divisional stages were found to be arrested and decreased. This promotive effect on mitosis was more pronounced with 4th internodal peptide extract. The effect of the fourth internodal peptide was tallied with ABA treatment in the same plant. It showed a strong positive effect on root growth expressed by mitotic cell count. The effect might be inhibitory, in prophase and other divisional stages, division percentage remained very low.

Key words : *Vigna catajang*, sonamung, internodal peptide extract, mitotic index

INTRODUCTION

Saab *et al.* (1990) noted that endogenous ABA increases root growth of maize. Das *et al.* (2001) also reported same type of activity with the internodal plant peptide on cell division. Miklashevichs *et al.* (1996) reported a plant peptide, with 12 to 22 amino acids, which had auxin-like activity in plants. Furthermore, hormone-like activity of a oligopeptide that is encoded by ENOD4O legume gene, consisting of 10 amino acid residues, enhanced auxin-response in tobacco protoplasts, at 10^{-12} to 10^{-16} M concentrations (Karin *et al.* 1996). Matsubayashi and Sakagami (2006) reviewed the literature on bio-reactive plant peptides. Still data are scanty on internodal plant peptides.

MATERIALS AND METHODS

Seeds of a dicotyledonous plant material sonamung (*Vigna catjing* var. K851) were weighed (200 g) and were allowed to grow up to 28 days when 4 internodes developed. These internodes were used for extraction of peptides, from each internode. The top internode was the 1st internode and the lowermost was the 4th internodal segment. They were weighed separately (95 g, 100 g, 55 g and 100 g respectively), and then crushed separately with liquid N_2 within cold room environment (4°C) with a grinder. The powdered internodal tissues were kept separately in a deepfreeze at -20°C. Sterile water was used for extraction as well as dilution of internodal peptides. These extracts were cold

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HIGH PROTON MEDIATED PHYTOTOXICITY, ALTERATION OF PEPTIDE PROFILE & SUGAR PARTITIONING IN GERMINATING SEEDLINGS OF *VIGNA RADIATA*(L.) WILCZEK

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INTRODUCTION

Plants are exposed to different kinds of stresses, which limit the normal growth of plants. Among them, soil acidity is one of the most important factors for plant growth and development. Acid soils significantly limit crop production worldwide because approximately 40% of the world's potentially arable soils are acidic (Kochian, 1995).

Nitrogen sources (fertilizers, manures, legumes) that contain or form ammonium ion, increases soil acidity. As ammonium is converted to nitrate in the soil (nitrification), H+ ions are released and soil acidity increases; and the industrial by-products such as sulphur dioxide and nitric acid, which ultimately enter the soil via rainfall. Though Nitrate uptake is largely unaffected at slightly acidic pH soils, the uptake of NH_4^+ generally decreases with decreasing external pH, probably as a result of increasing competition between H^+ and NH_4^+ (Kleiner, 1981). Acid soils, which are soils with pH of 5.5 or lower, are one of most important limitations to agricultural production worldwide. As the soil pH decreases from 5 to 3.5 aluminium solubility increases, which inhibits root growth rapidly, with the root apex being the primary target playing a central role in Al toxicity (Matsumoto, 2000). Increased soluble aluminium impairs the activity of H^+-ATPase in Plasma Membrane (Ahn *et al.*, 2002). The acid soil "syndrome" includes toxic levels of levels of Al, manganese (Mn) (Adams, 1981), and iron (Fe), as well as deficiencies of several essential mineral elements, with phosphorus (P) being the major limiting nutrient on acid soils (Kidd and Proctor, 2001). Low pH also leads to a change in root architecture, which in consequence, can reduce a plant ability to absorb water (and nutrients).

The subject of this paper is the study of morphological changes like paralyzing root & shoot growth (both length & weight), changes in anti-oxidative enzymes, total protein & low molecular wt. peptide profile along with sugar partitioning through which the status of oxidative, metabolic & transport process can be predicted in different parts of *Vigna radiata*(L.) Wilczek. under high proton stress.

MATERIALS AND METHODS

Plant Material: Sona Mung Seeds (*Vigna radiata* (L.) Wilczek. cv. Sonali).