CHAPTER-IX

GERMINATION INDUCED PEPTIDE POOL REGULATE WATER HOMEOSTASIS IN PLANTS

9.1 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 (Matsubayashi *et al.*, 2002; Lindsey *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 because 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 and osmolytes in different terminals and diameter of conductive tissues in association with number and distribution of root hairs. Stomata, each delimited by a pair of guard cells, are crucial for gas exchange and maintaining homeostatic control of water movement in plants. Stomatal movements are influenced by a variety of exogenous signals and also by

endogenous control of phytohormones like ABA, cytokinins and natriuretic peptides. Exogenous plant natriuretic peptides stimulate stomatal opening and activate the H^+-ATPase (Maryani *et al.*, 2001). Moreover, immunoreactive plant natriuretic peptides rapidly and specifically induce the transient elevation of cGMP levels in maize root stele tissue and stomatal guard cell protoplasts (Pharmawati *et al.*, 2001). Not only do immunoreactive peptides influence water movement via opening stomata, it also enhances osmoticum–dependent volume changes in leaf mesophyll protoplasts (Maryani *et al.*, 2001). Although the biological characterization of natriuretic peptides isolated from mature plants was carried in greater details, to date there is no report on the functional significance of total peptide pool isolated from germinating seedlings when homeostatic regulation of water is essential for maintaining cellular osmotic potential during different growth phases.

This report is an analysis of peptide profile of ultra low molecular weight range isolated from germinating seedlings of *Vigna radiata* (L) Wilczek. cv. Sonali B1 and an investigation of bioactivity of this peptide pool related to water homeostasis. The basic goal was to determine if these peptides affect absorption of solute, transpiration, stomatal opening and upward solute flow. Finally, the amino acid analysis of most potent bioactive peptide was carried out to correlate the amino acid profile of this unknown peptide with immunoreactive plant natriuretic peptides.

9.2 MATERIALS AND METHODS

9.2.1 Isolation, purification and characterization of peptides

One kg of 64 h old germinating seedlings of mung bean [*Vigna radiata* (L) Wilczek. cv *Sonali* B1] with emerging radicals and plumules were extracted with double distilled water in a cold room (4° C) with appropriate protease inhibitor and centrifuged in cold. It was passed through cation exchanger (Dowex 50) and anion exchanger resin (Dowex 1) followed by ether wash. During this procedure, acidic hormones like IAA, GA, ABA and basic compounds like cytokinin were removed through cation and anion resins respectively. Only amphoteric compounds, amino acids and peptides were present in the solution. The extract was then passed through Amicon's ultra filtration system with filter 10 kDa, 3 kDa and 0.5 kDa sequentially. It was further fractionated through Sephadex

LH-20 Column (80 cm X 3 cm) with 30% ethanol and 200 tubes were collected, each with 5 ml and after discarding the first 50 tubes, the remaining tubes were mixed, lyophilized and ultimately dissolved in 100 ml double distilled water. IAA, GA_3, 6-BAP, ABA – each of 2 ml solution (10^{-3} M), when passed through the same column separately, they appeared with in 45^{th} tube. So, probability of their free occurrence in bioactive fractions of LH-20 was ruled out. After lyophilization, tube number 51 to 200 were accumulated, concentrated through lyophilization and one-dimensional paper chromatography was performed after streaking peptide solution on one side of the paper through HPTLC sample application device and run through standard mobile phase solvents (isopropanol : ammonia : water :: 9 : 1: 1). After paper chromatographic separation, one narrow strip of paper parallel to solvent running front was cut and stained with ninhydrin reagent for spot development. In strip, peptide spots were recognized and accordingly the paper was cut parallel to sample application side in respect to their clustering pattern with different R_f values. The same process was repeated further for collecting more peptides required for experimentation. From paper, peptides were eluted with water and concentrated as desired.

Some biophysical aspects of these peptides were studied through paper chromatography and capillary electrophoresis. Markers of low molecular weight, like - insulin, insulin chain-A, insulin chain-B, somatostatin and CNBr treated lysozyme fraction A [all purchased from Sigma Chemical Co. USA] were used to determine the molecular weight of these peptides through capillary electrophoresis. Total peptide pool was hydrolyzed for amino acid analysis by RP-HPLC.

9.2.2 Measurement of transpiration

To investigate the effect of peptide pool on transpiration, freshly cut Jarul [*Lagerstroemia speciosa* (L.) Pers.] leaves with six leaves attached to one twig were placed in beakers containing 60 ml tap water with 20 ml vegetable cooking oil [olive oil] layered above. The layer of cooking oil prevents any evaporation of water so that any decrease in water has to be lost through the leaves. In addition to control leaves, to which no peptide was added to the water in the beaker, one more control beaker was kept with water and cooking oil but without a leaf bearing twig placed in it. This control lost no water when

observed for a ten-day period. The amount of water lost per beaker was recorded hourly for the first 12 h and then at 24 and 48 h. To determine the amount of water lost per unit area of leaf, an outline of the respective six leaves utilized were added together to define the total square inch leaf area. The amount of water lost in the respective beakers (with and without peptides) was then divided by the leaf area to determine the effect of water lost per unit area of leaf.

9.2.3 Protoplast isolation

Protoplasts were isolated from the leaves of one-week-old rice seedlings grown in dark according to standard methods by Lin (1980), with slight modifications. The surface layers were stripped from the coleoptiles of rice seedlings and they were incubated for 5 h with regular shaking (30 min) in 20 mM MES (Sigma) buffer solution adjusted to pH 5.5 and containing 2% (w/v) cellulase (Sigma), 400 mM mannitol, 100 mM glycine and 5 mM $CaCl_2$. After incubation, the protoplast suspension was filtered through a nylon filter with a 100 μm pore size and the protoplasts were washed several times in 20 mM Hepes (Sigma), pH 7.0, 500 mM mannitol, 2 mM $MgCl_2$ and 0.1 mM EGTA, followed by centrifugation at 200*g* for 3 min. After isolation, the protoplast concentration was adjusted to 2×10^5 cells ml^{-1} in the same solution that was used for washing. Protoplast viability was tested with the exclusion dye methylene blue (0.3 mM).

9.2.4 Determination of protoplast swelling

Protoplasts (5×10^5 in 0.5 ml) were kept in the dark for different time intervals at 22°C with or without the addition of peptides. Then, protoplasts were placed on a hemocytometer and photographed. The diameters of 100 protoplasts for each incubation time were determined and the mean volume was evaluated, making the assumption that the cells were spherical. All measurements of the changes in protoplast volume were made in the same medium.

9.2.5 Measurement of stomatal aperture

Epidermal peelings (abaxial surface) of *Vicia faba* L. leaves were rinsed and submerged in stomatal assay solution (50 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 6.5) at 25 °C

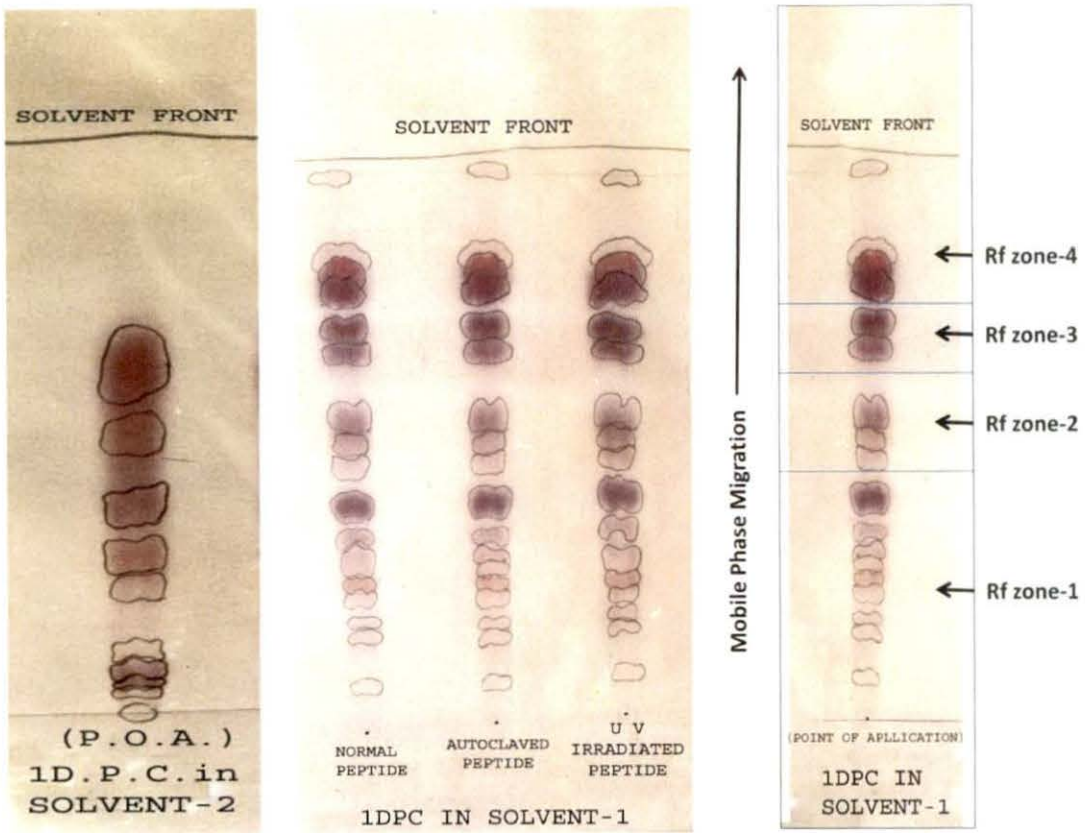

Figure 9.1 One dimension paper chromatographic separation of isolated peptides in both solvents

[1DPC= One dimension Paper Chromatography, POA=Point of Application]

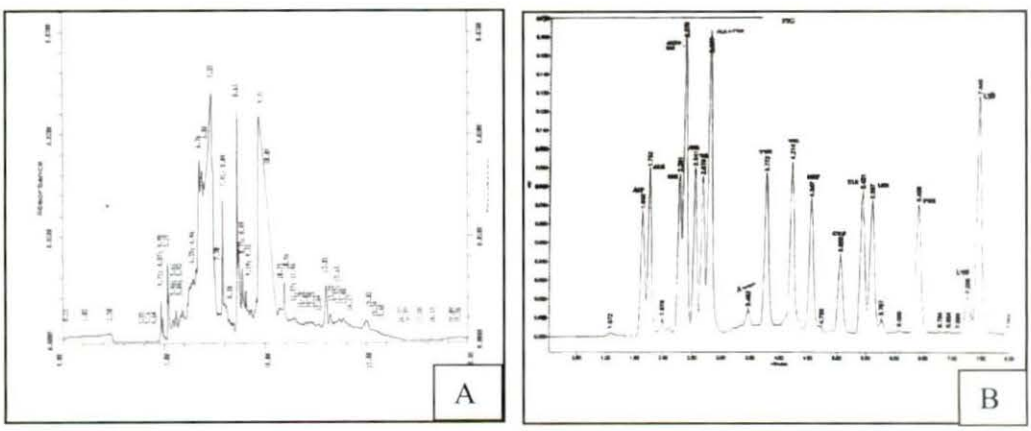

Figure 9.2 Capillary Electrophoresis (left) and Amino Acid Analysis (right) of peptide isolated from Rf zone 3

Table 9.1 Comparative amino acid profile of AtANP-A and chromatographically purified peptide of R_f zone 3 isolated from *Vigna radiata*

Amino Acid Code	Immunoreactive At-ANP A		Peptide of Vigna radiata		
	No of amino acid residue in the sequence	Percent composition	Retention Time in Chromatogram (min.)	Percent composition	Percent Deviation
Asp	4	3.1	1.632	3.6	+ 0.5
Gln + Glu	5 + 3	6.1	1.752	6.5	+ 0.4
Ser	9	6.9	2.281	2.1	- 4.8
Gly + His	12 + 1	10.0	2.370	10.4	+ 0.4
Arg	2	1.5	2.541	7.5	+ 6.0
Thr	10	7.6	2.678	7.8	+ 0.2
Ala + Pro	14 + 7	16.1	2.699	13.2	-2.9
Tyr	5	3.8	3.772	6.3	+ 2.5
Val	10	7.6	4.214	7.6	+ 0.0
Met	3	2.3	4.547	3.8	+ 1.5
Cys	7	5.3	5.050	5.1	- 0.2
Lle	10	7.6	5.431	7.9	+ 0.3
Leu	5	3.8	5.597	4.9	+ 1.1
Phe	4	3.1	6.406	4.4	+ 1.3
Lys	7	5.3	7.256 + 7.440	8.9	+ 3.6

under an incandescent light (430 nm at 35 W m^{-2}) in a microtiter plate and treated with various chromatographically isolated peptides at a final concentration of 500 ng/ml and 10 µg/ml of crude peptide from pool for an hour. As a control, an equivalent amount of sterile water was mixed with the assay buffer (90 µl) to cover the leaf peels under the above-mentioned condition. Stomatal guard cell pore widths were viewed using a microscope fitted with a calibrated ocular micrometer.

9.3 RESULTS

9.3.1 One dimension paper chromatography of peptides

From the Figure 9.1, it is clear that in case of solvent-1, the separation pattern is better than that of solvent-2, which indicates the nature of peptides. The overall expression showed that, majority of distinguishable peptides come within fraction-2 and 3; a few amount come within fraction – 4 and fraction – 1. In solvent-1 peptides were separated according to their R_f zones.

9.3.2 Capillary zone electrophoresis of four fractions of LH – 20 purified peptides

In capillary electrophoresis, major peaks were detected mainly in fraction-2 and fraction - 3. Peptides of fraction – 1, displayed some overlapping peaks with trailing effect and fraction – 4, contained lowest number of peaks (only one representation is documented in Figure 9.2 A). From capillary electrophoresis, five peptides were detected with MW of 850, 1050, 1200, 1800 and 2550 Da from germinating peptide pool.

9.3.3 Amino acid analysis

Nearly 16 amino acids were detected in isolated peptides (Figure 9.2 B). The amount of glycine-histidine and proline-alanine pool was found to be the highest. Amino acids like serine and threonine, which contain aliphatic hydroxyl group, were expressed moderately. Tyrosine and phenylalanine were present in low amounts. Cysteine is also present which indicates the probability of formation of disulphide bond. Methionine was found to be insignificant. Amino acid composition bears remarkable similarity with immunoreactive atrial natriuretic peptides of *Arabidopsis thaliana* and in most cases deviation is below 5% (Table 9.1).

Table 9.2 Bioactivity of *Vigna radiata* peptides related to flow of solute, absorption and transpiration

Sample	Solute Flow		Absorption Rate (ml.)	Transpiration Rate (ml)	Protoplast Volume μm^3	Root Length (mm)	Shoot Length (mm)
	Arrival time (min.) of dye coloured water at lowest flower of inflorescence						
	With leaf	Without leaf					
Control	19.5 ± 2.5	21.5 ± 2.8	2.8 ± 0.4	03 ± 1	4850 ± 50	38 ± 8	18 ± 5
Peptide Pool	18.2 ± 2.2	18.6 ± 2.0	5.0 ± 1.0	09 ± 1	5600 ± 50	67 ± 7	28 ± 6
R_f 1 Peptides	14.3 ± 1.3	15.5 ± 1.5	6.3 ± 0.5	10 ± 1	7100 ± 75	56 ± 4	31 ± 5
R_f 2 Peptides	20.4 ± 2.5	21.9 ± 2.2	2.5 ± 1.0	04 ± 1	4700 ± 50	48± 5	20 ± 6
R_f 3 Peptides	12.1 ± 3.0	14.5 ± 2.8	5.7 ± 0.5	11 ± 1	8700 ± 75	59 ± 4	33 ± 5
R_f 4 Peptides	27.5 ± 1.2	24.6 ± 1.5	2.9 ± 1.0	1.5 ± 1	5050 ± 75	47 ± 8	15 ± 3
LY 83583	-	-	-	-	4600 ± 50	-	-
8-Br-cGMP	-	-	-	-	5200 ± 50	-	-

9.3.4 Solute flow in plants

The isolated low molecular weight peptides of 0.5 to 3.0 KDa markedly increased the movement of solute up the stem of the plants. The peptide pool enhanced the arrival time of dye coloured water at the respective flowers by 19.5 ± 2.5 (SE) minutes compared with control in *Polianthes tuberosa* L. cv. 'Marginata' with all leaves present in the flower stalk (Figure 9.5). The picture was more prominent through one dimension paper chromatography, where peptides were separated according to their R_f zones. Purified peptides of R_f zone 1 and 3 improved the rate of water flow whereas the peptides representing R_f zone 4 could not mimic the same rate over control (Table 9.2). The peptides of R_f zone 2 did not significantly enhance the movement of dye coloured water to the respective flowers. The percent decrease in time for the dye coloured water to reach the flower varied between 26 and 38% for peptides of R_f zone 1 and 3 respectively. The elevated time taken by the dye coloured peptides for traveling the same path was 8 minutes more in case of peptides of R_f zone 4 in respect to control. The experiments were performed with all leaves removed from the stem and with all leaves present for observing the rate of transpiration. With all leaves removed, the peptides still markedly enhanced the flow of dye-coloured water into stems, reaching the flowers 15.5 ± 1.5 minutes before the control flowers.

9.3.5 Absorption

In addition to increasing the rate of flow of solute, the peptides increased the total volume of solution (i.e., water + solute) taken up by the plant. When the above experiments were continued for two days, pool of peptides caused 5 ± 1 ml of solution to be taken up as verses 2.8 ± 0.4 ml for the control side of the plant (Table 9.2). Peptides isolated from R_f zone 1 and 3 significantly enhanced absorption pattern up to 6.3 ± 0.5 ml and 5.7± 0.5 ml respectively, while this bioactivity is diminished in case of peptides from R_f zone 2 and 4. Peptides of R_f zone 2 and 4 caused 2.5 ± 1 and 2.9 ± 1 ml to be absorbed, which is almost similar to that of control.

Figure 9.3 Opened (left), closed (middle) and partially closed (right) stomata of *Vicia faba*

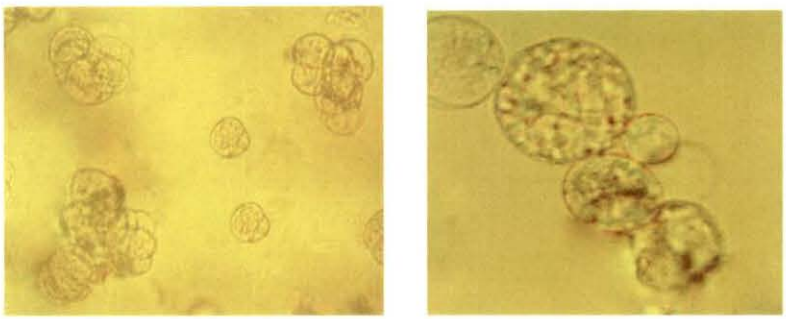

Figure 9.4 Isolated rice coleoptile's protoplast (left) under microscope and the same (right) after application of 500 ng/ml of peptides

Figure 9.5 Time course uneven distribution of colour on one side of the inflorescence and also in same flower due to accelerated movement of solute after peptide treatment

9.3.6 Transpiration

The isolated peptides caused a transpiration of 9 ± 1 ml of water in 12 h from Jarul leaf when 100 µg/ml fresh weight equivalents of peptides were present in 60 ml tap water. On the other hand, control leaves transpired 3 ± 1 ml of water during the same 12-h period. Purified peptides separated by preparative paper chromatography from R_f zone 1, 2, and 3 enhanced the transpiration rate with 10 ± 1, 4 ± 1 and 11 ± 1 ml, respectively in 12 h. Interestingly, the effect of peptides of R_f zone 4 on transpiration was less than control in Jarul leaves and transpired only 1 ± 1 ml in the same time (Table 9.2). The beakers without leaves lost no water through the cooking oil during these 12 h and did not lose any water, even when the experiments were prolonged for 10 days. The amount of water lost per area of the Jarul leaf is represented in Table 1 where it is clear that R_f zones 1 and 3 peptides increased transpiration three- and four-folds respectively, whereas R_f 2 peptides did not increase the rate of transpiration. Peptides isolated from R_f zone 4, when applied, decreased transpiration up to 2-fold. The weight of six Jarul leaves plus twig before placing in the beaker was 5.89 ± 0.28 g, whereas their weight after 12 h in 60 ml tap water with or without the peptides was 5.72 ± 0.46 g indicating that there is no weight loss in 12 h.

9.3.7 Protoplast volume enlargement

The experiment of protoplast swelling was started after equilibrium in a protoplast wash buffer for 20 minutes at 30° C. Figure 9.4 shows the time course of protoplast swelling caused by the application of peptides of different R_f zone and integrated peptide pool. Approximately 8% increase in protoplast volume was observed after 30 minutes when isolated protoplasts were treated with crude peptides (10 µg / ml) in mannitol isotonic buffer. According to our results, 15-min incubation was sufficient for the curves to reach a plateau corresponding to about a 12% to 16% increase in volume for peptides representing R_f zone 1 and 3, respectively, at a concentration range of 1 to 0.5 µg / ml (Table 9.2). No increase in volume was observed when peptides of R_f zone 2 and 4 were applied (Table 9.2). One important finding from the experiment is that the treatment of protoplast with a combination of 500 ng / ml of bioactive peptides of R_f zone 1 / 3 and 10 µM LY 83583 had no effect on protoplast volume but similar application with 20 µM LY

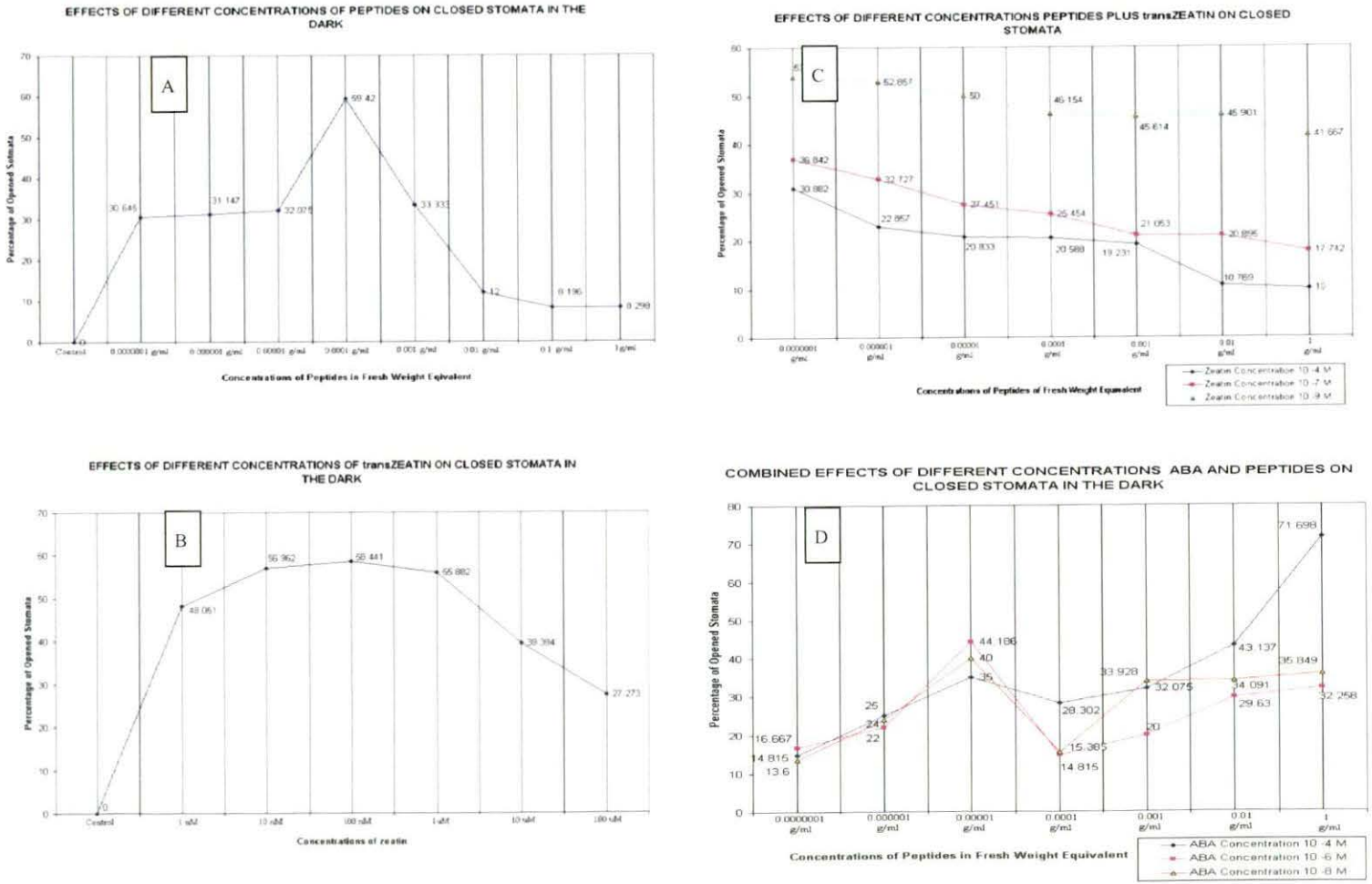

Figure 9.6 Interaction of peptides of *Vigna radiata* with Zeatin and ABA in dark; **[A]** Effect of peptides on close stomata; [B] Effect of zeatin on close stomata [C] Peptide Zeatin interaction on close stomata [D] Peptide-ABA interaction on close stomata

83583 reduced the protoplast volume up to 28%. Treatment of protoplasts with 10 μM 8-Br-cGMP, however, resulted in a slight increase in volume.

9.3.8 Stomatal aperture control

Stomata form pores on leaf surfaces that regulate uptake of CO_2 for photosynthesis and loss of water vapour during transpiration. In the sunlight all isolated peptides were effective in promoting the opening of stomatal aperture. Generally in dark, stomatal aperture closes but from this experiment it was found that these peptides have the potential to promote the opening of stomatal aperture (Figure 9.3). In dark, 500 ng / ml of peptide isolated from R_f zone 1 was most effective in promoting the opening of stomatal aperture (49.42%) with mean width of 3.85 ± 0.97 μm. Other peptides representing R_f zone 3 were also effective in opening 38.5 % stomatal pores. Peptides from R_f zone 1 and 4 had no effect on stomatal guard cell regulation. Again, higher concentrations of all peptides were rather inhibitory in the physiological process that generally promote stomatal opening.

Specific concentrations of the bioactive peptides were found to interact with hormones that regulate guard cell physiology - when applied to epidermal peelings in combination with cytokinin, a known promoter of stomatal opening, or ABA, a known promoter of stomatal closing. Though the effect of pure *trans*-zeatin was highest in opening of stomatal aperture in the dark at a concentration of 10^{-7} M (58.44% opened stomata) (Figure 9.6B), its activity was low at a concentration of 10^{-9} M (48.05 % opened stomata), in case of combined effects of *trans*-zeatin and bioactive peptides of R_f zone 1, opening of stomatal aperture was pronounced at 10^{-9} M *trans*-zeatin concentration. In spite of the fact that peptides promoted overall stomatal opening (Figure 9.6A), in combined application of *trans*-zeatin and peptides, peptides behaved as negative modulators of *trans*-zeatin action (Figure 9.6C). Opening of stomatal aperture was restricted to 10% when higher concentration of peptides (10 μg/L) and *trans*-zeatin (10^{-4} M) was applied in combination (Figure 9.6C), and the promotional effect of stomatal opening was normalized only after diluting either peptide solution or zeatin in cocktail. No synergistic effects were observed from these experiments.

In case of ABA, stomata were found to close almost linearly with the increase in concentrations of ABA. But when the peptides and ABA were interacted, peptides were able to enhance the opening of stomata significantly at a concentration of 10 μg/ml, even if the level of ABA was 10^{-4} M (Figure 9.6D). Though 10 μg/ml peptide was found as inhibitory dose, when treated alone, it could effectively minimize the closing effect of ABA. Here, optimal requirement of effector signals for stomatal opening could probably be achieved through peptide action.

9.3.9 Analysis of root and shoot length

Significant enhancement of root and shoot growth was observed after the application of appropriate dose of bioactive peptides of R_f zone 1 and 3. When root and shoot lengths were measured after six days of aqua-cultured seedlings of *Vigna radiata*, it was found that application of 10 μg/ml of isolated peptide pool increased the root and shoot length up to 76% and 58% respectively, over control. When these heterogeneous peptides were separated through paper chromatography, the peptides of R_f zone 1, 2, 3 and 4 at the rate of 500 ng/ml stimulated the growth of root up to 48, 25, 55 and 25% respectively, over control (Table 9.2). Shoot length was enhanced by the peptides of R_f zone 1 and 3, whereas growth retardation of shoot was noticed by the peptides of R_f zone 4 (Table 9.2). Hence, the peptide mediated growth responses of root and shoot of developing seedlings are different and probably under the influence of other hormonal control.

9.4 DISCUSSION

The separation profile of isolated peptides, in both separation techniques, confirms about their heterogeneous nature. Depending on partition co-efficient, pH range and solubility in that specific solvent mixture, peptides showed a better separation in solvent -1 than in solvent –2. As a consequence, it can be said that, a perceptible amount of peptides are with net basic and acidic charge but the main amount is amphoteric in nature in wide range of pH.

Earlier authors characterized the electrophoretic mobility of peptides on Whatman Paper through changing the polarity and pH of the solvent system. Recent reports indicated that potential of capillary electrophoresis can be explored in the field of

peptidomic research for identification of wheat cultivars or economically important lotus species through the analysis of storage proteins and peptides. In the present investigation, separation pattern of low molecular weight peptides isolated from germinating seedlings of mung bean execute characteristic 'map' or 'fingerprint' of peptide profile of that germination stage as revealed by capillary electrophoresis. Expressed peptides are mostly located in between 1500 to 3500 MW ranges within LH-20 fraction 1, 2 and 3. Our proposal is that, 64 h of germination is a quite mature and crucial phase for *Vigna radiata* when developing embryo reduces its dependence on cotyledonary food material and makes a balance between its heterotrophic and emerging autotrophic nature.

As already indicated in previous chapters, one of the most interesting findings is the thermo-stability and 'photo-stability' (against UV irradiation) of these peptides. Generally, it is believed that peptides are thermo-stable (Synge, 1955) and our finding supports that. They could stand for a temperature as high as 121°C and direct exposure under 2 germicidal UV lamps without any change in bioactivity.

Biological activity of the peptides was tested in the present study in a stomatal guard cell assay. Gehring *et al.,* 1996 demonstrated that rANP could cause stomatal opening at micromolar concentration in three plant species. From our results it is evident that the low molecular weight peptides, expressed during germination are able to alter water homeostasis by regulating the guard cell aperture. It was also demonstrated that these peptides have the capacity to modulate either positively or negatively in association or interact with other hormones as per the requirement of the plants. These positive or negative switch modulators in peptide pool may be mediated through secondary messenger like cGMP. The guanylate cyclase inhibitor LY 83583 has been extensively used as a tool in peptide research in animals even though there is considerable contradiction about its precise mode of action. LY 83583 inhibits different cGMP dependent processes such as GA dependent gene induction in barley aleurone layers and stomatal opening in *Tradescantia* sp. (Penson *et al.*, 1996; Pharmawati *et al.*, 1998). The treatment of protoplasts with the GA inhibitor LY 83583 resulted in a decrease in protoplast volume (as determined microscopically). These results are consistent with the previous reports where Maryani *et al.* (2001) showed that LY 83583 acts as an inhibitor

of soluble rather than particulate guanyl cyclase on natriuretic peptide dependent expansion of intact mesophyll cell protoplasts. The cell permeant cGMP analog 8-Br-cGMP (8-bromo-guanosine 3'5'-cyclic monophosphate) had the effect of protoplast volume expansion to mimic peptides when applied alone.

Present investigation demonstrated that isolated peptides can regulate transpiration stream in the leaves of phylogenetically unrelated groups. Weighing the plants before and after the transpiration experiments revealed that their weights were slightly less after completion of the experiments. Because weight of the plant did not increase during the experiment, this supports that the loss of water in the beaker was the result of transpiration. Removing the leaves of the plants, however, did not eliminate the solute movement through the stem. There was never an overlap in the flow of any of the solution from one side of the plant to the other in these experiments. When peptides were applied to one side of the coloured solution, the coloured solution was migrated at a faster rate to only one side of plant, and this pattern of movement of colour to only one-half of the flower at a certain time can be visualized dramatically in Figure 9.5. This was also found true for the sepals. This distinct flow of solute and nutrients would explain why one half of a plant may wither while the other half of the same plant may flourish.

It was reported earlier that amino terminal end of immunoreactive plant proANP significantly increased the flow of coloured solute through xylem vessel and amplified the rate of transpiration and absorption (Vesely *et al.*, 1993). In this study, amino acid analysis of isolated bioactive peptides of R_f zone 3 when compared with global sequence data of atrial natriuretic peptides, displayed striking similarities in amino acid composition. So it may be predicted that atrial natriuretic peptide like substances are also present in germinating *Vigna* peptide pool. Plant ANPs were found by Billington *et al.* (1997) using immunoaffinity chromatography to purify ANP anti-serum-specific epitope from the plant extract, followed by size exclusion separation of the eluate. But till now no feedback regulatory control of water homeostasis by peptides was assessed, because no investigation has performed with the bioactivity of individual peptides from peptide pool. Currently, the behaviour of unknown peptides can also be evaluated through *in silico* approach from unannoted secreted peptide database, a resource for plant peptidomics (Lease and Walker, 2006).

In animal systems, peptide hormones and specific receptors play a major role in cell-to-cell communication, as well as coordinating cell growth and differentiation in various organs. In contrast, most intracellular communications involved in plant growth and development have largely been explained on the basis of signaling by the six non-peptide plant hormones: auxin, cytokinin, ethylene, gibberellin, abscisic acid and brassinolides (Matsubayashi, 2003). There is no doubt about the significance of these hormones in plant homeostasis and growth, since discoveries over the past decade indicated that plant cell communication also made use of small peptide signals and specific receptors. Researchers to date have identified four peptide-ligand-receptor pairs in plants (Ryan *et al.*, 2002), which are involved in a number of processes, including wound responses, cellular differentiation and self incompatibility (Matsubayashi, 2003). Although most of the peptide signals in plants have no homologs in animals, and *vice versa* there are some similarities in the overall logic of peptide signaling systems in plants and animals (Takayama and Sakagami, 2002).

From our experiments it may be concluded that plant peptides, like animal peptides can also able to coordinate the physiology of seedlings during germination probably by mitigating turgor driven extensibility of cell wall and balancing water homeostasis. So, enhanced rate of elongation of root and shoot length after application of peptides is not a miracle. But the mode of action can not be ascertained based on present experiments. More intensive study will unravel the complex nature of signalling system of plant oligopeptides in near future.

REFERENCES

Billington T, Pharmawati M, Gehring CA. 1997. Isolation and immunoaffinity purification of biological active plant natriuretic peptide. *Biochem. Biophys. Res. Commun.* 235:722-725

Gehring CA. 1999. Natriuretic peptides-A new class of plant hormone. *Ann. Bot.* 83:329-334

Gehring CA, Md Khalid K, Toop T, Donald JA. 1996. Rat natruiretic peptide binds specifically to plant membranes and induces stomatal opening. *Biochemica. et. Biophys. Res. Commun.* 228:739-744

Higgins CF, Payne JW. 1982. Plant peptides. *Encyclopa plant physiol.* 14:438-458

Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, Dohmae N, Fukuda H. 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science.* 313:842-845

Lease KA, Walker JC. 2006. The Arabidopsis unannotated secreted peptide database, a resource for Plant Peptidomics. *Plant Physiol.* 142:831-838

Lin W. 1980. Corn root protoplasts: Isolation and general characterization of ion transport; *Plant Physiol.* 66:550-554

Lindsey K, Casson S, Chilley P. 2002. Peptides: new signaling molecules in plants. *Trends Plant Sci.* 7:78 - 83

Maryani MM, Bradley G, Cahill DM, Gehring CA. 2001. Natriuretic peptides and immunoreactants modify osmoticum-dependent volume changes in *Solanum tuberosum L.* mesophyll cell protoplasts. *Plant Sci.* 161: 443-452

Matsubayashi Y. 2003. Ligand-receptors pairs in plant peptide signaling; *J. Cell Sci.* 116:3863-3870

Matsubayashi Y, Ogawa M, Morita A, Sakagami Y. 2002. An LRR receptor-like kinase involved in perception of a peptide plant hormone, phytosulfokine. *Science.* 296:1470-1472

Penson SP, Schuurink RC, Fath A, Gubler F, Jacobsen JV, Jones RL. 1996. cGMP is required for gibberellic acid –induced gene expression in barley aleurone. *Plant Cell.* 8:2325-2333

Pharmawati M, Gehring CA, Irving HR. 1998. An immunoaffinity purified plant natriuretic peptide analogue modulates cGMP level in the *Zea mays* root stele. *Plant Sci.* 137:107-115

Pharmawati M, Maryani MM, Nikolakopoulos T, Gehring CA, Irving HR. 2001. Cyclic cGMP modulates stomatal opening induced by natriuretic peptides and immunoreactive analogues; *Plant Physiol. Biochem.* 39:385-394

Richer S, Lamppa GK. 1999. Stromal processing peptidase binds transit peptides and initiates their ATP-dependent turnover in chloroplasts. *J. Cell Biol.* 147:33-43

Ryan CA, Pearce G, Scheer J, Moura DS. 2002. Polypeptide hormones. *Plant Cell.* 14:251-264

Synge RLM. 1955. Peptides (Bound Amino Acids) and Free Amino Acids. In: *Modern Methods of Plant Analysis.* K Paech and MV Tracey (eds.), Springer-Verlag, Berlin, Germany, pp. 1-22.

Takayama S, Sakagami Y. 2002. Peptide signaling in plants; *Curr. Opin. Plant Biol.* 5:382-387

Vesely DL, Gower WR, Giordano AT. 1993. Atrial natriuretic peptides are present throughout the plant kingdom and enhance solute flow in plants. *Am. J. Physiol.* 265:465-477

Zyalalov AA. 2004. Water flows in higher plants: physiology, evolution and system analysis. *Russ. J. Plant Physiol.* 51:547-556