# CHAPTER-VII

## BASIPETAL ACTION PATTERN OF INTERNODAL PEPTIDES ISOLATED FROM *VIGNA RADIATA*

## 7.1 INTRODUCTION

All plant shoots can be interpreted as a series of developmental modules, which are produced from shoot apical meristem. Internodes are the crucial organs of the shoots, which carry water, hormones, and food materials from node to node in plants. Recent results indicate that the inputs from environments are channelled through internodes and execute complex interactions with hormones and transcription factors (Smith and Hake, 2003). In that sense each internode may be considered as a reservoir of signalling components associated with growth, development and senescence of lateral organs including leaves. Among several regulatory signals of senescence, phytohormone abscisic acid plays an important role as senescence inducer by breaking down of chlorophyll pigments (Aharoni and Richmond, 1978). Young leaves have the highest abscisic acid level although this is mainly originated and transported from the older leaves (Zeevaart and Creelman, 1988). Also dramatic increase in the endogenous abscisic acid level was observed in internodes and older leaves after initiation of chlorophyll degradation during senescence (Gan, 2007). Functionally senescence is the recycling process, by which nutrients accumulate in senescing tissues redirect to the other areas of the plant where they can be used for the production of new vegetative organs (Roberts *et al.*, 2012). Protein degradation, which allows recycling of amino acids, is probably the most important degradation process that occurs during senescence (Guo *et al.*, 2004). It was demonstrated that proteases that are involved in senescence are mainly active in chloroplast organelles (Thayer *et al.*, 1988). Also ubiquitin mediated protein degradation is observed in the cytosol of cellular compartments at the time of senescence. During proteolysis, lots of peptides are accumulated by sequential breakdown of larger proteins, but not all of these peptides are utilized by plant tissues for nutritional purposes (Huffaker, 1990). So it may be hypothesised that the peptides, which are generated during senescence or programmed cell death, may contribute in regulating the same process. Similarly it can also be assumed that some house-keeping peptides may perform as senescence inducer along with conventional phytohormone, through which the basipetal pattern of senescence may be regulated in different internodes along the stem. Unfortunately, till now no relevant data were obtained through which this type of interpretations can be drawn. But the bioactivity of these peptides should be checked in the perspective of senescence physiology for better understanding the

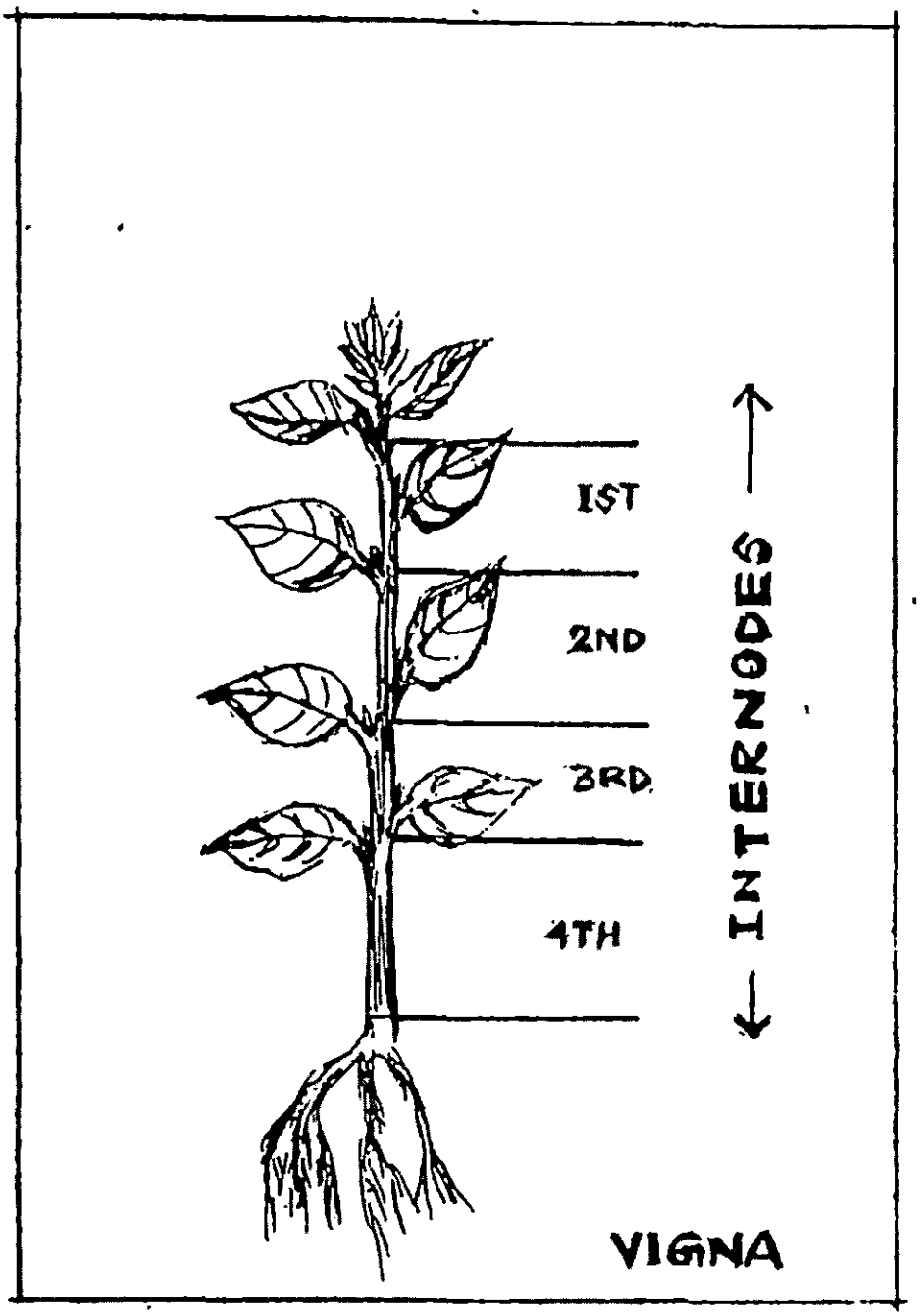


**Figure 7.1** Picture of *Vigna radiata* L. 28 days old plant showing four internodes. Apical node was considered as 1st and the basal one was considered as 4th internode

intricate role of these peptides. In present study, the bioactivity of different internodal peptides was assessed in the frame of their inhibitory roles related with vital physiological processes. Maturity of internodes generally approached towards basipetal direction with the main axis of the aerial parts (i.e. stem) of the plant. For isolation of peptides of different maturation stages of internodes, 28 days mature plants of *Vigna radiata* (vegetative stage) having four distinct internodes were selected. Numbering of internodes was assigned basipetally in ascending order i.e. the apical one was considered 1$^{st}$ and the basal was the 4$^{th}$ internode (Figure 7.1). Peptides were separately isolated from these internodes and bioassay related with senescence, stomatal closure, mitotic cell division and pattern of inhibition of coleoptile elongation was evaluated with the different doses of these peptides. In all cases, the activity of peptides was expressed with the performance of standard phytohormones.

## 7.2 MATERIAL AND METHODS

### 7.2.1 Isolation and purification of peptides

#### 7.2.1a *Plant Material*

Twenty-eight days old mature plants of mung bean [*Vigna radiata* (L) Wilczek.] at vegetative stage and having with four internodes

#### 7.2.1b *Isolation and purification of low-molecular weight peptides*

Four different internodes from apex to base were separated from plants. After surface sterilization, 250g of each internodal sections were separately taken and cryocrushed. After that, aqueous extraction and further purification of peptides up to the stage of ultrafiltration were performed in the same way as mentioned in Chapter III Section 3.2.2.

### 7.2.2 Chlorophyll degradation and senescence control

The isolated peptides were used to study their bioactivity on chlorophyll retention test using leaf discs of *Hibiscus rosa-sinensis*. Different concentrations of internodal extracts having peptides were tried on *Hibiscus* leaf discs after incubation with the same solution for 48 hours for evaluating their capacity to induce the degradation of chlorophyll in comparison to control. The chlorophyll content of *Hibiscus* leaf discs

were determined by the method of Arnon (1949) as specified in Chapter III Section 3.2.10b

### 7.2.3 Regulation of stomatal guard cell aperture

The stomata of *Colocasia* leaf epidermis were subjected to light treatment after peeling. The stomatal parameters like total number of stomata, number of opened stomata, its percentage of closure and pore width of guard cells were measured. It was under the treatment of water (untreated control) and different internodal semi-purified peptide extracts and different concentrations of abscisic acid in between $10^{-4}$ to $10^{-7}$ (M) for observing their effect on stomatal closure in presence of light. Aperture of stomatal guard cells was measured with the help of ocular and stage micrometer.

### 7.2.4 Study of mitotic index and cell division

**7.2.4a** *Plant Material:*

Root meristems of *Allium cepa* L. (2n = 16)

**7.2.4b** *Pre-treatment:*

Four different concentrations of isolated peptides [10, 25, 50 and 100 µg/ml] and ABA [$10^{-7}$, $10^{-6}$, $10^{-5}$ and $10^{-4}$ (M)] were prepared just before the root treatment. When the roots of *A. cepa* L. were about 2 cm long, they were aseptically exposed to freshly prepared test solution separately and incubated for 12h at 25° C. Control roots were simultaneously treated with water to compare with peptides and ABA.

**7.2.4c** *Analysis of Mitotic Index*:

After treatment, the root tips were fixed immediately in acetic-alcohol (1:3) for 24h and then transferred in 70% and stored in refrigerator until further use. Root tips were hydrolysed in 5 (N) HCl for 20 min at room temperature and then stained in 2% acetocarmine for 1 h. Root tips were then squashed in 2% aceto-orcein stain in 45% acetic acid as described in Lamsal *et al.*, 2010. Different stages of dividing cells were observed and photographed under Olympus research microscope.

### 7.2.5 Inhibition of coleoptile elongation

Surface sterilized barley (*Hordeum vulgare*) seeds were rinsed and soaked aseptically in sterile distilled water for 16 hrs at room temperature. The seeds were covered with

moist blotting paper and incubated at 25°C for 3 days in total darkness. Coleoptile segments, about 5mm in length were cut just below the apical cap. For each determination, eight such segments were partially submerged in 5ml of 10 mM potassium phosphate buffer pH 7.0 with the indicated addition of abscisic acid [$10^{-4}$ to $10^{-7}$ (M)] or different internodal peptides contained in small Petri dishes. The segments were incubated at 25°C for 48 hrs in darkness. The length of the coleoptile segments was measured with a dissecting microscope fitted with a calibrated ocular micrometer and their inhibition were determined in respect to untreated control.

## 7.3 RESULTS AND DISCUSSION

### 7.3.1 Senescence regulation and chlorophyll loss by internodal oligopeptides isolated from *Vigna radiata*

Senescence is an important developmental process in plants that eventually leads to a death through endogenously controlled degenerative process. This is a type of programmed cell death and in plants, cellular and molecular events associated with senescence include chlorophyll breakdown, chloroplast disintegration, a decline in photosynthesis, exposition of carotenoid pigments, stomatal closure, degradation of proteins, nucleic acids along with essential biopolymers and loss of plasma membrane integrity as a result of which increase of membrane permeability (Smart, 1994; Chandlee, 2001). From previous works on different internodes and whole stem, it appears that internal senescence-inducing factors are hormonal in nature (Kawa-Miszczak *et al.*, 1999). Though the senescence processes can be stimulated or retarded by low molecular weight plant growth regulators treatment, the initiation and progression of plant senescence can be influenced by various external factors like abiotic and biotic stresses, desiccation, temperature, wounding and detachment (Chandlee, 2001; Kawa-Miszczak *et al.*, 2005). So besides classical phytohormones, molecules like peptides having wide scale versatility are highly expected to play an important role in regulation of senescence as the process is influenced by so many environmental and pathogenic interactions. Among conventional phytohormones, abscisic acid is one of the important molecules for developing desiccation tolerance

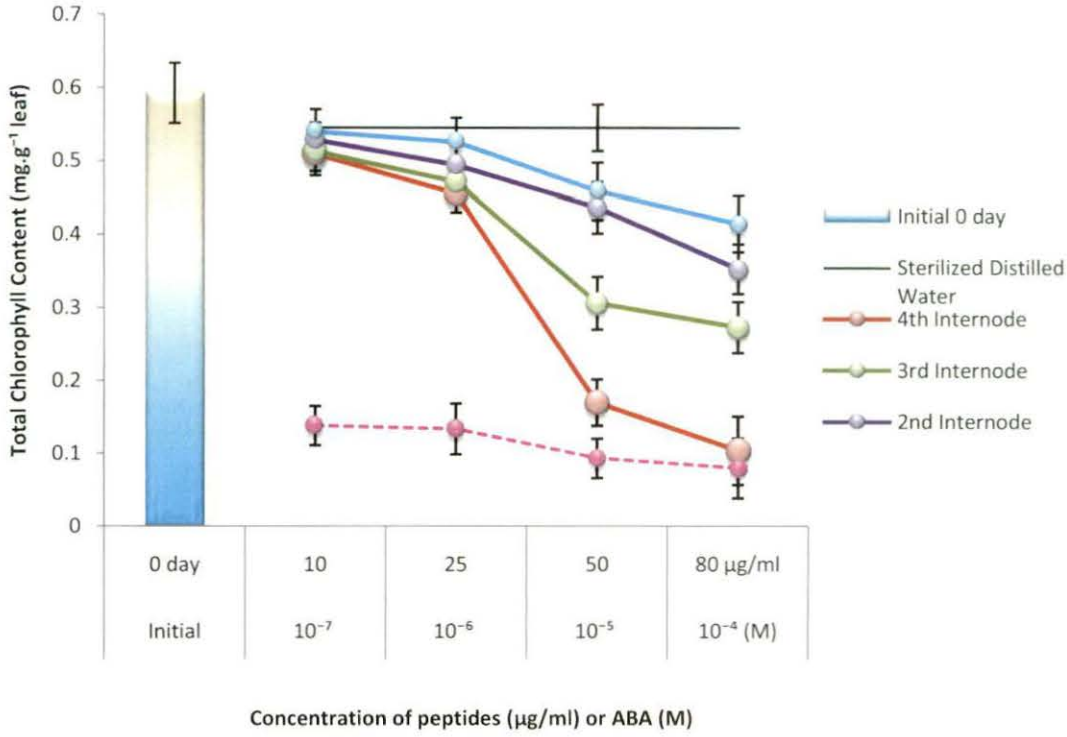


**Figure 7.2** Total Chlorophyll after 4 days incubation with internodal peptides

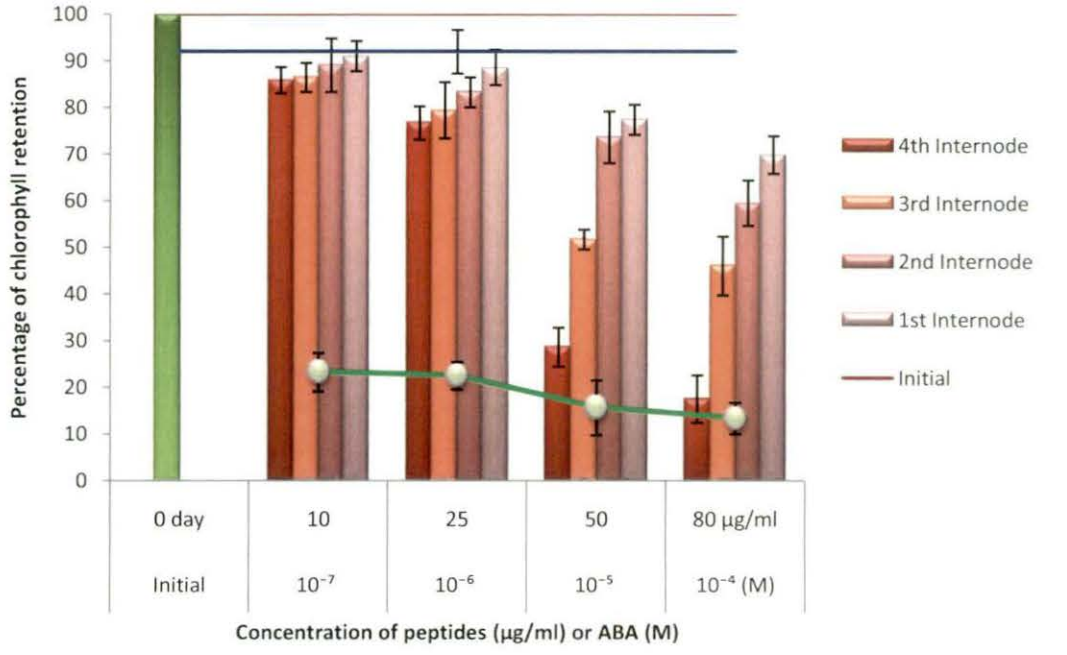


**Figure 7.3** Percentage of chlorophyll retention after application of internodal peptides

along with stress acclimation. ABA also accelerates senescence in several plant species (Lim *et al.*, 2007). Since ABA stimulates nodal senescence as evidenced from internodes and leaf discs application of the same in some genotypes of tulip (Kawa-Miszczak *et al.*, 2005), bioactivity of semi-purified plant peptides mimicking ABA action has been evaluated critically.

Different internodal extract of semi-purified peptides (0.5-3 KDa) were used to study their bioactivity on chlorophyll retention test using leaf discs of *Hibiscus rosa-sinensis*. The twenty eight days matured plants were selected with four internodes. The basal was the $4^{th}$ whereas the apical was the $1^{st}$ internode. These internodal extracts of peptides were tried on *Hibiscus* leaf discs and observed whether they could induce the degradation of chlorophyll pigments in respect to control. The effect was more pronounced with the application of peptides towards basal internodes which was optimized at $4^{th}$ internodal peptide treatment (about 8.53% in average) (Figure 7.2 & 7.3). The inhibition of chlorophyll retention was minimized gradually with the application of peptides towards upper internodal extracts *i.e.* $3^{rd}$, $2^{nd}$ and $1^{st}$ which was approximately 54%, 41% and 30% respectively at their highest applied concentrations (Figure 7.3). Again the bioactivity was dose-dependent *i.e.* higher concentrations of semi-purified peptide extracts showed high chlorophyll loss. At low concentration of peptides (10 µg/ml), sensitivity of bioassay was found to be insignificant, whereas the same inhibition was more prominent when the leaf discs were treated with higher dose of peptides (>25 µg/ml). Above 50 µg/ml peptide concentration, this inhibitory effect exhibited towards saturating trend (Figure 7.2). With 10 and 80 µg/ml peptide treatment, the difference between $1^{st}$ and $4^{th}$ internodal peptide bioactivity (chlorophyll retention) was found to be restricted approximately between 6% and 53% respectively (Figure 7.3). Different concentrations of ABA ($10^{-4}$ to $10^{-7}$ M) were also tried to observe its effect on chlorophyll loss. As expected, it enhanced the degradation of chlorophyll pigments. ABA at $10^{-4}$ M concentration produced the maximum loss of chlorophyll up to 87% or retention only up to 13%, whereas in case of control (treatment with sterile distilled water), this retention was as high as 92% (Figure 7.3). The extraction and purification process of these peptides clearly revealed the absence of endogenous ABA in the sample. However the effect of basal internodal plant peptides (especially $4^{th}$ internode) showed a similar induction of chlorophyll loss, which may suggest that these peptides were growth regulators or senescence promoter like abscisic acid.

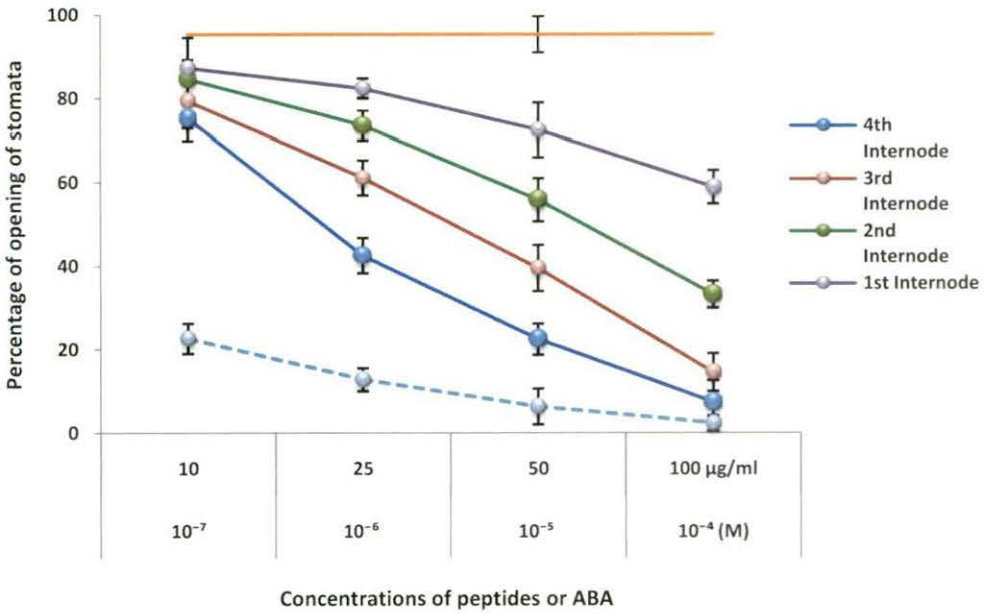


**Figure 7.4** Stomatal opening with internodal peptides

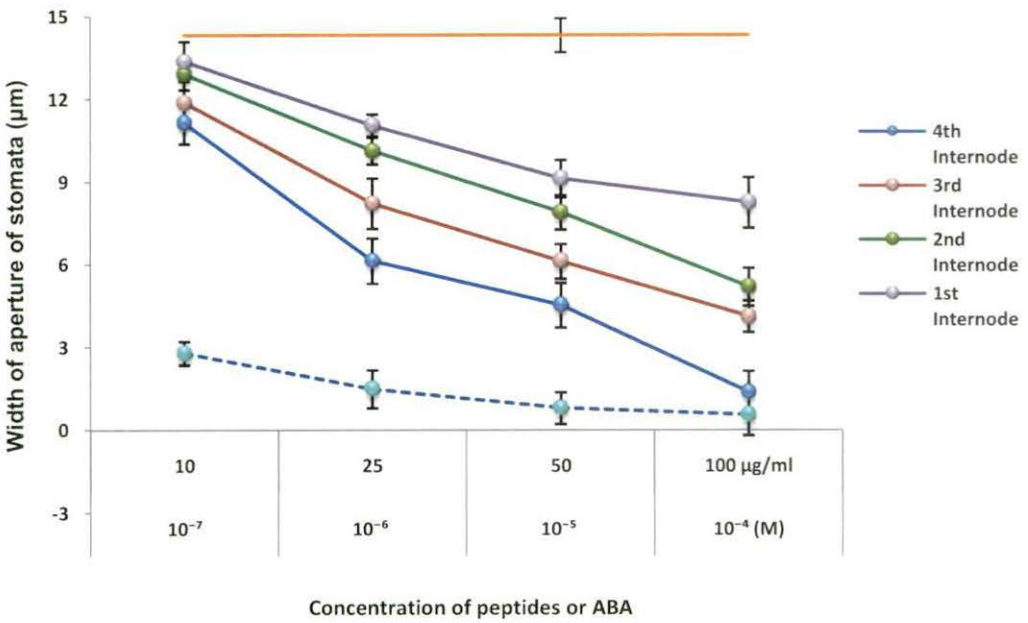


**Figure 7.6** Stomatal aperture control with internodal peptides

It is well known that different phytohormones play an important role in regulation of chloroplast degradation and senescence. Chlorophyll retention is strongly inhibited by ABA and ethephon, whereas greatly enhanced after application of IAA and benzyl adenine (Kawa-Miszczak *et al.*, 2005). In present study, response of abscisic acid in favour of senescence by chlorophyll breakdown is quite natural. During senescence proteins are degraded into large polypeptides, oligopeptides and amino acids by the enzymatic cleavage of peptide bonds (Schaller, 2004). Unsurprisingly, the proteolytic enzymes involved in degradation of chlorophyll and plastidial architecture are up-regulated in senescing stems and leaves (Fisher, 2000). Several oligopeptides transporters engaged in nutrient recycling are now well characterized (Lalonde *et al.*, 2004). Besides their nutritional role, other bioactivities of these plant derived oligopeptides are till date inadequately known. But the peptides derived from divergent pathogenic fungi or bacteria may induce senescence and necrosis in plants. One such peptide Nep1, produced by plant pathogen *Fusarium oxysporum* may induce ethylene and trigger cell death thereby generating necrotic spots during host-pathogen interaction (Veit *et al.*, 2001). Nep1 also caused the rapid disintegration of the cutin layer and integral chloroplast membrane architecture (Keates *et al.*, 2003). From our investigations, it may be concluded that the peptides isolated from 4$^{th}$ internode are functionally similar with Nep1 like peptides (NLP) produced by necrotrophs in plants.

### 7.3.2 Isolated internodal oligopeptides of *Vigna radiata* may regulate stomatal guard cell aperture

After observing inhibition of chlorophyll retention, the effect of peptides on stomatal guard cell aperture control was monitored. The stomata of *Colocasia* leaf epidermis were subjected to light treatment after peeling and the stomatal parameters like number and percentage of opened stomata and aperture width of guard cells were measured. The peelings were subsequently under the treatment of sterile distilled water as control and different internodal peptide extracts of mature *Vigna radiata* (1$^{st}$ to 4$^{th}$ internode in a basipetal direction) along with abscisic acid of $10^{-4}$ to $10^{-7}$ M concentrations, which was taken as standard for determining their consequence on stomatal opening/closing in presence of light. Sunlight mediated opening responses of stomatal guard cells are accomplished by harmonization of light signalling, energy conversion, ion transport and altered metabolic activity (Shimazaki *et al.*, 2007). In

the present experiment, the percentage of opening of stomatal guard cells of *Colocasia esculenta* under bright sunlight was very high, almost 95%. When these stomatal guard cells were subjected to different concentrations of internodal peptides under sunlight, surprisingly in most cases significant closure of stomata was observed. This stomatal closure was reached at maximum point with the application of $4^{th}$ internodal peptides (approximately up to 93% at 100 ppm concentration) whereas the same inhibitory potency was minimized towards $1^{st}$ internode (41% closure by peptide treatment of same concentration) (Figure 7.4). So there is a gradient of inhibitory bioactivity in basipetal direction or reversibly it can be stated that the closure effect falls towards the apex in acropetal order. Inhibition of stomatal opening by peptides was also dose dependent and the percentage of closure was enhanced (up to 68%) with the application of higher doses (from 10 to 100 μg/ml) of peptides (Figure 7.5). Inhibitory activity of $4^{th}$ internodal peptides (93%) at their optimized doses is comparable with the bioactivity of ABA (98%) at $10^{-4}$ (M) concentration. Average width of aperture of opened stomata also significantly reduced in basipetal order, i.e. the diameter of aperture between two guard cells of opened stomata decreased with the treatment of internodal peptides isolated towards basal direction of stem. Width of stomatal aperture after application of apical $1^{st}$ internodal peptide was only reduced up to 42% (8.25 μm width) at its optimized dose from control (14.32 μm), whereas the treatment with same dose of $4^{th}$ internodal peptide decreased this reduction up to 90.5% (1.36 μm) (Figure 7.6). In this trial, abscisic acid at $10^{-4}$ (M) concentration reduced the aperture of opened stomata nearly 96% (0.54 μm width) from control. ABA induces stomatal closure by activation of inward $Ca^{2+}$ channels leading to inhibition of $K^+$ influx and subsequent drop in turgor pressure of guard cells (Blatt and Grabov, 1997). Recent findings suggested that plant peptides can also regulate stomatal aperture through adjusting cytosolic calcium ion concentration in guard cells. Secretary peptides like extracellular calmodulin (ExtCaM) found in many plant species are reported to activate heterotrimeric G-protein, $H_2O_2$ generation in guard cells and alteration in regulation of stomatal movement (Chen *et al.*, 2004). Epidermal strip bioassay in *Vicia faba* suggested that ExtCaM induces the enhancement of cytosolic $Ca^{2+}$ leading to reduction in aperture of stomatal guard cells (Chen *et al.*, 2003). As mentioned in earlier chapters, reversal of guard cell closure *i.e.* stomatal opening is induced by another group of peptides, originally isolated from rat atrium (atrial natriuretic peptides, and immune-reactive analogues of these peptides

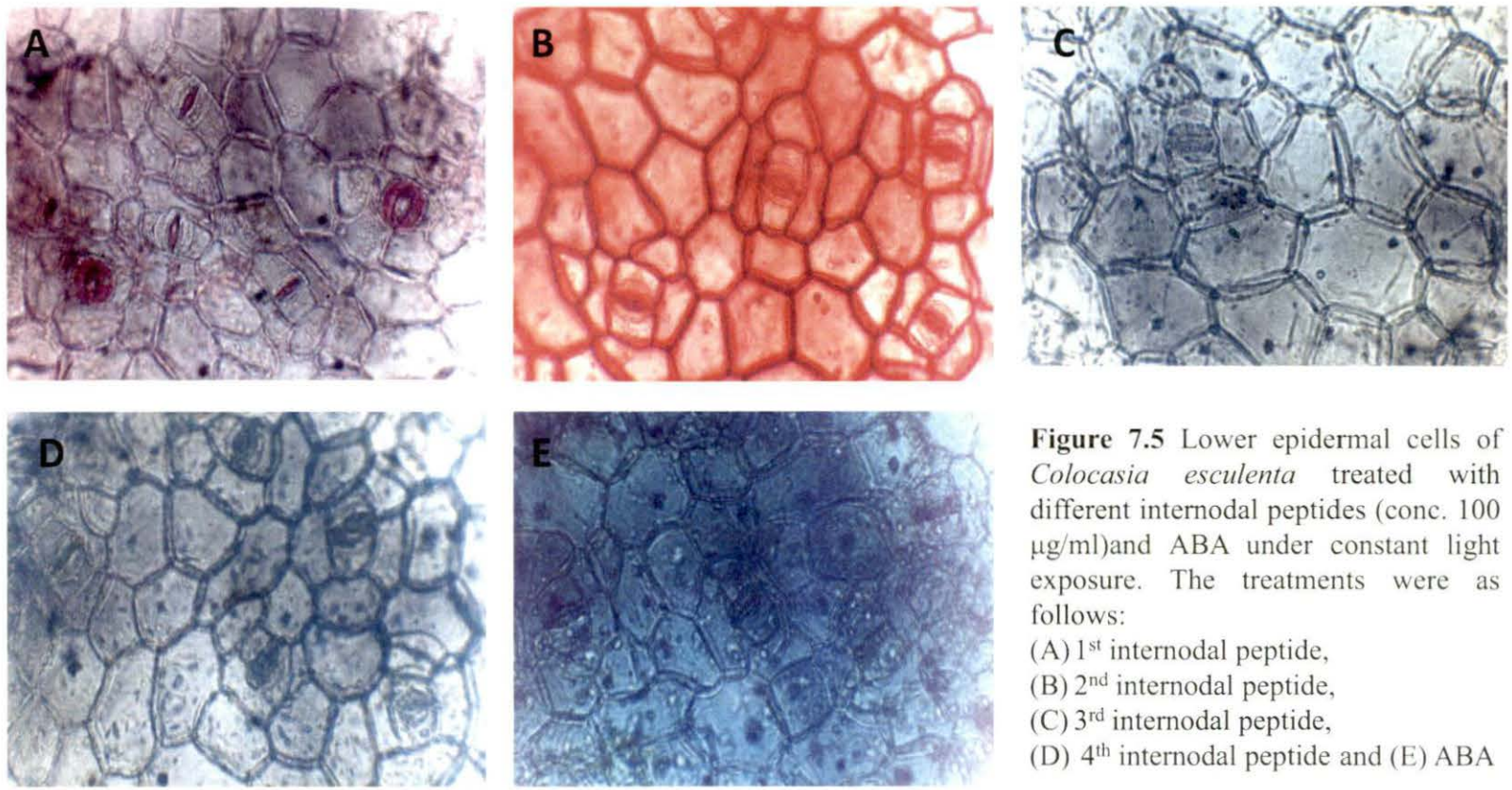


**Figure 7.5** Lower epidermal cells of *Colocasia esculenta* treated with different internodal peptides (conc. 100 µg/ml)and ABA under constant light exposure. The treatments were as follows:
(A) 1st internodal peptide,
(B) 2nd internodal peptide,
(C) 3rd internodal peptide,
(D) 4th internodal peptide and (E) ABA

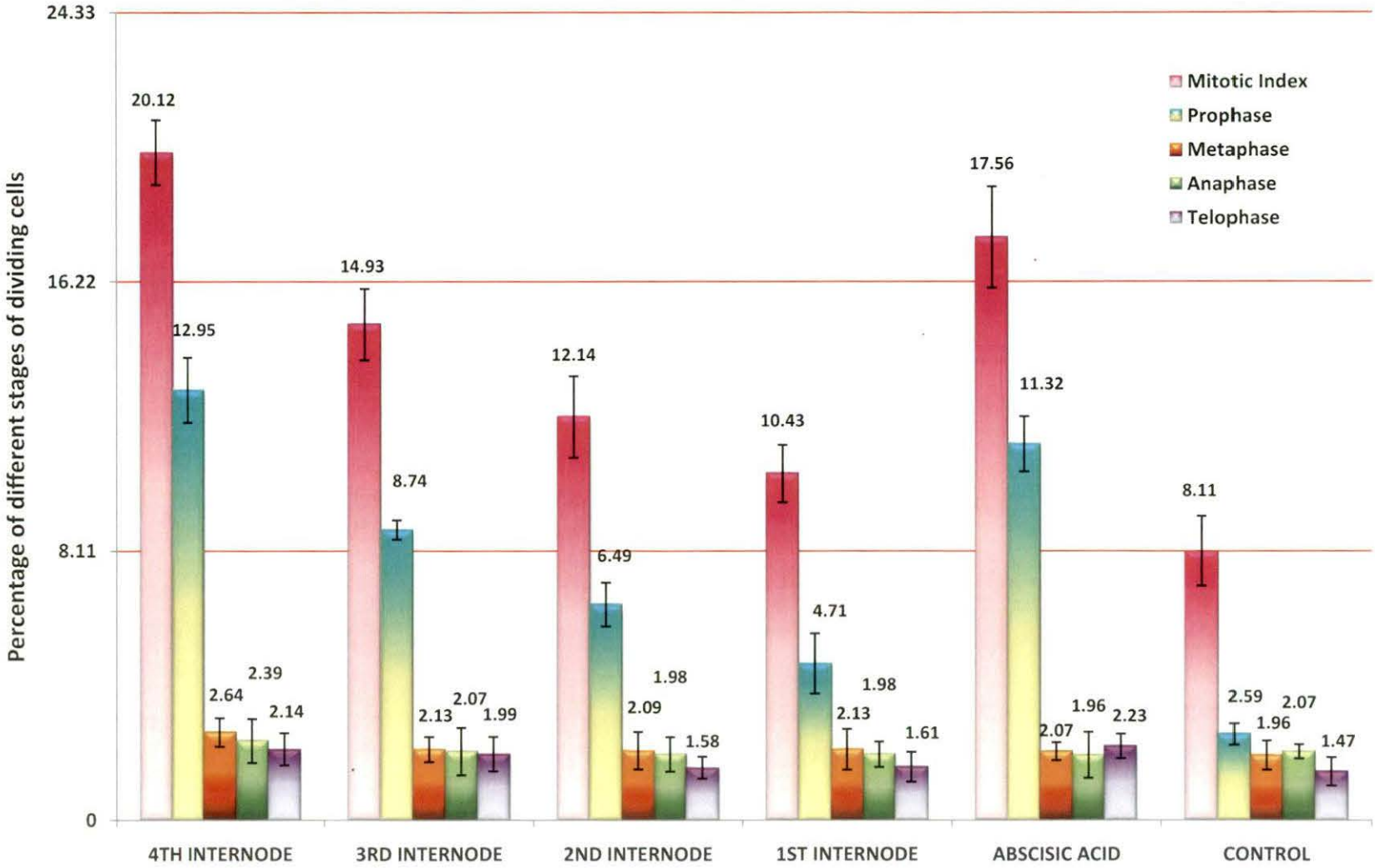


**Figure 7.7** Elicitation of cell division with internodal peptides in root tips of *Allium cepa*

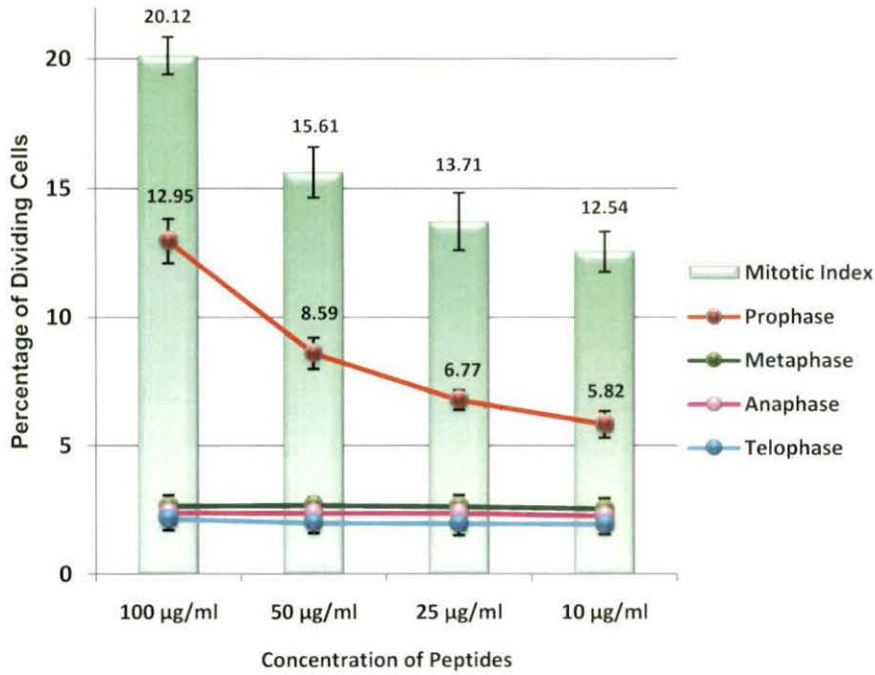


**Figure 7.8** Elicitation of cell division with 4th internodal peptides in root tips of *Allium cepa*

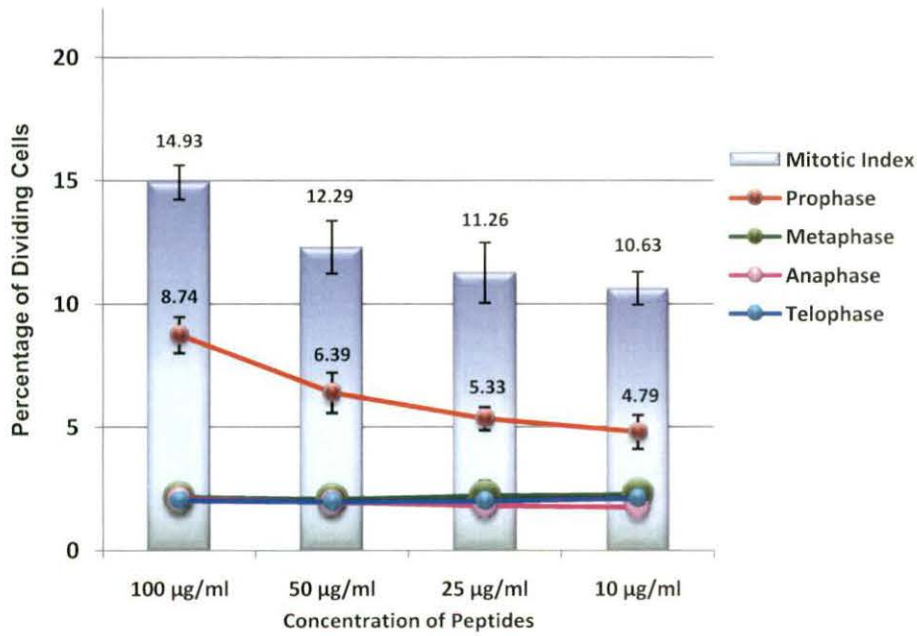


**Figure 7.9** Elicitation of cell division with 3rd internodal peptides in root tips of *Allium cepa*

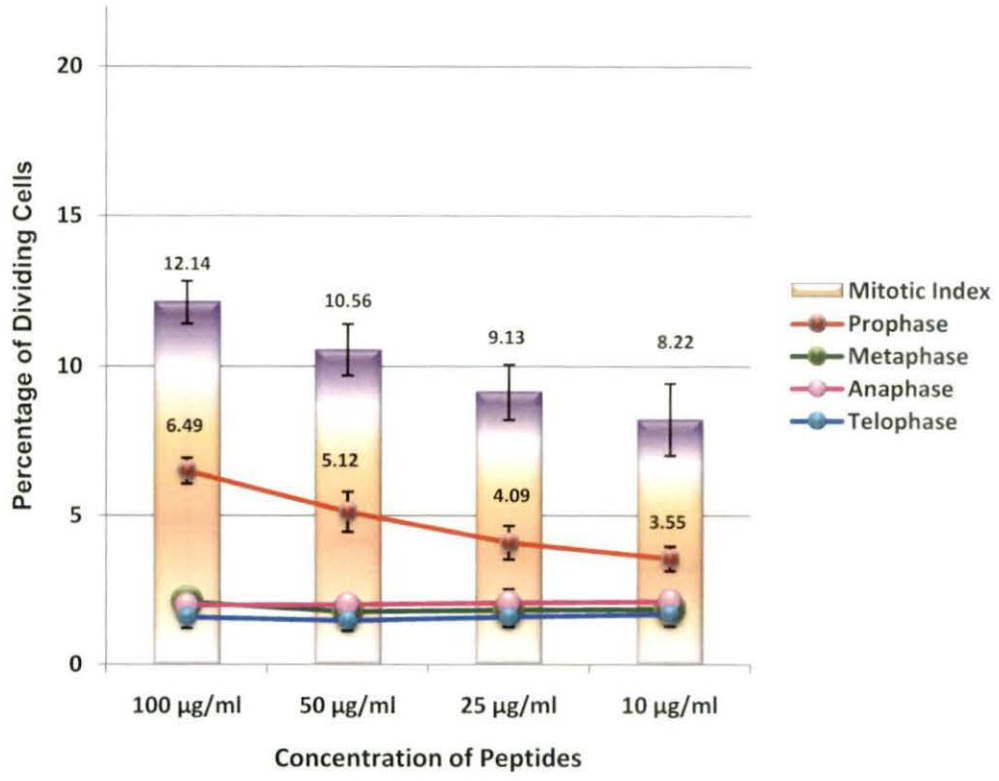


**Figure 7.10** Elicitation of cell division with 2nd internodal peptides in root tips of *Allium cepa*

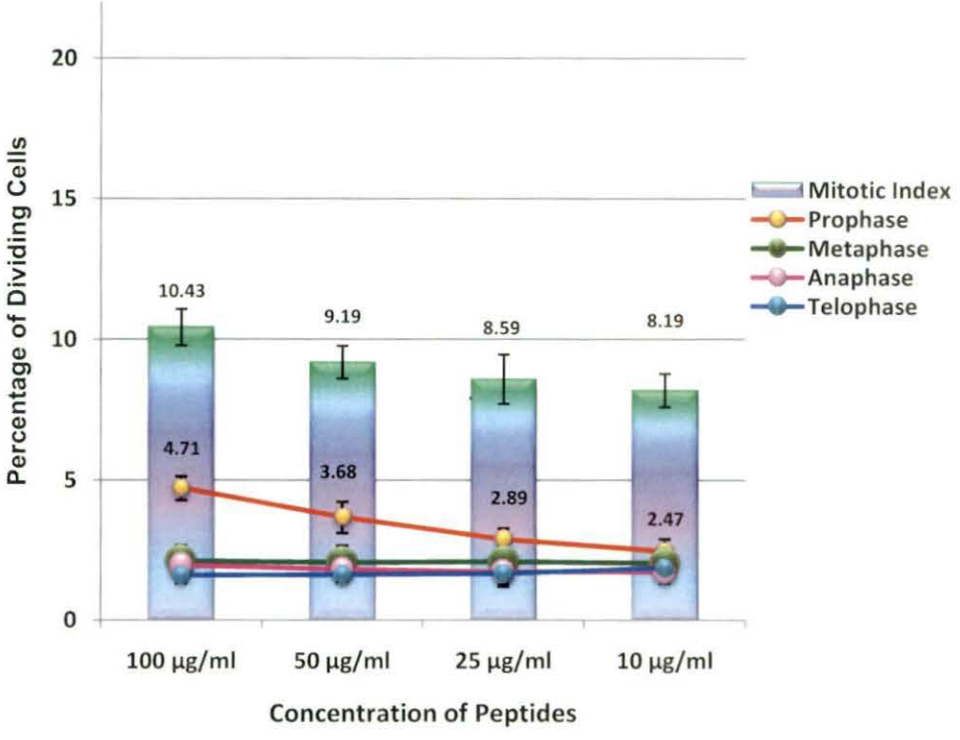


**Figure 7.11** Elicitation of cell division with 1st internodal peptides in root tips of *Allium cepa*

were also isolated from plants) by up-regulating guanylate cyclase enzyme, which is a downstream component of G-protein mediated signalling pathway (Pharmawati *et. al.*, 1998) and mimicking kinetin action. Our experiments suggest that 4$^{th}$ internodal peptide(s) of 0.5-3.0 KDa molecular weight range in plant system mimic the bioactivity of abscisic acid.

### 7.3.3 Effect of oligopeptides of *Vigna radiata* on the mitotic cell division of root tip of *Allium cepa*

In higher eukaryotes, the mitotic division involves the chronological division of nucleus, i.e. karyokinesis followed by partitioning of cytoplasm, i.e. cytokinesis (Fujisawa *et al.*, 2001). On mitotically dividing cells of *Allium* root tip, different internodal peptide extracts isolated from twenty-eight days mature *Vigna radiata* plant showed interesting result. First to fourth internodal semipurified peptides (in a basipetal direction) showed a promotive effect by increasing mitotic index (MI) and also by the significant increase in prophase percentage over three other divisional phases i.e. metaphase, anaphase and telophase. Later stages were arrested and final cell division is less. The promotive effect on mitotic index was more pronounced with 4$^{th}$ internodal semipure peptide extract (20% in comparison to control 8%) (Figure 7.7). This was, however, not observed by the treatment of semipure peptide extract of acropetal internodes, i.e. 3$^{rd}$, 2$^{nd}$ and the topmost; 1$^{st}$. MI was gradually reduced in acropetal direction (from 20.12% in basal 4$^{th}$ to 10.43% in apical 1$^{st}$ internode), along with significant decline of prophase percentage (from base to apex downfall of 12.95% to 4.71%) by the application of oligopeptides (Figure 7.7). This elicitation of cell division by peptides is also dose-dependent. MI declined up to 38%, 29%, 32% and 21% in case of 4$^{th}$, 3$^{rd}$, 2$^{nd}$ and 1$^{st}$ internodal peptide application respectively, if the doses of peptides were decreased from 100 to 10 ppm (Figure 7.8-7.11). With similar reduction in applied peptide doses, the prophase percentage was dropped up to 55%, 45%, 45% and 47% for 4$^{th}$, 3$^{rd}$, 2$^{nd}$ and 1$^{st}$ internodes respectively (Figure 7.8-7.11). In contrast, there were no significant dose dependent alteration in the percentages of metaphase, anaphase and telophase in all cases, indicating that the isolated peptides can only enhance prophase, but not the other phases of mitosis. This was more pronounced when the contribution of prophase in mitotic index after application of different internodal peptides were evaluated (Figure 7.12). Basal internodal (3$^{rd}$ and 4$^{th}$) peptides with higher applied doses considerably augment the

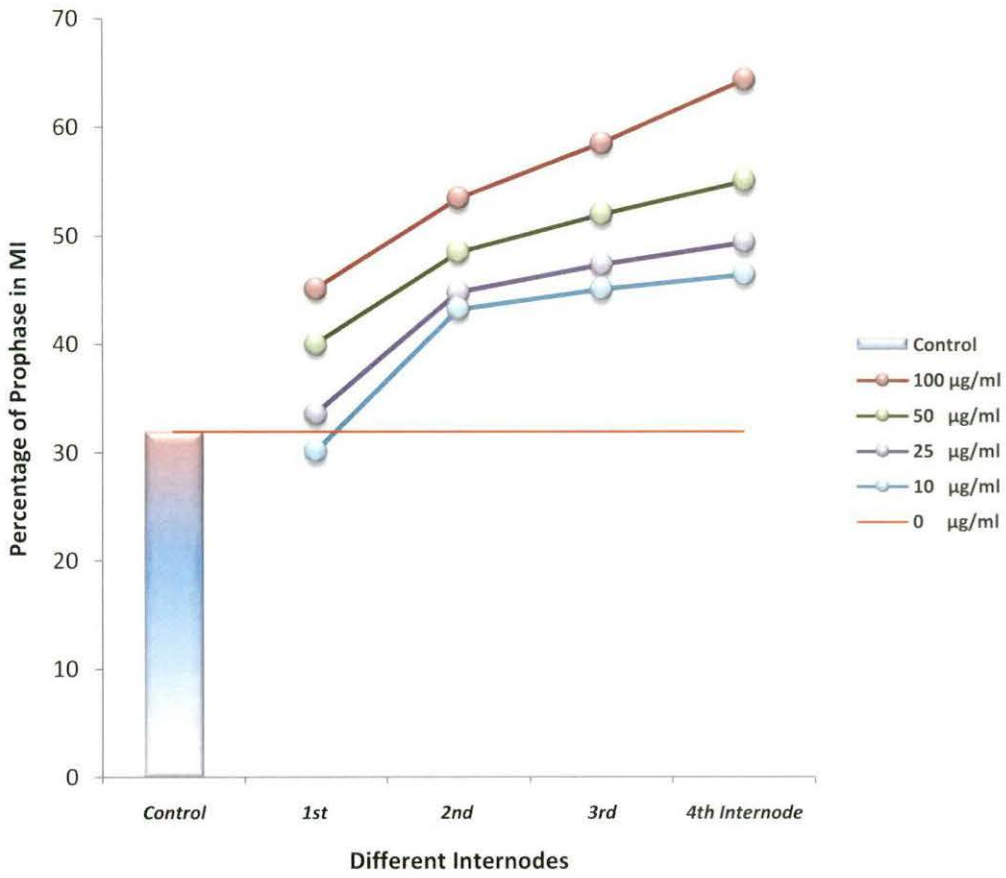


**Figure 7.12** Contribution of Prophase in Mitotic Index after *Vigna radiata* internodal peptide application

percentage of prophase, when compared with peptides of apical internodes (1st and 2nd). The result with higher MI under ABA treatment ($10^{-4}$ M) on the same root tip was also observed (17.56% in ABA in comparison to control 8.11%) (Figure 7.7). Interestingly like basal internodal peptides, ABA also enhanced the percentage of prophase (11.32%) than control (2.59%) (Figure 7.7). Endogenous ABA at low water potential exerts a strong positive effect on root growth and induces mitotic cell division in maize seedlings (Saab *et al.*, 1990). Endogenous ABA appears to promote mitotic cell division in roots by suppressing ethylene biosynthesis during water stress (Taiz and Zeiger, 2006). The obtained data of peptide treatment and ABA with increasing mitotic index are in agreement with the above statements. In contrary, some authors claimed that ABA inhibited mitosis in a dose-dependent manner in root tips of *Allium cepa* (Mahajan and Sharma, 2008). They suggested that the cell division was inhibited mainly due to enhanced catalase activity, an indicator of redox metabolism. Polyamines, on the other hand, appear to enhance MI by reducing oxidative stress. Possibly time and duration of treatment with ABA alters the pattern of MI in *Allium* root tip. In this experiment also, our findings suggested that internodal peptides mimic the action of ABA, and both the components enhance MI by increasing prophase stage only. Similar observations were obtained by earlier authors who noticed that peptides from germinating seeds of *Vigna radiata* increased MI by augmenting prophase, whereas the other mitotic stages were significantly inhibited (Sarkar *et al.*, 2011). During interphase, the cell roughly doubles its mass; synthesizes DNA and waits for specific signal to enter into mitosis, especially prophase. The peptides isolated from plants may act as signalling molecules and direct the active cells of meristematic tissue to enter into prophase (Sarkar *et al.*, 2011). Phytosulfokine-α (PSK), a sulphated pentapeptide isolated from mesophyll culture of asparagus promoted cell division at nanomolar range at very low initial cell densities (Matsubayashi and Sakagami, 1996). Researchers have also identified other different oligopeptides like Polaris, Clavata3 etc. which may influence the meristem organization and cell division significantly (Farrokhi *et al.*, 2008). All these information are again tempting to speculate the existence of similar kind of peptides in different internodes which may stimulate inductive signals on mitotic prophase.

### 7.3.4 Effect of internodal oligopeptides on barley coleoptile elongation

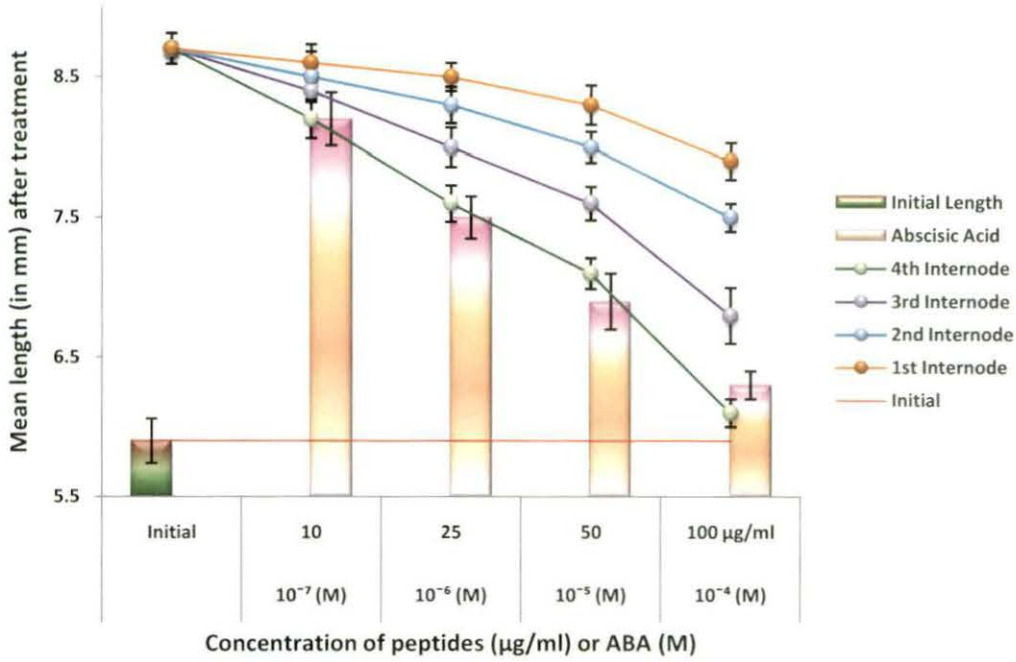


**Figure 7.13** Mean length of coleoptiles after internodal peptide treatment

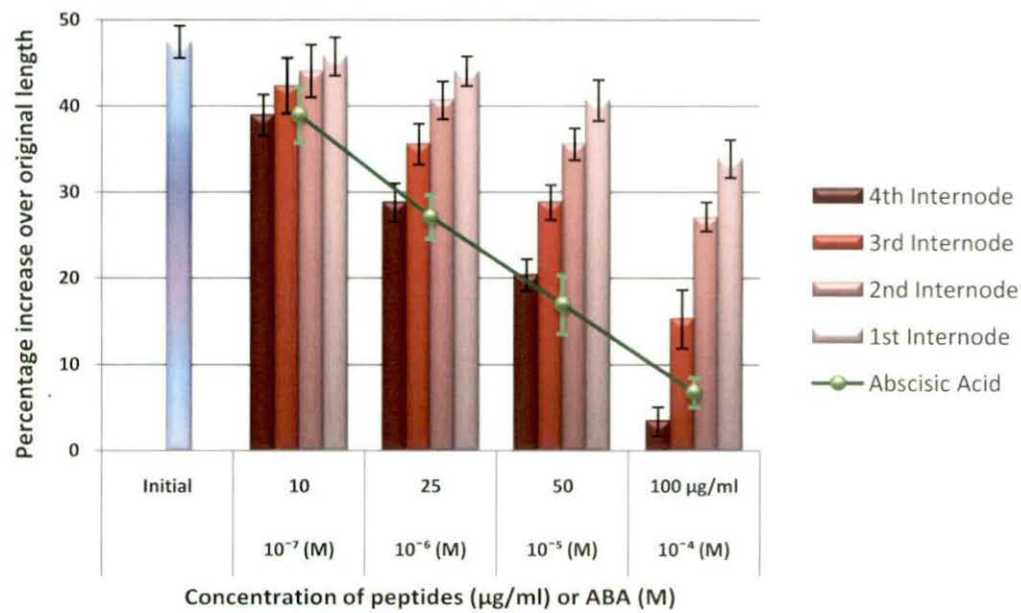


**Figure 7.14** Percentage of increase over original length after application of internodal peptides

Charles Darwin with the assistance of Francis Darwin was the first, who investigated on physiological behaviour and elongation pattern of intact grass coleoptiles in response to light mediated external stimuli (Darwin, 1880). During the past few decades, the mode of action of phytohormones, which stimulate cell elongation (hypertrophy) in excised segments of coleoptiles of different monocot cereals like oats, barley, maize, rye, etc., has been critically analyzed in details (Woodward and Bartel, 2005; Schopfer, 2006). However, the fundamental biochemical processes involved in the cessation of cell elongation and inhibition of cell division in the excised coleoptile segments has not yet been addressed properly (Kutschera *et al.*, 2010). It is well established that IAA promoted the elongation of coleoptiles rapidly. Zene (1970) first reported that IAA promoted elongation of coleoptiles of *Avena sativa* was inhibited by $10^{-4}$ (M) abscisic acid (ABA). ABA also strongly inhibited low pH induced elongation of *Avena* coleoptiles and this inhibition may involve an interaction with metabolic energy expensive processes (Rehm and Cline, 1973). In this study, dose dependent ABA-induced inhibition of coleoptiles' length was observed in *Hordeum vulgare* (Figure 7.13). Percentage increase of coleoptiles' length from initial was only negligible (6.78%) when ABA was treated with high doses ($10^{-4}$ M), whereas the same growth with comparatively lower dose of ABA ($10^{-7}$ M) was significant (38.98%) (Figure 7.14). Inhibition of coleoptiles elongation was more pronounced in Figure 7.15, where 27.5% inhibition of coleoptiles was observed by the application of $10^{-4}$ (M) ABA in respect to control. Previous workers also investigated the response of transcriptional inhibitors like actinomycin-D or inhibitors of ribosomal RNA synthesis like α-amanitin and found no effect on inhibition of coleoptiles elongation; whereas the treatment with cyclohexamide, a protein synthesis inhibitor causes inhibition of coleoptiles elongation with latent period coincided exactly within the range required for inhibition by ABA (Rehm and Cline, 1973). This indicates that the participation of newly synthesized proteins are probably essential in ABA mediated signals associated with inhibition of coleoptiles elongation. But so far very few investigations were only performed for detecting the role of small proteins or oligopeptides regulating coleoptile growth. Cessation of coleoptile elongation and loss of auxin sensitivity was associated with down-regulation of a peptide (eighteen amino acid long) corresponds to subunit E of V-type $H^+$-ATPase, as recognized through two dimensional gel electrophoresis of microsomal and cytosolic protein fraction of coleoptiles, where the growth was slowed down due to emergence of primary leaf

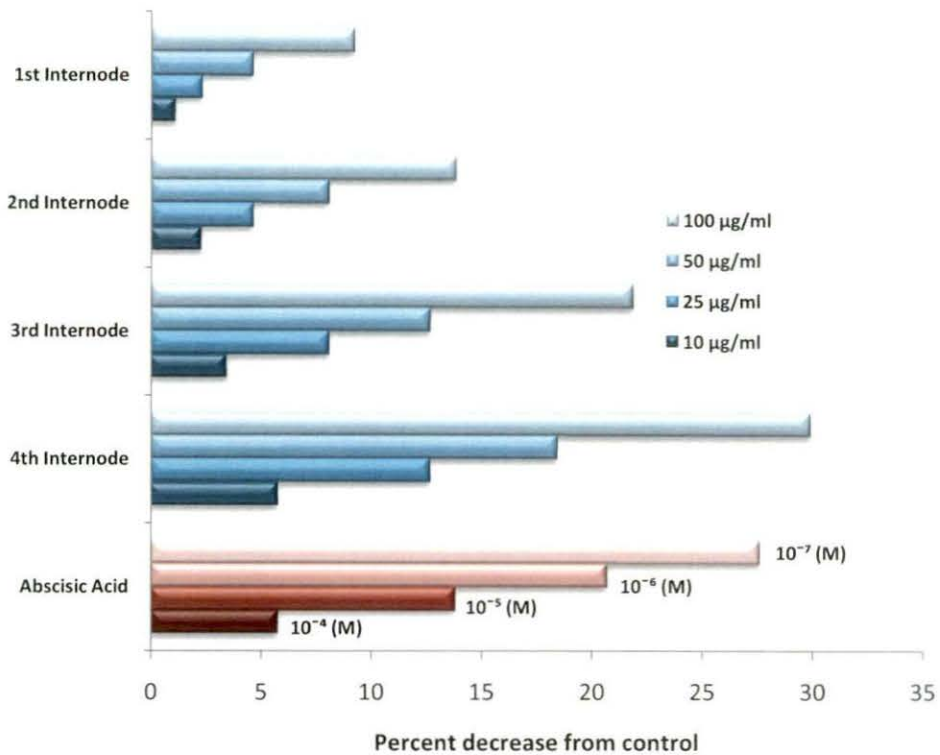


**Figure 7.15** Inhibition of coleoptile elongation by internodal peptide application

(Kutschera *et al.*, 2010). The LC-MS_MS spectrum and corresponding library matching also depicted that the down-regulated peptides isolated from cytosolic fractions of coleoptiles of rye during their cessation phase exhibited high degree of sequence homology with V-type $H^+$-ATPase (Kutschera *et al.*, 2010). The above investigations also suggest that few peptides are up-regulated during coleoptile inhibition but those peptides were not characterized in details. From our experiments it was observed that the coleoptile growth was inhibited by the application of internodal oligopeptides and the gradient of bioactivity was again basipetal in nature. Figure 7.13 indicated the dose dependent inhibition of coleoptile elongation after treatment with peptides from 1st to 4th internode. Also from figure 3.10, it is clear that the dose-dependent inhibition of coleoptile was most prominent with the peptides of 4th internode followed by 3rd, 2nd and 1st internode. Figure 7.14 depicted the percentage increase of original length of coleoptiles from initial with peptide application and here also enhancement was reduced with the increment of applied peptide doses and the increase was least by 4th internodal peptide application followed by 3rd, 2nd and 1st internodal peptides. Figure 7.15 illustrated how much inhibition of coleoptile elongation was happened due to internodal peptide application. Inhibition percentage was most prominent when the applied peptide dose was 100 µg/ml, followed by 50, 25 and 10 µg/ml. Obviously the maximum inhibition was observed from peptides of basal internodes (4th and 3rd), followed by apical internodes (2nd and 1st). Previous reports suggested that the naturally occurring cyclic tetrapeptides like HC toxin and tentoxin, isolated from fungal culture filtrates may inhibit root growth or coleoptile inhibition in susceptible maize hybrids (Walton *et al.*, 1982). Even synthetically derived simple acyclic tri- or tetrapeptides may contain basic structural functionalities and have excellent plant growth regulating properties as evidenced from inhibition of elongation of detached wheat coleoptiles in assay medium containing these peptides (Edwards and Lax, 1988). The peptides isolated from basal internodes of *Vigna radiata* may act as growth regulators by mimicking the functions performed by abscisic acid in plants. As already stated, similar phytotoxic functions may be performed by the released peptides from culture filtrate of fungal plant pathogen but till now the exact mechanism behind this action is obscure.

In conclusion, it can be stated that the peptides isolated from internodes may accelerate senescence by promoting chlorophyll degradation, closing stomatal aperture, enhancing mitotic index of root tip by promoting only prophase and

retarding coleoptile growth. Similar actions were also performed by abscisic acid, when applied at optimal concentrations. Isolated peptides from different internodes execute a clear functional gradient in basipetal order which was more pronounced at higher applied doses. Probably oligopeptides involved in regulation of senescence are accumulated gradually with ageing. During different physiological stages peptides may be accumulated in plant tissues through proteolytic processing of precursors by either proteases or peptidases (Farrokhi *et al.*, 2008). These proteases modify endogenous substrates; specifically produce short peptides which may bear important roles in different cellular processes like signalling, defence and biogenesis (Schaller, 2004). Earlier findings suggest that some extracellular subtilisin proteases are likely to perform degradative function at the time of fruit ripening and internodal senescence. Senescence specific subtilisin-like proteases were also induced in detached wheat leaves incubated in dark for three days (Roberts *et al.*, 2003). In mammalian and bacterial system, these proteases act as pre-protein convertases which are critically involved in the processing of polypeptide precursors (Schaller, 2001). Most probably, these types of proteases, which are expressed during senescence of plant organs may involve in degradation of large polypeptides into shorter bioactive forms. Till now the biological role of oligopeptides generated during senescence are not focused appropriately. Recently several oligopeptide transporters, specifically expressed during senescence are characterized through transcript analysis (van der Graaff *et al.*, 2006) and these transporters are thought to promote nitrogen mobilization during senescence by transporting oligopeptides from senescent region. Besides nutritional role, our experiments suggest that the oligopeptides accumulated in internodes during ageing may also modulate other important functional parameters of senescence, which mimics phytohormone action.

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