RESEARCH ARTICLE

Cross talk among hormones during growth of Mung Bean [*Vigna radiata* (L.) Wilczek] seedlings under water stress

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Abstract

Plant growth, most sensitive to drought stress, showed differential responses when subjected to simulated water stress. Present study was aimed to elucidate the hormonal basis and their possible cross talk involved in differential responses of plants under water stress induced by PEG-6000 solution by using *Vigna radiata* seedlings. Plant hormones like ABA, GA and ethylene (ethrel) and their biosynthesis inhibitors- fluridone, paclobutrazole and cobalt chloride, respectively, were used for such study. Both exogenous ABA (10µM) and mild PEG-induced stress (ψ_w -0.5MPa) promoted root growth, which was not due to suppression of ethylene biosynthesis. On the other hand GA, though inhibited root growth, was associated with shoot (hypocotyl) growth but could not overcome stress-induced shoot growth inhibition. Ethylene appeared to have no role in water stress-induced growth responses either in roots or hypocotyls.

Introduction

Drought is a worldwide problem that limits global crop production and quality more than any other environmental factors (Boyer, 1982; Shao et al., 2009). Recent global climate change has made drought situation more serious. It affects the growth, dry matter and harvestable yield in plants (Anjum et al., 2011). In case of most of the crop species including maize and *Vigna radiata*, the growth of roots and shoots is inhibited during water stress; however, roots continue growing at low water potentials (ψ_w) that are completely inhibitory to shoot growth (Spollen et al., 1993; Das and Kar, 2013). Earlier observation on *Vigna radiata* seedlings showed that water stress simulated by exposing seedlings to PEG 6000 solutions of water potential (-0.5, -1.0 and -1.5MPa) induced growth inhibition except low level of stress (-0.5MPa), which promoted only root growth (Das and Kar, 2013). It has now become important to understand how growth is regulated in response to water deficit in order to find ways to improves productivity of crop plants under marginal conditions. ABA application to well watered plants inhibited both root and shoot growth (Sharp et al., 1994; Sharp, 2002). However, we observed that lower concentrations (less than 100µM) of ABA promote only root growth significantly even under non-stress condition in case of *Vigna radiata* seedlings (Das and Kar, 2013). Foliar application of ABA on *Pisum sativum* plants decreases the adverse effect of water-deficit stress (Latif, 2014). Thus it appears that endogenous ABA accumulation is necessary for maintenance of primary root elongation under water stress condition as was observed in *Zea mays* seedling (Sabb et al., 1990; Sharp et al., 1994). Indeed, low water potential causes significant decrease in IAA and GA concentrations while increased endogenous ABA level in leaves of *Pisum sativum* plants (Latif, 2014). It is possible that root growth promotion under mild water stress (-0.5MPa) may be due to endogenous ABA in *Vigna radiata* seedling (Das and Kar, 2013). Endogenous ABA level can be reduced by treating seedling with carotenoid biosynthesis inhibitor i.e. fluridone or by using ABA deficient mutant (Sabb et al., 1990; Sharp et al., 1994; Sharp, 2002). However,

exogenous ABA can restore root growth of fluridone treated seedling under water stress condition (Sharp, 2002; Das and Kar, 2013). The exact mechanism of ABA that determined differential growth responses between root and shoot under water stress is not well understood till date. Hormones, other than ABA, may also be involved in root growth regulation under water deficit conditions (Ober and Sharp, 2007). The effects of ethylene on plant growth are complex; very low concentrations of ethylene can stimulate growth while higher concentrations inhibit growth (Pierik et al., 2006). In case of deepwater rice ethylene functions as growth promoter by increasing the responsiveness of the intermodal tissue to GA (Benning and Kende, 1992). During maize seedling development at low water potential, ethylene shifts from growth promotion of the mesocotyl to growth inhibition (Sharp, 2002; Sharp and LeNoble, 2002). Gibberellin (GA) apparently does not show any effect under water stress. However, it modulates root growth in a synergistic manner together with ethylene in deep water rice (Steffens et al., 2006). Endogenous ABA function as growth modulator under water stress by keeping ethylene production under control (Spollen at al., 2000). ABA accumulation in root tissues is sufficient to prevent ethylene action thus root growth continues, but insufficient to prevent ethylene action in shoot, thus inhibits growth in shoot at low water potential (Sharp, 2002). Increase in ethylene evolution was also observed in ABA deficient mutant *vp*5 when exposed to water deficit condition (Sharp, 2002). Some additional factors modulate maize leaf growth under water deficit conditions and these factors may be independent of ABA/ethylene interactions (Voisin et al., 2006). GA promotes growth by stimulating the breakdown of DELLA proteins (Ober and Sharp, 2007). It was suggested that ethylene may inhibit growth by stabilizing the activity of DELLA proteins, which restrict root growth (Achard et al., 2006). It is possible that under low water potential ABA accumulation is prevented in shoot, thus ethylene increased and this ethylene may inhibit GA, which then protects DELLAs and inhibition of shoot growth occur (Ober and Sharp, 2007).

In the present study the involvement of different hormones (Ethylene, ABA and GA) and their possible cross talk in regulation of growth of *Vigna radiata* seedlings under well watered as well as water stress (ψ_w - 0.5MPa) condition has been investigated to understand differential growth responses of shoot and root to water stress. Our results clearly show that under normal (non-stress) condition ABA and GA play major role in root and hypocotyl growth, respectively. However, under water stress ABA is mainly responsible for differential growth responses of root and hypocotyl. Ethylene can interact with both GA and ABA in controlling root and hypocotyl growth.

Materials and Methods:

Materials: Seeds of mung bean [*Vigna radiata* (L.) Wilczek var. B1] were collected from Pulses Research Centre, Baharampur, West Bengal, India. 2-Chloroethylphosphonic Acid (Ethrel) equivalent to Ethylene (C_2H_4), Abscisic Acid (ABA), Gibberellic Acid (GA) were purchased from SRL (Sisco Research Laboratories), Mumbai, India. Poly Ethylene Glycol 6000 (PEG 6000), Cobalt Chloride ($CoCl_2$) were from E. Merck, Mumbai, India whereas Fluridone (Flu) and Paclobutrazol (Pac) were from Sigma-Aldrich Chemicals, USA.

Incubation and measurements: Seeds were first surface sterilized by 1% sodium hypochlorite solution followed by twice rinsing in distilled water and finally incubated on the moistened Whatman No. 1 filter paper placed in a 9 cm diameter Petri dish and kept in a Germinator at 30^oC ($\pm 2^\circ C$) for 20 h. After 20 h, the seedlings were transferred into transparent (for light treatment) and black coated (for dark treatment) plastic boxes containing Whatman No. 1 filter paper which was soaked with either distilled water (control) or test solutions under stress or non-stress condition. Boxes were then kept under the same conditions as mentioned above. In case of stress conditions PEG solutions of water potential -0.5 MPa (mild stress) was used to simulate water stress. Water potential was adjusted by using particular concentration of polyethylene glycol (PEG 6000) solution (Michel and Kaufman, 1973). The test solutions consisted of either combination of hormones or their inhibitors or both the hormones and inhibitors. Growth of seedlings was monitored by measuring root (radicle) and shoot (hypocotyl) length at one day intervals up to three days. For root growth studies germinated seeds were maintained under continuous dark condition (in black coated plastic box) while for shoot growth these were kept under continuous light (transparent plastic box) condition.

Treatments: To establish the crosstalk among phytohormones (Ethylene, ABA and GA) in controlling growth under mild water stress (-0.5 MPa) as well as non-stress condition, germinated seeds of *Vigna radiata* were treated with hormones (ABA, 10 µM; ethylene, 50 µM; GA, 100 µM), inhibitors of hormone biosynthesis [fluridone, ABA biosynthesis inhibitor (10 µM); paclobutrazol, a GA biosynthesis inhibitor (40 µM); cobalt chloride ($CoCl_2$), an ethylene biosynthesis inhibitor (10 µM)] and their combinations. For such treatments optimum concentrations of hormones and their inhibitors were selected from range of concentrations tested in non-stress condition.

Statistical analysis: Growth of root and hypocotyl was monitored by measuring length of ten seedlings for each treatment and average data were presented in the figures. Data were statistically analysed by subjecting to ANOVA and probability values were determined for significance. Standard errors (SE) around mean were shown in the figures as error bars.

Results:

ABA-Ethylene interaction under stress and non-stress condition:
Germinated seeds of *Vigna radiata* were treated with ABA, ethylene, fluridone (ABA biosynthesis inhibitor), $CoCl_2$ (Ethylene synthesis inhibitor) and their combinations (Fig. 1) under non-stress and PEG-induced stress conditions. Significant root growth inhibition was observed in ethylene (50 μM) treated seedlings, whereas, in case of ABA (10μM) treatment, root growth promotion was observed under non stress condition (Fig. 1A). Moreover combination treatment of ethylene and ABA showed inhibition of growth though the rate of inhibition was somewhat less compared to those treated with ethylene only. In case of shoot (hypocotyl), all the treatments i.e. ethylene, ABA and their combination, inhibited hypocotyl growth significantly (Fig.1B). Under non-stress condition $CoCl_2$ did not have much effect on root growth while fluridone suppressed root growth (Fig. 1C). Water stress simulated by PEG-treatment caused root growth promotion; however, under water stress condition combination of fluridone and $CoCl_2$ showed severe root growth inhibition (Fig.1C). On the other hand, in case of hypocotyls both $CoCl_2$ and fluridone had least effect on growth (Fig. 1D). PEG-treatment retarded growth considerably and treatment with fluridone and $CoCl_2$ combination under stress could not recover growth over PEG-induced stress (Fig.1D).

GA-ABA interaction under stress and non-stress condition:
Seedlings of *Vigna radiata* treated with GA, ABA, paclobutrazole (GA biosynthesis inhibitor), fluridone and their combinations under non-stress condition showed promotion of root growth by ABA (10μM) while GA inhibited growth; however, their combination also inhibited root growth but the rate of inhibition was somewhat less compared to GA alone under non-stress condition (Fig.2A). On the other hand, shoot growth promotion was found in case of GA treated seedlings and significant inhibition in ABA treated seedlings but their combination showed promotion of shoot growth almost near to the values attained in case of GA treatment alone (Fig.2B). On the other hand, seedlings treated with fluridone, paclobutrazol and their combination under non-stress condition showed root growth inhibition in all cases (Fig.2C). In case of shoot, although fluridone was almost ineffective, paclobutrazol severely inhibited growth and combination with fluridone also showed similar rate of inhibition (Fig.2D). Under mild water stress, GA and fluridone combination showed pronounced inhibition of root growth than their individual treatment (Fig.2E). Similarly shoot growth was also inhibited considerably with combination treatment with GA and fluridone under PEG-induced water stress (Fig.2F).

GA-Ethylene interaction under non-stress condition:
Germinated seeds of *Vigna radiata* were incubated with GA, ethylene (ethrel) and their combination. Results showed an inhibition of root growth both by GA (100 μM) and ethylene (50μM), latter being more effective and combination of GA and ethylene also inhibited root growth at the level of ethylene treatment alone (Fig. 3A). In case of shoot (hypocotyl) again ethylene was inhibitory for growth in contrast to GA and a combination of ethylene with GA also inhibited growth (Fig. 3B)

Fig 1: Role of ethylene (C_2H_4) and ABA and their possible cross talk in seedling growth of *Vigna radiata* under stress and non-stress condition. Germinated seeds were incubated in presence of C_2H_4 (50μM), and ABA (10 μM) and combinations of ABA (10 μM) with C_2H_4 (50μM) under non-stress condition and length (mm) of root (A) and hypocotyl (B) was measured at 1 day intervals. Length of root (C) and hypocotyl (D) was also measured under PEG 6000-induced stress (-0.5 MPa) in presence of $CoCl_2$ (10μM) and fluridone (10μM) combination along with $CoCl_2$, fluridone and PEG independently. SE shown as vertical bars.
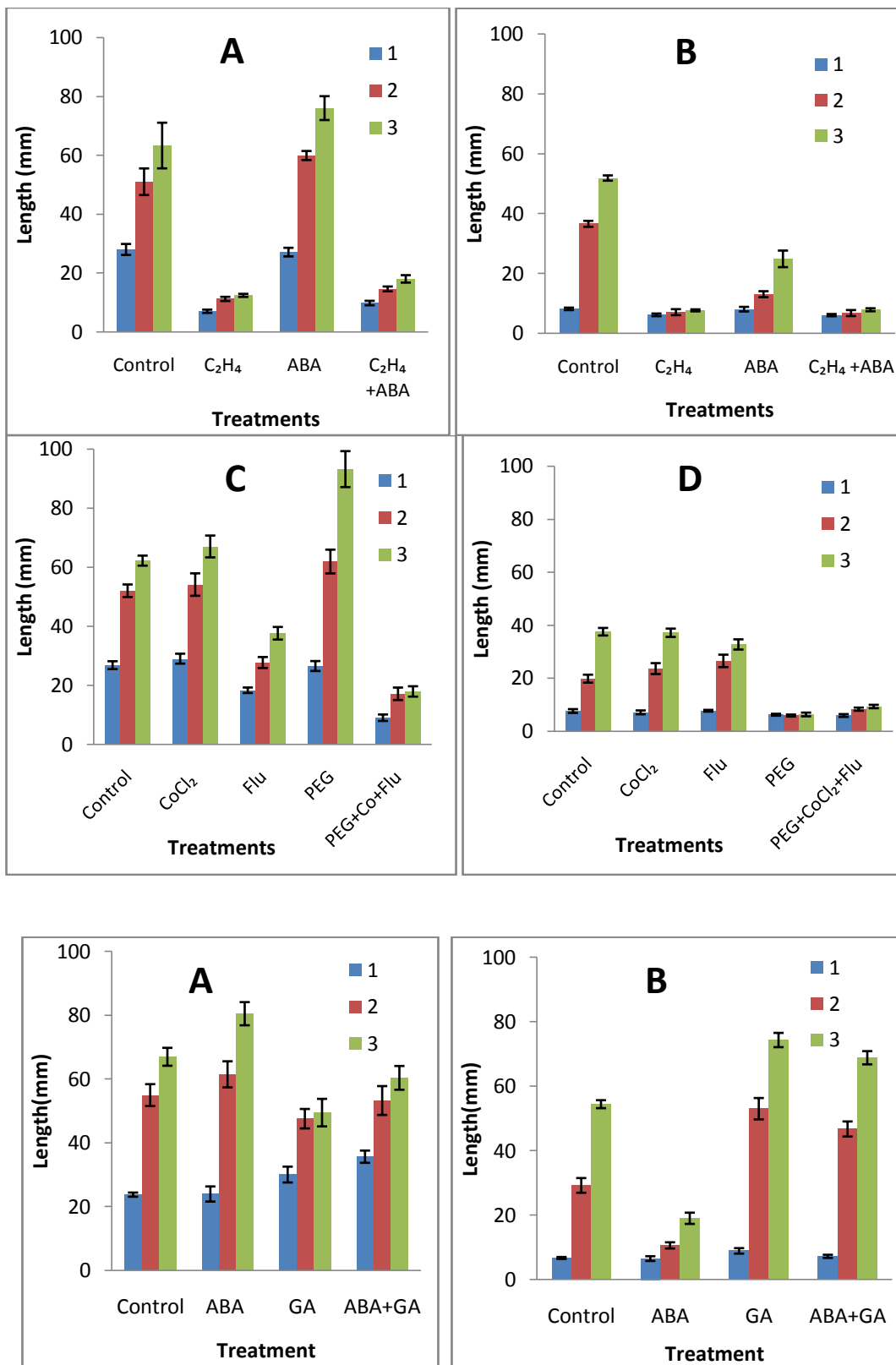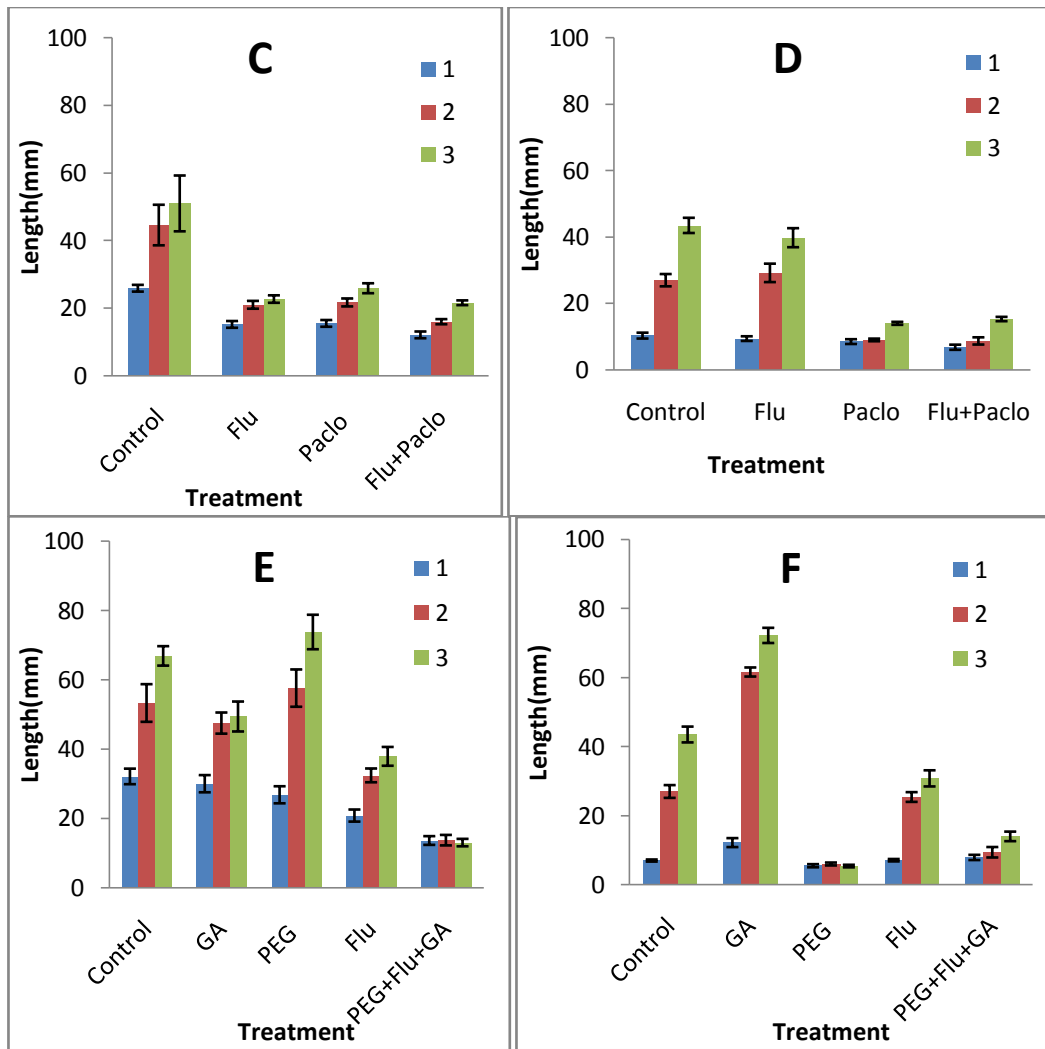
Fig.2: Role of ABA and GA and their possible cross talk in seedling growth of *Vigna radiata* under non-stress and stress condition. Germinated seeds were incubated in presence of GA (100µM) and ABA (10µM) and their combination under non stress condition and length (mm) of root (A) and hypocotyl (B) was measured at 1 day intervals. Length of root (C) and hypocotyl (D) was measured under treatment of fluridone (10µM) and paclobutrazol (40µM) and their combination under non stress condition. Length of root (E) and hypocotyl (F) was also measured under PEG-induced stress (-0.5MPa) in presence of fluridone (10µM) and GA (100µM) combination along with fluridone (10µM), GA (100µM) and PEG independently. SE shown as vertical bars.
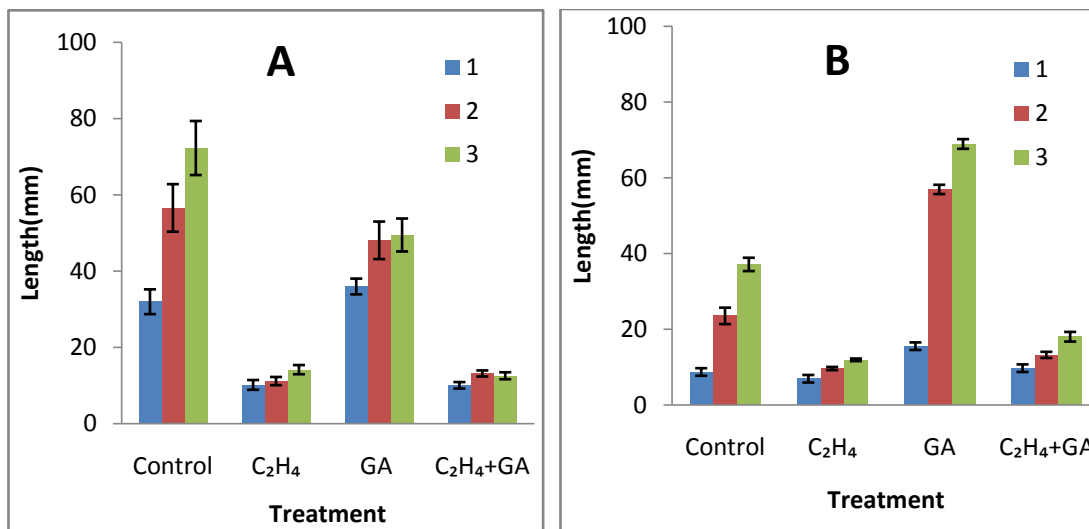
Fig. 3: Role of GA and ethylene (C₂H₄) and their possible cross talk in seedling growth of *Vigna radiata* under non-stress condition. Germinated seeds were incubated in presence of GA (100μM) and C₂H₄ (50μM) and their combination and length of root (A) and hypocotyl (B) length was measured at 1 day intervals. SE shown as vertical bars.

Discussion:

Drought is one of the major abiotic stress factors that limit crop productivity throughout the world (Shao et al., 2009). Among the physiological processes, growth is the most sensitive to drought stress being affected first. In case of a number of crop species including *Vigna radiata* and maize, root and shoot growth is normally inhibited by water stress, although roots may continue growing at low water potentials (ψ_w) that are completely inhibitory to shoot growth (Spollen et al., 1993; Das and Kar, 2013), particularly at mild water stress (ψ_w -0.5MPa), as was found in seedlings of *Vigna radiata* (Das and Kar, 2013). Such shift in balance between root growth and shoot growth is considered as an adaptive response of plants under limiting water supply. Abscisic Acid (ABA), a stress-associated plant hormone, is reported to be mainly responsible for water stress-induced differential responses in maize plants (Sharp et al., 1994). In our previous study with *Vigna radiata* seedlings it was observed that fluridone, an ABA biosynthesis inhibitor, inhibited root growth to a minimum while slightly recovered shoot growth under water stress; conversely, exogenous supply of ABA (10μM) could marginally counteract the inhibitory effect of fluridone on root under water stress (Das and Kar, 2013). Interestingly, exogenous application of ABA to non-stressed seedlings also augmented root growth while the same was retarded by fluridone treatment (Fig. 1A and C) apparently suggesting a natural role of ABA in root growth at least in early seedling (post-germinative) stage. On the other hand, both ABA and fluridone retarded hypocotyl growth indicating a requirement of an optimal level of ABA for shoot growth (Fig. 1B and C). Saab et al. (1990) have also demonstrated promotion of both shoot and root growth by endogenous ABA using ABA-deficient maize plants. This was explained as due to ABA-induced suppression of synthesis of ethylene, which could otherwise inhibited growth. Alternatively, growth inhibition may result from uncontrolled water loss by deregulated stomata in ABA-deficient mutants (Sharp 2002). However, the latter possibility can be ruled out in the present study as seedlings from early developmental stage, used for growth experiments, were devoid of effective stomata. Moreover, water stress-induced or ABA-induced (non-stress) root growth does not appear to be due to suppression of ethylene synthesis by ABA, since treatment with $CoCl_2$, ethylene synthesis inhibitor, was not much effective in promoting root growth during either in non-stress condition or PEG-induced water stress (earlier observation, Das and Kar, 2013); rather ABA synthesis is essential under stress, as fluridone suppress growth even though ethylene synthesis was blocked by $CoCl_2$ in stressed seedlings (Fig. 1C). However, exogenous ethylene application overrides the ABA-induced promotive effect on root growth of non-stressed seedlings (Fig. 1A). In case of shoot growth both ABA and ethylene are inhibitory (Fig. 1B) and water stress-induced growth inhibition involves

some other factors, since a combination of $CoCl_2$ and fluridone treatment could not recover growth under PEG-induced water stress (Fig. 1D). Some additional factors independent of ABA/ethylene interaction have also been reported to be involved in modulating maize leaf growth under water deficit conditions (Voisin et al., 2006) supporting the notion that ABA and ethylene cross talk is not only responsible for stress induced differential responses in *Vigna radiata* seedlings.

Gibberellins (GA) are well recognised as growth promoter and GA application enhances both shoot and root elongation (Fu and Harberd, 2003). However, in case of *Vigna radiata* seedlings exogenous GA application inhibited root growth although treatment with paclobutrazole, GA synthesis inhibitor, also inhibited root growth (Fig. 2A and C). This may be indicative of requirement of an optimal low level of GA for root growth and any additional amount of GA becomes supra-optimal inhibiting growth. In case of shoots, however, GA was found to be essential for growth while ABA was inhibitory. Regarding ABA-GA interaction, under non-stress condition ABA significantly recovered root growth by suppressing inhibitory effect of GA while in case of shoot GA recovered growth considerably by suppressing the inhibitory effect of ABA (Fig. 2A and B) leading to the inference that ABA counteracts the effect of GA in case of root and GA counteracts the effect of ABA in shoot under non-stress condition. Experiments on combined treatment of fluridone and paclobutrazol also support the view that ABA and possibly an optimal level of GA induce root growth whereas GA is predominantly involved in shoot growth. Under PEG-induced stress enhancement of root growth is dependent on endogenous synthesis of ABA while GA is still inhibitory as revealed from combined treatment with fluridone and GA with PEG solutions (Fig. 2E). On the other hand, in case of shoot (hypocotyl) PEG-induced growth inhibition could not be overcome even by blocking ABA and adding exogenous GA together (Fig. 2F) corroborating our earlier statement that some additional factor is also involved in water stress-induced inhibition of shoot growth.

Regarding ethylene-GA interaction for controlling seedling growth, ethylene possibly restricts GA by stabilizing DELLA proteins at least at high concentration leading to growth inhibition, though low concentration of ethylene may allow GA to breakdown DELLA proteins thus promoting growth (Ober and Sharp, 2007). In the present experiment with *Vigna radiata* seedlings data supports the above notion only in case of shoot where GA can somewhat recover growth from ethylene induced inhibition under non-stress condition (Fig.3B). However, in case of root no such recovery was apparent because both the hormones inhibited root growth when applied exogenously (Fig.3A). The reason for root growth inhibition by exogenous GA is not very clear and required further research to reveal the exact mechanism.

Conclusions:
Finally, it may be concluded that endogenous ABA is mainly responsible for normal root growth as well as water stress-induced root growth promotion and partially responsible for shoot growth inhibition due to water stress. On the other hand, although shoot growth is mainly controlled by GA under non-stress condition, stress-induced shoot growth inhibition involves ABA and some other factors. Although ethylene interacts with ABA and GA in controlling root growth and hypocotyl growth under non-stress condition, it appeared to have no role in water stress-induced growth responses either in roots or hypocotyls. Therefore the role of hormones in modulating seedling growth under non stress condition is somehow different from that in water stress grown seedling.

Acknowledgment:

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