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Effect of Ethylene Synthesis and Perception Inhibitor and ABA on Seed Germination of *Vigna radiata*

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Abstract: In the present investigation the effect of cobalt chloride ($CoCl_2$), ethylene biosynthesis inhibitor and silver nitrate ($AgNO_3$), ethylene perception inhibitor was studied on the seed germination of *Vigna radiata*. While ethylene slightly promoted germination, both the inhibitors decreased the germination percentage. However, Ag^{2+} was more effective in retarding germination than Co^{2+}. A combined treatment with silver nitrate and cobalt chloride overcame inhibition with reference to silver nitrate treatment,whereas it was almost without effect compared to the treatment with cobalt chloride alone. Ethylene treatment could somewhat rescue germination from inhibition by Ag^{2+} but was ineffective in case of Co^{2+}. Abscisic acid (ABA) caused a lower germination percentage at a very high concentration. Ethylene treatment following ABA treatment showed rescue effect to some extent on germination of *Vigna radiata* seeds.

Key words: ABA · Ethylene perception inhibitor · Ethylene synthesis inhibitor · Germination · *Vigna radiata*

INTRODUCTION

Germination and seedling growth are complex physiological processes under the control of plant hormones that play important and manifold roles [1]. Among the plant hormones ethylene is regarded as one of the key regulators in the process of seed germination apart from its role in seed development [2, 3]. Ethylene has been implicated in the seed germination of many species based on the evidences either with ethylene-insensitive mutants [4, 5] or using ethylene inhibitors [2, 6]. However, most studies with ethylene synthesis and perception inhibitors have not demonstrated a clear requirement for ethylene production for initiation of radicle protrusion [2, 3, 6]. In fact, far less is known about the molecular basis of ethylene perception and biosynthesis in germinating seeds [7]. Besides, strong interaction between ethylene and ABA suggests that ethylene may promote germination directly by interfering with ABA signaling [8, 9]. In the present study, non-dormant seeds of mungbean (*Vigna radiata*) were tested for germination under the treatment of ethylene synthesis and perception inhibitors and also for interaction between ethylene and ABA in this regard.

MATERIALS AND METHODS

Seeds of mung bean [*Vigna radiata* (L.) Wilczek var B1], collected from Pulses and Oilseeds Research Station, Berhampur, Murshidabad, West Bengal, India, were used as experimental material. Seeds were first sterilized in sodium hypochlorite solution, rinsed in distilled water several times and incubated in 9 cm petridishes on Whatman no.1 filter paper moistened with distilled water or test solutions under controlled temperature (30°C) and darkness in a seed germinator. Among the test solutions, ethrel (0.01, 0.05 and 0.1mM), commercial preparation of ethylene, was used as ethylene treatment. On the other hand, cobalt chloride ($CoCl_2$; 1, 10 and 50mM or 50mM only) was used as ethylene synthesis inhibitor and silver nitrate ($AgNO_3$; 0.5, 1 and 5mM or 5mM only) as ethylene perception inhibitor. Abscisic acid (ABA) was used at 0.001, 0.01, 0.1 and 1mM concentrations for germination inhibition study. In case of all test solutions for treatment, pH was adjusted to neutrality using 1N NaOH or 1N HCl. At intervals, germination counts were taken out of 100 seeds (germination percentage) in triplicate for each treatment. In case of interaction experiments, seeds were first pretreated with ABA (1mM) for 8, 10 and 12 h followed by

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transfer to ethrel solution (0.1mM) and germination percentage was recorded at the end of 12, 24 and 48 h of incubation. Average values were presented in the form of figures showing the standard errors around means as vertical bars.

RESULTS

Effect of ethylene of different concentrations (0.01, 0.05 and 0.1mM) on germination percentage of mung bean seeds (*V. radiata*) during incubation at 30ºC in darkness has been depicted in the Fig. 1. It shows that ethylene of all concentrations enhanced germination at early stage of incubation. However, at the end of 24 h incubation germination percentage almost reached to the maximum value (90-95%) along with the control seeds (incubated in distilled water).

Fig.2 includes data on the effect of cobalt chloride (1, 10 and 50mM) and silver nitrate (0.5, 1 and 5mM), inhibitors of ethylene biosynthesis and perception, respectively on germination percentage. Germination percentage was retarded at 10mM and 50mM cobalt chloride proportionately, while 1mM concentration of the same was ineffective (Fig. 2A). However, after 24 h of incubation germination percentage of seeds at all concentrations of cobalt chloride reached to the level of control. On the other hand, silver nitrate solutions of 0.5 and 1mM concentrations were almost effect less, while 5mM reduced the germination percentage remarkably (Fig. 2B).

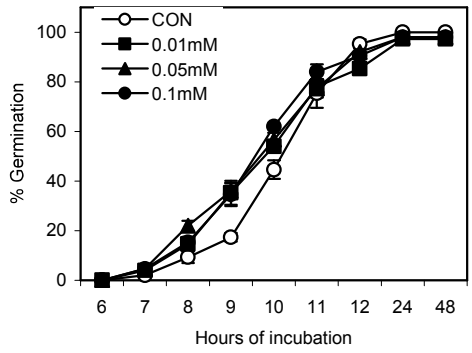

Fig. 1: Effect of different concentrations ethylene (0.01, 0.05, 0.1mM) on the percentage germination of Vigna radiata seeds. SE indicated as vertical bars.

In recovery experiments, seeds of mung bean were treated in combination of silver nitrate (5mM) and cobalt chloride (50mM) and ethylene (0.05mM) and germination at intervals was monitored (Fig. 3A and B). Combined treatment with silver nitrate and cobalt chloride could not overcome the germination inhibition by cobalt chloride alone (Fig. 3A). Also, no significant change was noticed in combined treatment of ethylene and cobalt chloride in comparison to cobalt chloride treatment alone. However, germination percentage was high in case of combined treatment of silver nitrate and cobalt chloride compared to silver nitrate alone and the recovery effect was more pronounced at later hours (Fig. 3B). Similarly, combined treatment of silver nitrate and ethylene showed an improvement of germination over the percentage under treatment with silver nitrate alone.

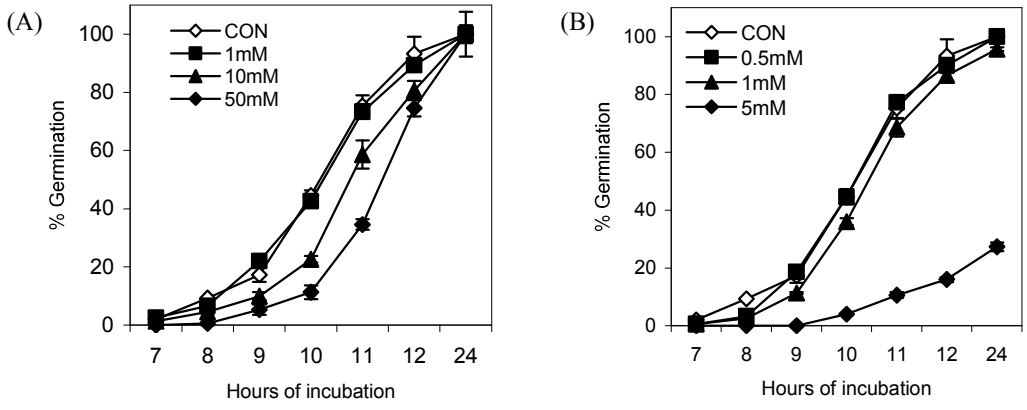

Fig. 2: Effect of different concentrations of (A) cobalt chloride (1, 10, 50mM) and (B) silver nitrate (0.5, 1, 5mM) on the germination percentage of V.radiata seeds. SE indicated as vertical bars

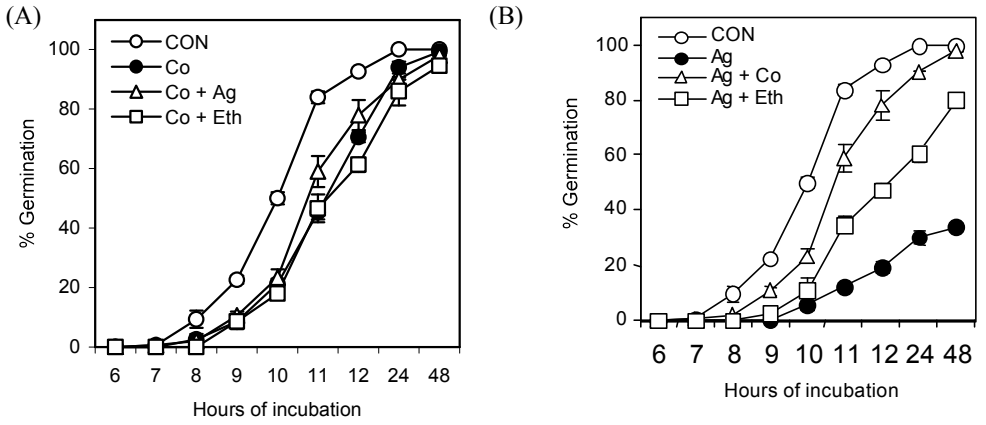

Fig. 3: Effect of combined treatment of (A) cobalt chloride (50mM) + silver nitrate (5mM) and cobalt chloride (50mM) + Ethrel (0.05mM) compared to cobalt chloride (50mM) alone and (B) silver nitrate (5mM) + cobalt chloride (50mM) and silver nitrate (5mM) + Ethrel (0.05mM) compared to silver nitrate (5mM) alone on the germination percentage of V.radiata seeds. SE indicated as vertical bars

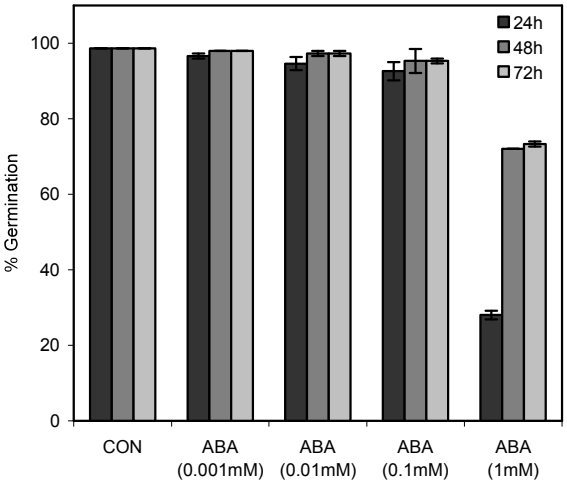

Fig. 4: Effect of different concentrations of ABA (0.001, 0.01, 0.1, 1mM) on the germination percentage of V.radiata seeds. SE indicated as vertical bars.

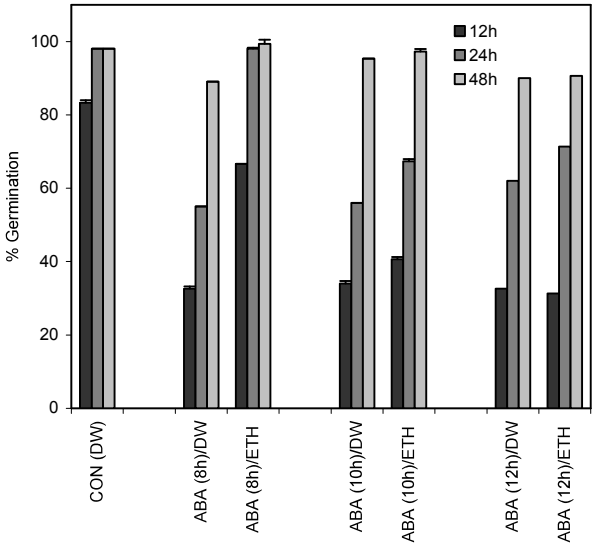

Fig. 5: Effect of ETH (0.1mM) following ABA (1mM) pretreatment for different hours (8, 10 and 12 h) on the germination percentage of V.radiata seeds. SE indicated as vertical bars

Fig. 4 shows the effect of abscisic acid (ABA) on the germination percentage of seeds of *Vigna radiata.* Seeds were treated with ABA of 0.001, 0.01 and 0.1mM showed no inhibition compared to the control set. However, germination was reduced compared to control only at 1mM ABA and this inhibitory effect was somewhat ameliorated after 48 h and 72 h of incubation.

Fig. 5 shows the effect of ABA pretreatment for different hours (8, 10, 12 h) followed by distilled water (DW) or ethrel (0.1mM). In case of ABA pretreated seeds for 8 h, ethrel treatment could recover the inhibition imposed on germination and this recovery could be clearly noticed from 12 h through 48 h of incubation compared to control (DW treated seeds). Recovery was not so much significant in case of 10 h and 12 h ABA pretreated seeds followed by ethrel treatment with reference to control.

DISCUSSION

It is now established in several species that ethylene plays an important role in germination [10, 11]. In the present investigation, seeds of *Vigna radiata* showed a

marginal improvement by ethylene over control only during early hours of incubation. The reason may be that the system is already producing required amount ethylene and thus a little added effect was noted with exogenous ethylene treatment. As a negative approach, treatment with ethylene inhibitors (for synthesis and perception) were rather tested to verify the role of ethylene in germination, though inhibitor studies require careful interpretation, since any given inhibitor may be specific or may be non-specific or may not complete its intended action. First we tried with cobalt chloride (Co^{2+} ions) that inhibits ACC oxidase thus blocking ethylene synthesis [12]. But such treatment was not much effective in inhibiting germination as was also observed by Lalonde and Saini [13]. On the other hand, silver nitrate (5mM) inhibited germination significantly. Silver ions are potent, specific, non-competitive inhibitors of ethylene binding [14]. There are also reports in literature that ethylene synthesis inhibitors like AVG and Co^{2+} ions failed to inhibit seed germination [15, 16] while ethylene perception inhibitor could inhibit germination[17, 18]. Apparently both ethylene synthesis and perception inhibitors should give essentially the same results. It may be that cobalt ions could not block ethylene biosynthesis completely and as a result the amount of ethylene still synthesized in presence of inhibitor might be sufficient to stimulate germination.

Interestingly, ethylene treatment along with cobalt chloride could not overcome the inhibition shown by cobalt chloride alone. However, a significant finding was that cobalt ions would overcome the inhibitory effects of silver ions on germination and growth. A similar situation was encountered in case of barley seed germination where Ag^{2+} effectively inhibited germination while Co^{2+} was effect less and Co^{2+} could overcome Ag^{2+} inhibition [6]. It was proposed that polyamines formed as a result of blockade of both ethylene synthesis and perception (combined treatment of Co^{2+} and Ag^{2+}) complemented the role of ethylene on germination [6]. However, it is difficult to explain ethylene recovery from inhibition by ethylene perception inhibitor (Ag^{2+}). Others also observed such recovery of germination by ethylene from inhibition by ethylene perception inhibitors [13, 19]. A complex positive feed back loop for ethylene biosynthesis might be operative in controlling germination [7].

ABA, another important phytohormone regulates various aspects of germination and is reported to inhibit ethylene production and germination in chickpea seeds [20]. In the present investigation with mung bean seeds ABA could inhibit germination only at a very high concentration (1 μM) While far lower concentration (1μM) has been reported to exert a complete inhibition of germination [21]. The reason may be that either ABA can not reach fully to the active site or the system used is not very sensitive to ABA. In case of seeds pretreated with ABA, ethylene could recover marginally germination only when duration of ABA pretreatment was not long enough (up to 10 h). Such recovery may be attributed to an interaction between ethylene and ABA signal transduction pathways and ethylene can promote germination by directly interfering with the ABA signaling pathway [6, 21].

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REFERENCES

1. Bewley, J.D., 1997. Seed germination and dormancy. Plant Cell, 9: 1055-1066.
2. Gallardo, M., M.E. Gallardo, A.J. Matilla, P.M. De Rueda and I.M. Sánchez-Calle, 1994. Inhibition of polyamine synthesis by cyclohexylamine stimulates the ethylene pathway and accelerates the germination of *Cicer arietinum* seeds. Physiologia Plantarum, 91: 9-16.
3. Matilla, A.J., 2000. Ethylene in seed formation and germination. Seed Science Research, 10: 111-126.
4. Johnson, P.R. and J.R. Ecker, 1998. The ethylene gas signal transduction pathway: a molecular perspective. Annual Rev. Genetics, 32: 227-254.
5. Siriwitayawan, G., R.L. Geneve and A.B. Downie, 2003. Seed germination of ethylene perception mutants of tomato and Arabidopsis. Seed Science Research, 13: 303-314.
6. Locke, J.M., J.H. Bryce and P.C. Morris, 2000. Contrasting effects of ethylene perception and biosynthesis inhibitors on germination and seedling growth of barley (*Hordeum vulgare* L.). Journal of Experimental Botany, 51: 1843-1849.
7. Petruzzelli, L., I. Coraggio and G. Leubner-Metzger, 2000. Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclo-propane-1-carboxylic acid oxidase. Planta, 211: 144-149.

8. Beaudoin, N., C. Serizet, F. Gosti and J. Giraudat, 2000. Interactions between abscisic acid and ethylene signaling cascades. The Plant Cell, 12: 1103-1115.
9. Kucera, B., M.A. Cohn and G. Leubner-Metzger, 2005. Plant hormone interactions during seed dormancy release and germination. Seed Science Research, 15: 281-307.
10. Kepczynski, J. and E. Kepczynska, 1997. Ethylene in seed dormancy and germination. Physiologia Plantarum, 101: 720-726.
11. Dunlap, J.R. and P.W. Morgan, 1997. Reversal of induced dormancy in lettuce by Ethylene, Kinetin and Gibberellic acid. Plant Physiol., 60: 222-224.
12. Lau, O.L. and S.F. Yang, 1976. Inhibition of ethylene production by cobaltous ion. Plant Physiology, 58: 114-117.
13. Lalonde, S. and H.S. Saini, 1992. Comparative Requirement for endogenous Ethylene during Seed Germination. Annals of Botany, 69: 423-428.
14. Beyer, E.M., 1976. A potent inhibitor of ethylene action in plants. Plant Physiology, 58: 268-271.
15. Kepczynski, J. and C.M. Karssen, 1985. Requirement for the action of endogenous ethylene during germination of non-dormant seeds of *Amaranthus caudatus*. Physiologia Plantarum, 63: 49-52.
16. Pertuzzelli, L., F. Harren, E. Perrone and J. Reuss, 1995. On the role of ethylene in seed germination and early of *Pisum sativum*. Journal of Plant Physiology, 145: 83-86.
17. Sisler, E.C. and A. Pian, 1973. Effect of ethylene and cyclic olefins on tobacco leaves. Tobacco Science, 17: 68-72.
18. Reid, M.S., 1995. Ethylene in plant growth, development and senescence. In Plant Hormones: Physiology, Biochemistry and Molecular Biology. Davis, P.J. (Ed.). Kluwer Academic, Dordrecht, pp: 486-508.
19. Abeles, F.B., 1986. Role of Ethylene in *Lactuca sativa* cv 'Grand Rapids' seed germination. Plant Physiology, 81: 780-787.
20. Gallardo, M., M.M. Delgado, I.M. Sánchez-Calle and A. Matilla, 1991. Ethylene production and 1-amino-cyclopropane-1-carboxylic acid conjugation in thermo inhibited *Cicer arietinum* seeds. Plant Physiology, 97: 122-127.
21. Ghassemian, M., E. Nambara, S. Cutler, H. Kawaide, Y. Kamiya and P. McCourt, 2000. Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell, 12: 1117-1126.