Journal of Plant Biochemistry & Physiology

Chaudhuri et al., J Plant Biochem Physiol 2013, 1:1
Doi: 10.4172/jpbp.1000103

Research Article Open Access

# Interaction of Hormones with Reactive Oxygen Species in Regulating Seed Germination of *Vigna radiata* (L.) Wilczek

**Chaudhuri A, Singh KL and Kar RK***

*Plant Physiology & Biochemistry Laboratory, Department of Botany, Visva-Bharati University Santiniketan- 731235, West Bengal, India*

## Abstract

Regulation of seed germination is quite complex and is further complicated by interaction of hormones like gibberellin (GA), abscisic acid (ABA) and ethylene. Moreover, the involvement of reactive oxygen species (ROS) in hormone signaling for such regulation is still less understood. The aim of the present study was to explore the interactive role of GA, ABA and ethylene and possible involvement of ROS in mediation of hormone action during seed germination of *Vigna radiata*. Seeds of *V. radiata* were germinated in presence of hormones, their biosynthesis or action inhibitors and in combinations with hydrogen peroxide and at intervals germination percentages were determined. Treated seeds were also tested for production of superoxide by NBT staining and analysed for NADPH oxidase (NOX) activity by in-gel assay. ABA and paclobutrazole (GA biosynthesis inhibitor) inhibited germination of non-dormant *V. radiata* seeds. GA recovered germination from inhibition by Ag+ (ethylene action inhibitor) whereas paclobutrazole-induced inhibition could not be recovered by ethylene. But ethylene could recover significantly ABA-inhibited germination. Treatment with $H_2O_2$ rescued germination from inhibition by ABA, paclobutrazole and Ag+ with efficiency in the order ABA> Ag+>paclobutrazole. Superoxide ($O_2$·-) production, as revealed by NBT staining, was found mostly in the apical part of the axis in control and ethylene treated seeds and to a less extent in GA treated seeds while almost no stain was found in case of treatments with ABA, Ag+ and paclobutrazole. In-gel assay of NOX activity showed three bands in control and in case of treatments with ethylene, GA and fluridone while intensity became less for one or more bands in case of paclobutrazole, ABA and Ag+ treatment. It appears that ethylene and ABA is antagonistic to each other while GA is partially independent in regulating germination of *Vigna radiata* seeds through mediation of ROS.

**Keywords:** ABA; Ethylene; GA; NOX; Paclobutrazole; ROS; Seed germination; *Vigna radiata*

## Introduction

Seed germination, an early developmental event, starts with imbibitional water uptake and culminates into radicle protrusion [1-3]. Seed germination involves activation of embryonic growth following hydration. Underlying this activation is a metabolic upregulation that includes some subtle intangible regulatory components like reactive oxygen species (ROS) which are connected with messengers like hormones through complex signaling chains [4]. Among phytohormones, GA and ABA are well known for their antagonistic action for seed dormancy and germination. They play a contrasting role - GA breaks dormancy and promotes germination while ABA maintains dormancy and inhibits germination. Role of GA and ABA has been firmly established particularly by studying through mutational analysis [5]. Thus seeds of GA-deficient mutants do not germinate in absence of exogenous GAs and also germination can be prevented by GA biosynthesis inhibitors. On the other hand, seeds of an ABA-deficient mutant can germinate even in absence of GAs [5]. However, the exact mode of action of these hormones at molecular level in the process of germination (radicle emergence) is not clear. Ethylene, another plant hormone involved in the control of growth and developmental processes, has also been reported to break dormancy and promote germination of seeds in a number of species [2,6,7]. Ethylene mimics the action of GAs since seeds of GA deficient mutant germinate upon application of ethylene [8]. Also, ethylene mutants show phenotypes that resemble ABA action as these mutants do not germinate well and are hypersensitive to ABA [9]. However, the exact mechanism of ethylene action in promoting germination is not established, although an ethylene-ABA interaction has been demonstrated in controlling germination [10].

Recently, reactive oxygen species (ROS) have been demonstrated to play roles in growth and development either directly by participating in the process of cellular growth or differentiation or indirectly by signaling for induction of processes or reactions related to growth and differentiation. Seeds are reported to generate ROS either in dry condition or even when imbibed [11] and ROS generation has been associated with a positive role in the process of germination [12-14]. Attempts have been made to integrate phytohormones and ROS for their interaction either through cross talk in signaling or influencing metabolism in regulating germination [10,15,16]. However, the results are variable leading to no clear cut order of their position in the cascade of action. Present study is aimed to explore the interactive role of GA, ABA and ethylene and possible involvement of ROS in mediation of hormone action during seed germination of *Vigna radiata*.

## 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 Petri dishes on Whatman no.1 filter

***Corresponding author:** Rup Kumar Kar, Plant Physiology & Biochemistry Laboratory, Department of Botany, Visva-Bharati University, Santiniketan 731235, West Bengal, India, Tel: 91-3463-262752(382); E-mail: rupkumar.kar@visva-bharati.ac.in

**Received** January 21, 2013; **Accepted** February 13, 2013; **Published** February 20, 2013

**Citation:** Chaudhuri A, Singh KL, Kar RK (2013) Interaction of Hormones with Reactive Oxygen Species in Regulating Seed Germination of *Vigna radiata* (L.) Wilczek. J Plant Biochem Physiol 1: 103. doi:10.4172/jpbp.1000103

paper moistened with 2.5 $cm^3$ distilled water or test solutions under controlled temperature (30 ± 2°C) and darkness in a seed germinator.

To establish the roles of phytohormones, seeds of *V. radiata* were directly treated with various concentrations of Gibberellic acid (GA; 0.01, 0.1 and 1 mM), Abscisic Acid (ABA; 0.01, 0.1 and 1 mM) and paclobutrazole (PAC; 0.01, 0.1 and 1 mM), a GA biosynthesis inhibitor and incubated as mentioned earlier. Germination percentage was monitored at hourly intervals after 6 h of incubation.

For any possible interaction among hormones seeds were subjected to combined treatments and assessed for recovery of germination. Combinations used were GA (1 mM) with $AgNO_3$ (5 mM), ethrel (0.05 mM) with PAC (1 mM) and ABA (1 mM) with ethrel (0.05 mM). Similarly, for a possible interaction between ROS and hormones, seeds were treated with combination of $H_2O_2$ (10 mM) with ABA (1 mM), with paclobutrazole (1 mM) and with $AgNO_3$ (5 mM) for any rescue from inhibition.

Superoxide generation by axes during germination under the effect of different treatments was detected by histochemical staining according to a modified method of Jabs et al. [17]. Whole seeds were either incubated in distilled water or treatments like Ethrel (0.05 mM), $AgNO_3$ (5 mM), GA (mM), paclobutrazole (mM) and ABA (mM) for 12 h followed by incubation in 0.5 mM nitroblue tetrazolium (NBT) in 50 mM phosphate buffer (pH 6.8) for 20 min and observed for intensity of staining. NOX activity in axes of germinating seeds under different treatments was determined by an in-gel assay method. In this assay seeds of *Vigna radiata* were incubated in DW or treatment solutions for 12 h. The enzyme was then extracted from embryonic axes isolated from seeds and the samples were run on non-denaturing polyacrylamide gels to detect NOX activity following the method described in Carter et al. [18]. The activity was graded by studying the band width and band intensity.

Each experiment was conducted with three replicates and the mean values were presented. Germination percentage was calculated by counting germinated seeds (radicle emerged 2 mm) out of 100 seeds for each set. The data were expressed in figures and the standard errors (SE) of the means were calculated [19] and plotted in the figures as vertical bars.

## Results

Germination percentages of seeds of *V. radiata* incubated at different concentrations of GA (0.01, 0.1 and 1 mM), ABA (0.01, 0.1 and 1 mM) and paclobutrazole (0.01, 0.1 and 1 mM) for different durations along with a control have been shown in figure 1. In case of treatments with GA, germination percentage was found to be almost similar to control at all concentrations of GA (Figure 1A), whereas in case of seeds treated with different concentrations of ABA significant inhibition (reaching maximum of 40%) was found only at 1 mM, lower concentrations (0.01 and 0.1 mM) being almost ineffective (Figure 1B). Paclobutrazole was also effective in preventing germination almost completely only at high concentration (1 mM) while lower concentrations (0.01 and 0.1 mM) could not alter much the germination percentage (Figure 1C).

Figure 2 is related to the hormonal interaction in regulating germination. To study the effect of GA in absence of ethylene action, seeds treated with a combination of $AgNO_3$ (5 mM) and GA (1 mM) showed a good recovery of germination as the germination percentage in case of combined treatment reached very near to control (almost 90%) compared to $Ag^+$-treated seeds (about 25%) after 24 h (Figure 2A). Conversely, for the action of ethylene in absence of GA biosynthesis seeds were treated with a combination of paclobutrazole (1 mM) and Ethrel (0.05 mM). Ethrel could marginally improve germination only after 24 h of incubation (Figure 2B). To study ethylene-ABA interaction during germination, seeds were incubated in a mixture of ABA (1 mM) and Ethrel (0.05 mM) along with ABA and Ethrel treatments alone and control (DW). In the combined treatment germination percentage increased over ABA alone from the beginning (near control value) and reached almost 90% after 24 h of incubation (Figure 2C).

To study the interaction of ROS with ABA, ethylene and GA, seeds treated with ABA (1 mM), $AgNO_3$ (5 mM) and PAC (1 mM) were supplemented in all cases with $H_2O_2$ (10 mM) (Figure 3). Results showed that germination percentage of the seeds treated with the combination of ABA and $H_2O_2$ was recovered almost fully reaching almost to the level of control (Figure 3A). On the other hand, a significant recovery of germination was noted by combining $AgNO_3$ with $H_2O_2$ (Figure 3B) and paclobutrazole with $H_2O_2$ (Figure 3C) compared to $AgNO_3$ and PAC alone. Thus germination percentage reached nearly 80% in case of former combination while it reached up to 60% in the latter.

Results of NBT staining for localization of superoxide producing tissues of *V. radiata* seeds subjected to treatments with ethylene (0.05 mM), $AgNO_3$ (5 mM), GA (1 mM), paclobutrazole (1 mM) and ABA (1 mM) for 12 h along with a control have been depicted in figure 4. Seeds treated with ethylene and GA as well as control seeds showed stain of differential intensity in the apical region and part of the subapical region of the germinated axis. On the other hand, seeds treated with $AgNO_3$, paclobutrazole and ABA remained unstained for any portion and axes also did not grow.

Figure 5 shows the photographs of in-gel assay for NOX activity in axes from *V. radiata* seeds treated with different hormones and their inhibitors. Three NBT-sensitive bands of almost equal intensity were found in the control set (Figure 5A). Axes from seeds treated with fluridone, ABA biosynthesis inhibitor and somewhat axes from GA-treated seeds also showed a similar NOX profile. However, in case of paclobutrazole treatment the upper band was of less intensity, while in case of axes from ABA-treated seeds all three bands became faint (Figure 5A). On the other hand, axes from ethylene-treated seeds showed a NOX profile similar to control, whereas bands almost disappeared in case of $Ag^+$ treatment.

## Discussion

Phytohormones like GA and ABA and also ethylene play a major role in regulating the processes of dormancy release and germination [2,20]. Seed dormancy release and germination of seeds having coat dormancy result from the increased growth potential of the embryo that overcomes the constraint exerted by the covering layers like testa and endosperm [20]. ABA is a positive regulator of dormancy induction and its maintenance, while it is a negative regulator of germination. On the other hand, GA releases dormancy, promotes germination and counteracts ABA effects. Seeds of *Vigna radiata* are non-dormant having no residual endosperm and germination percentage started to increase upon imbibition after a lag of 6 h reaching maximum (nearly 100%) at about 12 h of incubation. Exogenous application of GA was hardly effective in promoting germination further over control (Figure 1A). It can be assumed that endogenous GA biosynthesis in the radicle is sufficient enough to cause axis elongation associated with germination. On the contrary, exogenous application of ABA inhibited germination only at a very high concentration (1 mM) (Figure 1B) indicating a low sensitivity of seeds of this species (or variety) to ABA. Role of endogenous GA in germination of *V. radiata* seeds was further

proved by the experiment with paclobutrazole, a GA biosynthesis inhibitor that showed a marked decrease in percentage of germination particularly at high concentration (1 mM) (Figure 1C). Besides GA and ABA, ethylene has also important role in seed germination what we have already shown using ethylene action inhibitor, $Ag^+$ ($AgNO_3$) in case of *V. radiata* seeds [7]. Therefore, the role of these three hormones in controlling seed germination has been re-established in a model species, *Vigna radiata*.

Phenotypic analysis of mutants on hormone biosynthesis and action suggests a cross talk resulted from influence on synthesis and sharing of signalling components for control of most physiological and developmental processes [21]. Germination is a complex response expected to have converging signal inputs from several hormones. Indeed, antagonistic role of GA and ABA based on cross talk in signalling for seed germination is well established [22]. However, little was known about the interaction or cross talk of ethylene with GA and ABA until recently [10,20]. To study ethylene-GA interaction, $AgNO_3$ was used to stop ethylene action and exogenous GA was applied. It was observed, that GA could overcome the inhibitory effect of $Ag^+$ after a short lag period (Figure 2A). On the contrary, the inhibition imposed on germination by paclobutrazole (Figure 2B) was marginally overcome by ethylene. Earlier observation that GA at high concentration restores germination of *etr1* seeds while ethylene could not break dormancy in *gib-1* mutant seeds [23,24] also leads to the same inference that GA has possibly a more direct action than ethylene. Weiss and Ori [25] proposed for both the positive and negative interaction between ethylene and GA. Other observations suggest that GA-ethylene interaction in case of seed germination seems to be complementary [2,26]. Apparently, GA might have its own signalling pathway or components apart from its interactive response pathway with ethylene that may counteract the effects of ABA. In case of recovery experiments with ethylene over ABA inhibition on germination of *V. radiata* seeds, a significant recovery of germination was noted (Figure 2C), though recovery was far less or almost nil when ABA treatment given alone for

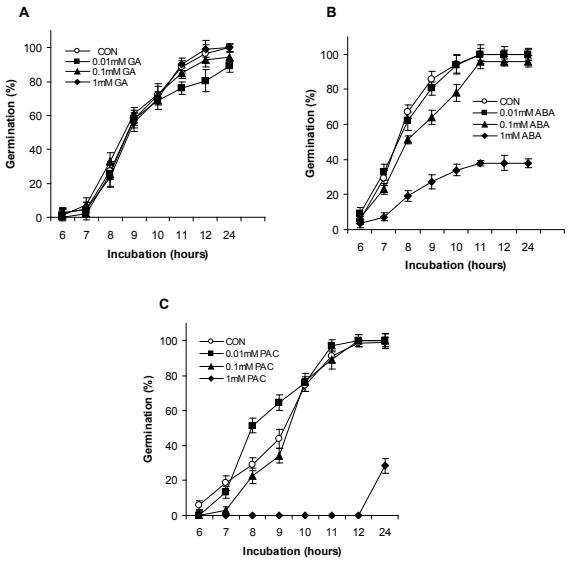


**Figure 1:** Effect of hormones and their inhibitors on germination percentage of *Vigna radiata* seeds.
Seeds were incubated with medium containing (A) GA, (B) ABA and (C) paclobutrazole (PAC) of different concentrations as mentioned in the figure and germination percentage was recorded at intervals. Data were mean of three replicate values and ± SE has been indicated as vertical error bars.

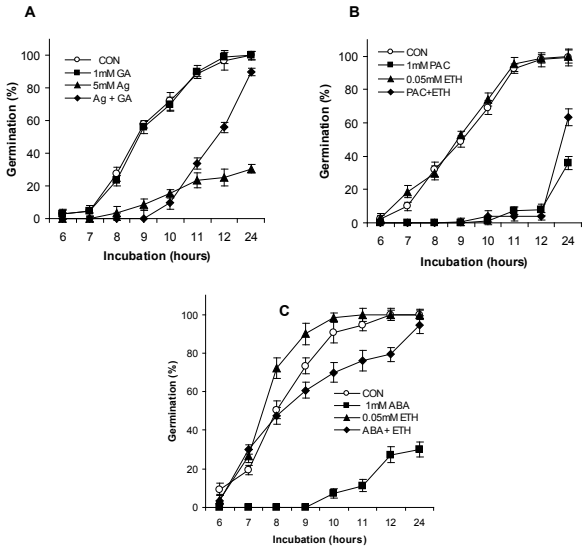


**Figure 2:** Effect of combined treatments of hormones and their inhibitors on germination percentage of Vigna radiata seeds.
Seeds were incubated with medium containing (A) $AgNO_3$ (Ag) + GA, (B) paclobutrazole (PAC) + ethylene (ETH) and (C) ABA + ethylene (ETH) at concentrations as mentioned in the figure and germination percentage was recorded at intervals. Data were mean of three replicate values and ± SE has been indicated as vertical error bars.

a considerable period followed by ethylene, as observed by us earlier in the same species [7]. Such recovery could be attributed to a strong interaction between ethylene and ABA signal transduction pathways and ethylene can promote germination by directly interfering with ABA signalling [9,21,27]. Linkies et al. [10] demonstrated that ethylene counteracts the inhibitory action of ABA on endosperm cap weakening and rupture during germination of *Lepidium* seeds. It was also observed by some workers that there was an increase in sensitivity to ABA with respect to germination and seedling establishment for wild type seeds of Arabidopsis in presence of $AgNO_3$, which further demonstrates the negative regulation of ABA sensitivity by the action of ethylene [28].

It has recently emerged that besides their role in stress and pathogenic defence ROS can act as signalling molecule for several plant processes like growth and development [29-31] and that it may act downstream of plant hormones [32]. Therefore, it is quite reasonable to assume for a similar action of ROS action for GA and ABA during seed germination. Our recovery experiments in this study showed that $H_2O_2$ rescued germination almost fully from the inhibition imposed by ABA (Figure 3A). ABA might have affected ROS metabolism either directly or by interfering with ethylene action on ROS metabolism as exogenous $H_2O_2$ suppressed the action of ABA. Sarath et al. [33] also demonstrated a reversal of ABA-induced inhibition of germination by $H_2O_2$ and they explained this as due to interference of $H_2O_2$ in ABA signalling possibly involving nitric oxide. On the other hand, action of GA on seed germination of *V. radiata* is in line with $H_2O_2$, as $H_2O_2$ can partially overcome the inhibition by paclobutrazole on germination (Figure 3C). Roles of ROS in GA signalling in the aleurone layer and programmed cell death (PCD) in *Hordeum vulgare* has already been established, where GA initiates cell death of aleurone cells, whereas ABA inhibits cell death [34]. However, GA may have some other way of signalling in use for germination as $H_2O_2$ could not fully rescue when GA synthesis was blocked by paclobutrazole. Liu et al. [15] demonstrated that $H_2O_2$ upregulates ABA catabolism through NO signalling while promotes

GA synthesis thus favouring germination. On the other hand, Bahin et al. [16] proposed that $H_2O_2$ alleviates dormancy by activating GA signalling and synthesis, not by repressing ABA signalling.

In order to verify the ethylene-ROS cross talk, seeds of *V. radiata* were tested for recovery by exogenous ROS from inhibition by ethylene action inhibitor ($AgNO_3$). Significant recovery in germination was noted by the application of exogenous ROS supplied in the form of $H_2O_2$ along with $AgNO_3$ (Figure 3B). Conversely, treatment with ethylene was not that much effective in recovery of seed germination when added along with propyl gallate, a potent ROS scavenger, as observed earlier [12]. It appears that ROS are acting downstream, while ethylene is an upstream component for the signalling cascade regulating germination as was found in case of ROS action for elongation growth [35-37].

Whether these hormones (GA, ABA and ethylene) influence germination via ROS production was further tested by NBT staining for superoxide accumulation (Figure 4). Specifically the tip region and a part of subapical region of axis corresponding to the growing region were found to accumulate superoxide corroborating the view that apoplastic synthesis of ROS is involved in the cell elongation process associated with germination [37]. Treatment of seeds with GA and ethylene showed $O_2$.- accumulation almost similar to the control set whereas treatments with ABA, paclobutrazole and $Ag^+$ prevented such accumulation. This observation supports the view that these hormones interact with ROS for controlling germination [38]. Among the different possible pathways, ROS generation through a transmembrane enzyme, NAD(P)H oxidase (NOX), which transfers electrons from cytoplasmic NAD(P)H to $O_2$ to form $O_2$•– in the apoplast has been proposed to play important role in developmental phenomena [39,40]. NADPH oxidases from plants have been reported to be encoded by *Rboh* (respiratory burst oxidase homologs) gene family [41,42]. It is proposed that NOX-generated $O_2$•– is metabolized further to $H_2O_2$ and OH· that ultimately initiates the cell elongation process [37,43]. In-gel assay for NOX activity in the axes from germinating seeds showed three NBT-sensitive bands (Figure 5) which indicates $O_2$•– producing activity possibly through NOX enzyme during germination of *V. radiata* seeds. Sagi and Fluhr [44] carried out the activity gel assay of NOX based on protein fractionation in native or sodium dodecyl sulphate (SDS) - polyacrylamide gels that also gave significant results. In their native-PAGE analysis, one or two major $O_2$- producing formazan bands were detected in tomato (*Lycopersicum esculentum*) and tobacco (*Nicotiana tabacum*) plasma membranes, respectively. In contrast to the mammalian gp91$^{phox}$, the plant homolog can produce $O_2$•– in the absence of additional cytosolic components and is stimulated directly by $Ca^{2+}$. NOX activity was clearly affected by ABA and $Ag^+$ thus indicating the possible interaction of ABA and ethylene with ROS production through NOX activity in a contrasting way. GA is probably not totally dependent on NOX activity for controlling germination as paclobutrazole partially affected NOX activity.

Finally, it can be concluded that ethylene essentially plays a positive role in seed germination of *Vigna radiata* with a possible interaction with ROS, the latter may be placed downstream of ethylene action. ABA can interfere with ROS generation or ROS signalling, which is counteracted by ethylene, while GA may partially rely upon ROS action independently or via interfering with ABA for its role in seed germination.

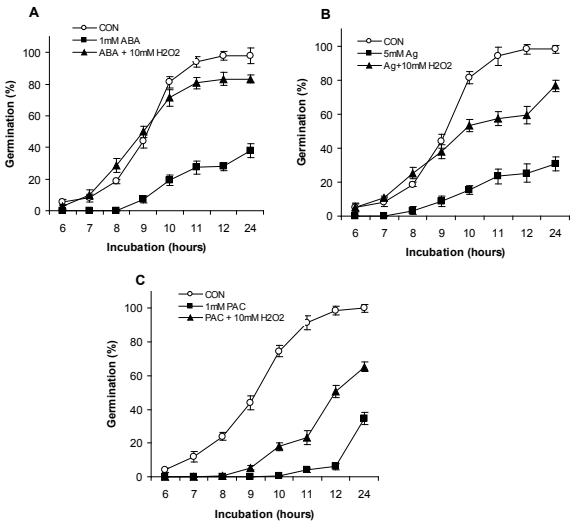


**Figure 3:** Effect of combined treatments of hormones or their inhibitors and hydrogen peroxide ($H_2O_2$) on germination percentage of *Vigna radiata* seeds.
Seeds were incubated with medium containing (A) ABA, (B) $AgNO_3$ (Ag) and (C) paclobutrazole (PAC) along with $H_2O_2$ in each case at concentrations as mentioned in the figure and germination percentage was recorded at intervals. Data were mean of three replicate values and ± SE has been indicated as vertical error bars.

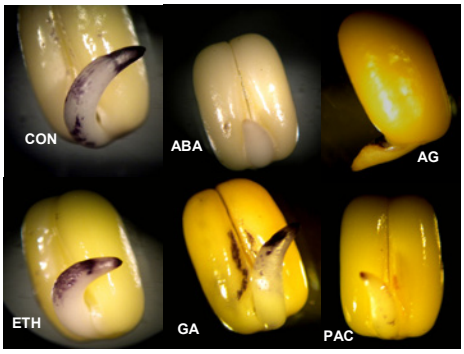


**Figure 4:** Histochemical staining of Vigna radiata seeds for superoxide accumulation.
Seeds were germinated for 12 h in the medium containing ABA (1 mM), $AgNO_3$ (AG, 5 mM), ethylene (ETH, 0.05 mM), GA (1 mM) and paclobutrazole (PAC, 1 mM) along with a control (CON) followed by staining in NBT solution for localization of superoxide producing zones. Photographs are of representative seeds showing the average effect for respective treatments.

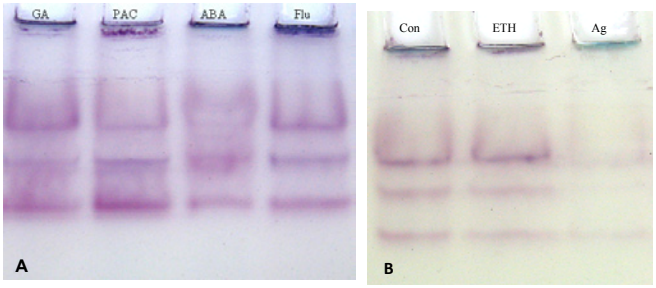


**Figure 5:** In-gel assay for NADPH oxidase (NOX) activity profile of axes from seeds of *Vigna radiata* subjected to treatments with hormones and their inhibitors.
Seeds were germinated for 12 h in the medium containing (A) GA (1 mM), paclobutrazole (PAC, 1 mM), ABA (1 mM), fluridone (Flu, 1 mM) and (B) ethylene (ETH, 0.05 mM), $AgNO_3$ (AG, 5 mM) along with control (Con). Axes were extracted with Triton X100 for membrane proteins which was then run in native gel followed by NBT staining for NOX activity specific bands.

## Acknowledgement

Authors acknowledge the funding for the present investigation from research grants by UGC under the scheme of major research project [No. 32-406/2006(SR) and No. 39-375/2010 (SR)].

## References

1. Bewley JD (1997) Seed Germination and Dormancy. Plant Cell 9: 1055-1066.
2. Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15: 281-307.
3. Holdsworth MJ, Bentsink L, Soppe WJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol 179: 33-54.
4. Kar RK (2007) Physiology and metabolic regulation of seed germination. In: Trivedi PC (ed) Plant Physiology: Current Trends, Pointer Publishers, Jaipur, India, 290-304.
5. Bentsink L, Kooenneef M (2002) Seed dormancy and germination. In: Somerville CR, Meyerowitz EM (eds) The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, doi/10.1199/tab.0009.
6. Baskin CC, Baskin JM, Chester EW, Smith M (2003) Ethylene as a possible cue for seed germination of Schoenoplectus hallii (Cyperaceae), a rare summer annual of occasionally flooded sites. Am J Bot 90: 620-627.
7. Chaudhuri A, Kar RK (2008a) Effect of ethylene synthesis and perception inhibitor and ABA on seed germination of *Vigna radiata*. World J Agri Sci 4: 914-921.
8. Karssen CM, Zagorski S, Kepczynski J, Groot SPC (1989) Key role for endogenous gibberellins in the control of seed germination. Ann Bot 63: 71-80.
9. Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interactions between abscisic acid and ethylene signaling cascades. Plant Cell 12: 1103-1115.
10. Linkies A, Muller K, Morris K, Tureckova V, Wenk M, et al. (2009) Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using Lepidium sativum and Arabidopsis thaliana. Plant Cell 21: 3803-3822.
11. Bailly C (2004) Active oxygen species and antioxidants in seed biology. Seed Sci Res 14: 93-107.
12. Chaudhuri A, Kar RK (2008b) Inhibition of seed germination by propyl gallate, a free radical scavenger and recovery of germination by hydrogen peroxide and ethylene in *Vigna radiata*. World J Agri Sci 4: 914-921.
13. Garnczarska M, Wojtyla L (2008) Differential response of antioxidative enzymes in embryonic axes and cotyledons of germinating lupine seeds. Acta Physiol Plant 30: 427-432.
14. Ishibashi Y, Tawaratsumida T, Zheng S-H, Yuasa T, Iwaya-Inoue M (2010) NADPH oxidase act as key enzyme on germination and seedling growth in barley (*Hordeum vulgare* L.). Plant Physiol 13: 45-52.
15. Liu Y, Ye N, Liu R, Chen M, Zhang J (2010) $H_2O_2$ mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis seed dormancy and germination. J Exp Bot 61: 2979-2990.
16. Bahin E, Bailly C, Sotta B, Kranner I, Corbineau F, et al. (2011) Crosstalk between reactive oxygen species and hormonal signalling pathways regulates grain dormancy in barley. Plant Cell Environ 34: 980-993.
17. Jabs T, Dietrich RA, Dangl JL (1996) Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Science 273: 1853-1856.
18. Carter C, Healy R, O'Tool NM, Naqvi SM, Ren G, et al. (2007) Tobacco nectaries express a novel NADPH oxidase implicated in the defense of floral reproductive tissues against microorganisms. Plant Physiol 143: 389-399.
19. Clarke, GM (1969) Statistics and Exprimental Design. (1st edn), Edward Arnold, London, pp. 91-100.
20. Linkies A, Leubner-Metzger G (2012) Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. Plant Cell Rep 31: 253-270.
21. Gazzarrini S, McCourt P (2003) Cross-talk in plant hormone signalling: what Arabidopsis mutants are telling us. Ann Bot 91: 605-612.
22. Leubner-Metzger G (2002) Seed after-ripening and over-expression of class I beta-1,3-glucanase confer maternal effects on tobacco testa rupture and dormancy release. Planta 215: 959-968.
23. Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to Ethylene Conferred by a Dominant Mutation in Arabidopsis thaliana. Science 241: 1086-1089.
24. Groot SPC, Karssen CM (1987) Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-de? cient mutants. Planta 71: 525–531.
25. Weiss D, Ori N (2007) Mechanisms of cross talk between gibberellin and other hormones. Plant Physiol 144: 1240-1246.
26. Dugardeyn J, Vandenbussche F, Van Der Straeten D (2008) To grow or not to grow: what can we learn on ethylene-gibberellin cross-talk by in silico gene expression analysis? J Exp Bot 59: 1-16.
27. Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, et al. (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell 12: 1117-1126.
28. Subbiah V, Reddy KJ (2010) Interactions between ethylene, abscisic acid and cytokinin during germination and seedling establishment in Arabidopsis. J Biosci 35: 451-458.
29. Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. Curr Opin Plant Biol 5: 388-395.
30. Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55: 373-399.
31. MÃ¸ller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58: 459-481.
32. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, et al. (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 22: 2623-2633.
33. Sarath G, Hou G, Baird LM, Mitchell RB (2007) Reactive oxygen species, ABA and nitric oxide interactions on the germination of warm-season C4-grasses. Planta 226: 697-708.
34. Ishibashi Y, Koda Y, Zheng SH, Yuasa T, Iwaya-Inoue M (2013) Regulation of soybean seed germination through ethylene production in response to reactive oxygen species. Ann Bot 111: 95-102.
35. Rodriguez AA, Grunberg KA, Taleisnik EL (2002) Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension. Plant Physiol 129: 1627-1632.
36. Liszkay A, van der Zalm E, Schopfer P (2004) Production of reactive oxygen intermediates (O(2)(.-), H(2)O(2), and (.)OH) by maize roots and their role in wall loosening and elongation growth. Plant Physiol 136: 3114-3123.
37. Muller K, Linkies A, Vreeburg RA, Fry SC, Krieger-Liszkay A, et al. (2009) In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiol 150: 1855-1865.
38. El-Maarouf-Bouteau H, Bailly C (2008) Oxidative signaling in seed germination and dormancy. Plant Signal Behav 3: 175-182.
39. Murphy TM, Auh CK (1996) The Superoxide Synthases of Plasma Membrane Preparations from Cultured Rose Cells. Plant Physiol 110: 621-629.
40. Van Gestelen P, Asard H, Caubergs RJ (1997) Solubilization and Separation of a Plant Plasma Membrane NADPH-$O_2$- Synthase from Other NAD(P)H Oxidoreductases. Plant Physiol 115: 543-550.
41. Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8: 397-403.
42. Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 141: 336-340.
43. Schopfer P, Plachy C, Frahry G (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiol 125: 1591-1602.
44. Sagi M, Fluhr R (2001) Superoxide production by plant homologues of the gp91(phox) NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol 126: 1281-1290.