World Journal of Agricultural Sciences 4 (S): 914-921, 2008
ISSN 1817-3047

Inhibition of Seed Germination By Propyl Gallate, A Free Radical Scavenger and Recovery of Germination By Hydrogen Peroxide and Ethylene in *Vigna Radiata*

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Abstract: In the present investigation, effect of propyl gallate, a free radical scavenger, was studied on germination of *Vigna radiata* seeds to verify the role of active oxygen species in the regulation of seed germination. Propyl gallate inhibited germination and such inhibition was not due to osmotic effect as verified by the treatment with sucrose solution of similar concentrations. Treatments were also effective in a similar way on water uptake and growth kinetics of isolated embryonic axes. Increasing duration of pretreatment with propyl gallate also retarded seed germination as well as water uptake and growth of axes proportionately. Conversely, incubation in water followed by treatment with propyl gallate caused inhibition of germination immediately after the transfer. Same pattern was noted in case of water uptake and growth kinetics of isolated axes. When the seeds were simultaneously treated with propyl gallate and hydrogen peroxide, germination was restored to a great extent. Such recovery, though to a far lesser extent, was also found with ethylene (in the form of ethrel) given simultaneously with propyl gallate. No added effect was found when seeds were treated with both ethylene and hydrogen peroxide together in presence of propyl gallate. However, recovery effect by these combined treatments was not reflected in case of water uptake and growth kinetics of isolated axes.

Key words: Propyl gallate . Hydrogen peroxide . Ethylene . Active oxygen species . Seed germination . Water uptake . Growth kinetics . Vigna radiata

INTRODUCTION

Active Oxygen Species (AOS) or Reactive Oxygen Intermediates (ROI) are usually considered as toxic cellular metabolites, the accumulation of which leads to cell injury and deleterious changes like seed deterioration [1]. Hydrogen peroxide (HP), a potential free radical, being produced under cellular conditions or stress may accumulate leading to deteriorative processes like Programmed Cell Death (PCD) or senescence. However, there are now compelling evidences that HP may act as a signaling molecule in plants mediating several hormone-regulated processes [2, 3]. Thus, it is likely that HP may have cross talk with the signaling pathways of plant hormones [3].

Other studies showed that seed germination is accompanied by HP generation in the embryonic axes as well as the seed coat [4]. As seed germination represents a developmental period most sensitive to pathogen infection, it is argued that HP release in this stage might play a role in protecting the emerging embryo against invasion by parasites [4]. However, promotion of germination by exogenous application of HP [5] indicates a positive role of this AOS in germination. A recent observation that ETR1, the ethylene receptor, is involved in HP signaling during stomatal closure [6] suggesting a possibility of similar cross talk between HP and ethylene (Eth), the latter being well known promoter of seed germination [7].

The present investigation is aimed to justify the role of AOS in seed germination by assessing the effect of propyl gallate (PG), an established potential free radical scavenger or antioxidant [8] and also to explore any possible cross talk between HP and Eth in controlling germination.

MATERIALS AND METHODS

Seeds of mungbean [*Vigna radiata* (L.) Wilczek var B1], collected from Pulses and Oilseeds Research Station, Behrampur, Murshidabad, West Bengal, India, were used as experimental material. Seeds were first sterilized in 1% sodium hypochlorite solution followed by rinsing in distilled water several times and then incubated in 9 cm petri dishes on Whatman no. 1 filter paper, moistened with distilled water or test solutions under controlled temperature (30^{o}C) and darkness in a seed germinator.

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To assess the role of AOS in germination seeds were incubated with PG, a free radical scavenger, of different concentrations (1mM, 10mM and 50mM). A similar experiment was also done with sucrose (Suc; 10mM and 50mM) to check whether the effect of PG was osmotic. At definite intervals germination percentage of seeds was determined. For studying water uptake and growth kinetics of embryonic axes, seeds were dissected after 6 h of incubation in water or treatment solutions to isolate embryonic axes, which were again incubated in water or respective treatment solutions and assessed at definite intervals for water uptake and growth kinetics. Water uptake kinetics was measured in terms of fresh weight change and growth kinetics was measured in terms of increase in length.

In a different set of experiment, seeds were either treated with PG for different durations (6, 8, 10 and 12h) followed by incubation in water or just reverse, i.e. incubated in water for different durations (6, 8, 10 and 12h) followed by treatment with PG (10mM). At intervals (12, 24 and 48h), germination percentage was noted. Also, under similar conditions changes in fresh weight and length of isolated embryonic axis were measured at intervals (6, 8, 10, 12 and 30h).

Whether AOS treatment can rescue germination from inhibition by scavengers was determined by assessing the effect of combined treatment of PG (10mM) and HP (5mM, 10mM, 50mM) on germination percentage. Effect of such combined treatment was also examined in case of water uptake and growth kinetics of isolated axes. To check any possible interaction of AOS with Eth, which is also a germination promoter, seeds were treated simultaneously with PG (10mM) and Eth, in the form of ethrel (10μM, 50 μM, 100μM) and germination percentage was recorded. Again, the same experiment was also done for any effect on water uptake and growth kinetics of isolated axes. Lastly, effect of a combination of PG (10mM), HP (10mM) and Eth (50μM) was tested for any effect on germination percentage.

For germination experiments, three replicates, each containing 50 seeds, were used, while for determination of fresh weight and lengths of axes, ten replicates were used. Mean values were presented in figures with standard errors of mean values shown as vertical bars.

RESULTS

Germination percentage of seeds of *V. radiata* incubated at different concentrations (1mM, 10mM, 50mM) of PG has been shown in Fig. 1. It was observed that incubation with higher concentrations of PG (10mM, 50mM) caused total inhibition of germination even up to 48 h of incubation. However,

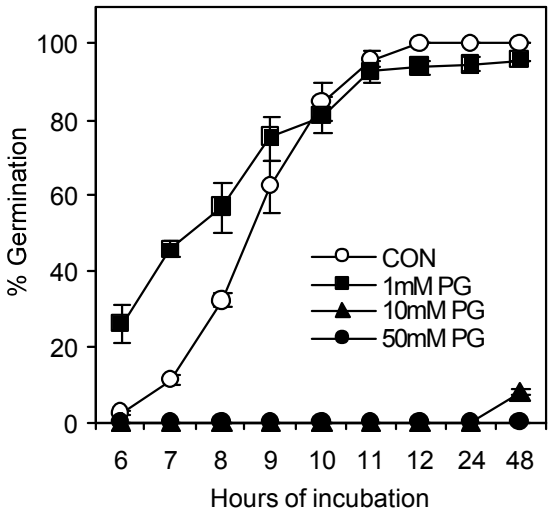


Fig. 1: Effect of propyl gallate (1, 10 and 50 mM) on germination percentage of *Vigna radiata* seeds. SE indicated as vertical bars

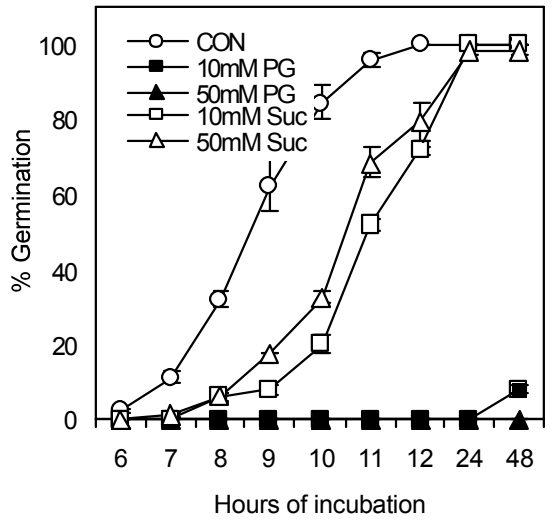
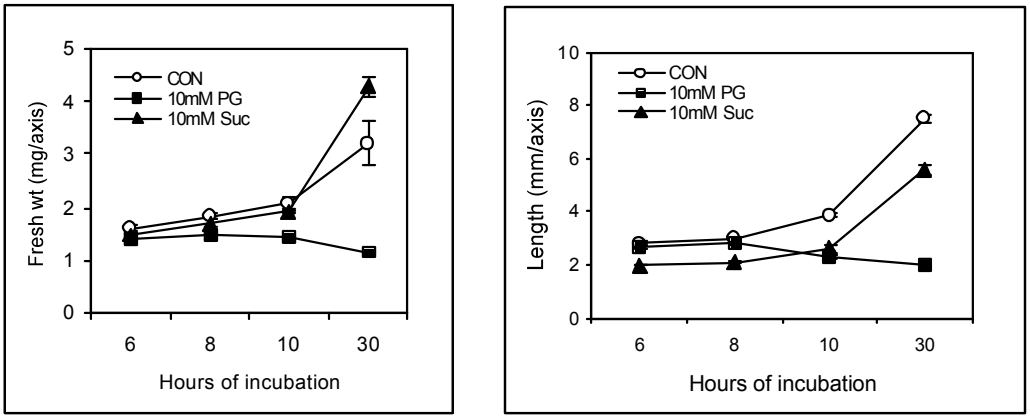


Fig. 2: Comparative effect of propyl gallate (10 and 50 mM) and sucrose (10 and 50 mM) on germination percentage of *V. radiata* seeds. SE indicated as vertical bars

1mM PG showed rather a promotion of germination as compared to control at least during the early hours (up to 9 h). To crosscheck whether inhibitory effect of high concentration PG was osmotic or not, treatment was also done with the same concentrations (10mM, 50mM) of Suc solution as an osmoticum and monitored for germination (Fig. 2). Although the germination percentage in Suc solutions was initially somewhat lower than in water, later the percentage reached to 100%. Studies on the comparative changes in fresh



A B

Fig. 3: Comparative effect of propyl gallate (10 mM) and sucrose (10 mM) on water uptake and growth kinetics of isolated embryonic axes of *V.radiata* seeds. SE indicated as vertical bars

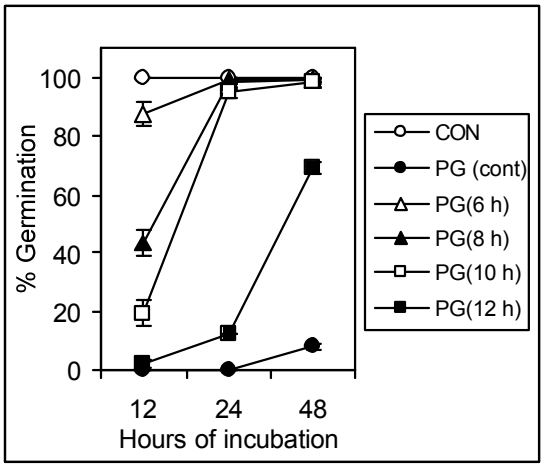
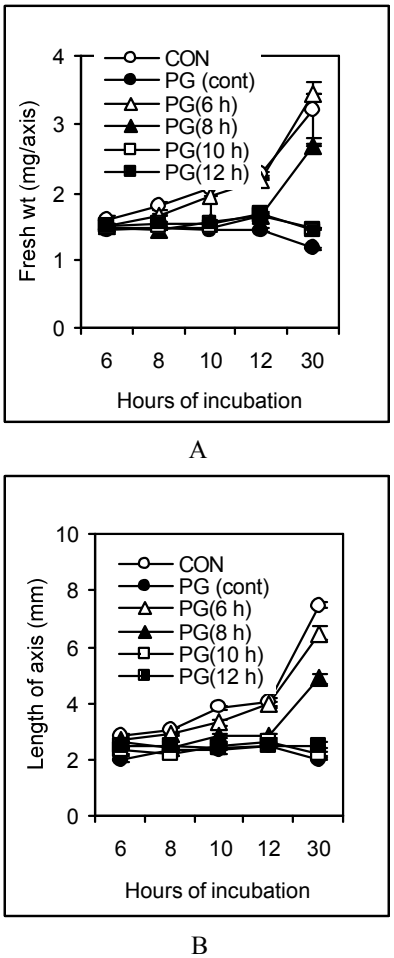


Fig. 4: Effect of different durations of pretreatment with propyl gallate (10 mM) followed by incubation in water on germination percentage of *V. radiata* seeds. SE indicated as vertical bars



A

B

Fig. 5: Effect of different durations of pretreatment with propyl gallate (10 mM) followed by incubation in water on water uptake and growth kinetics of isolated embryonic axes of *V. radiata* seeds. SE indicated as vertical bars

weight and length of isolated axis in PG (10mM) and Suc (10mM) (Fig. 3) showed that both fresh weight (Fig. 3A) and length of axis (Fig. 3B) was increased gradually in water as well as in Suc solution, while treatment with PG inhibited such increases.

When the seeds were pretreated with PG (10mM) for 6, 8, 10 and 12h followed by transfer to distilled water (Fig. 4), at 12h germination percentage reached 100% in control while the percentage was lower in seeds that received PG treatment, the value being proportionate to the duration of PG treatment. After 24h, percentage reached almost to the level of control in seeds incubated in PG for 6, 8 and 10h. However, the

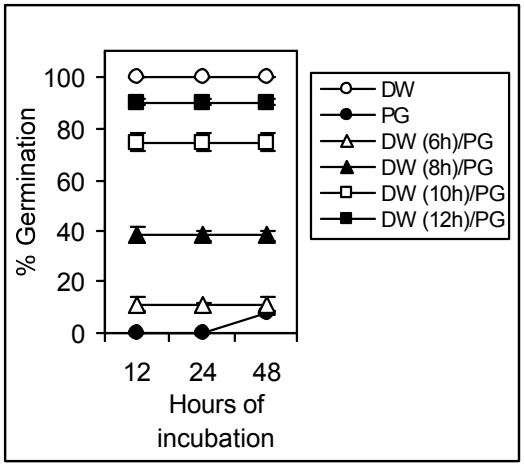
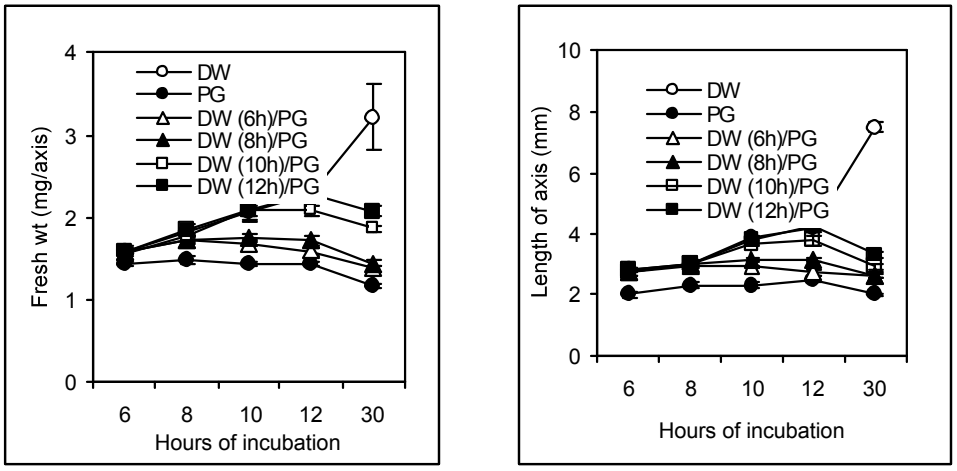


Fig. 6: Effect of different durations of incubation in water followed by treatment with propyl gallate (10 mM) on germination percentage of *V. radiata* seeds. SE indicated as vertical bars



A B

Fig. 7: Effect of different durations of incubation in water followed by treatment with propyl gallate (10 mM) on water uptake and growth kinetics of isolated embryonic axes of *V. radiata* seeds. SE indicated as vertical bars

percentage in case of seeds treated in PG for 12h reached only around 70% even after 48 h. Studies on changes in fresh weight and length of isolated embryonic axes from seeds received PG treatment for above-mentioned hours (Fig. 5) showed that fresh weight of axis in control was increased gradually and axes treated with PG for 6h almost followed the same trend (Fig. 5A). However, PG treatment for longer duration (8, 10 and 12h) almost inhibited increase in fresh weight up to 12h. After 30h fresh weight of axes was increased only in axes treated with PG for 8h, but not in those treated for 10 or 12h. Similar changes were noted for length of axes also (Fig. 5B).

In another experiment where the seeds were first incubated in distilled water for 6, 8, 10, 12h followed by continuous incubation in PG (Fig. 6), germination percentage of the treated seeds at 12h was accordingly lower than control (100%), shorter the duration of incubation in water lower the percentage. Germination percentage value during the subsequent period (up to 48 h) of incubation in PG was maintained in each case, i.e. no additional germination occurred once transferred to PG. Under similar treatment pattern, changes in fresh weight and length of isolated axes were also studied (Fig. 7). Fresh weight records (Fig. 7A) show that axis weight was gradually increased in water while continuous PG treatment arrested this increase. Axes incubated in water for 6 and 8h followed by PG incubation also showed a similar inhibition. In case of axes incubated in water for 10 and 12h showed an

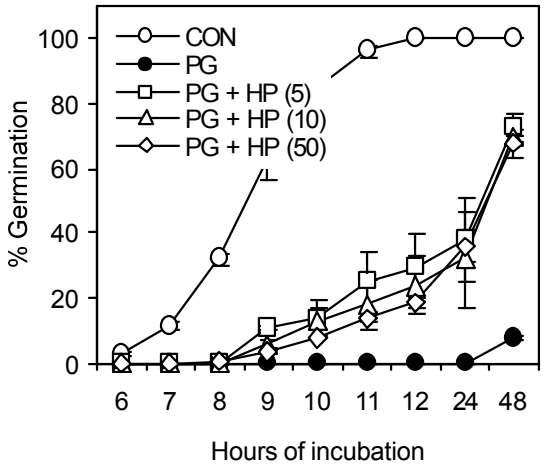


Fig. 8: Effect of combined treatment with propyl gallate (10 mM) and hydrogen peroxide (HP; 5, 10 and 50mM) on germination percentage of *V. radiata* seeds. SE indicated as vertical bars

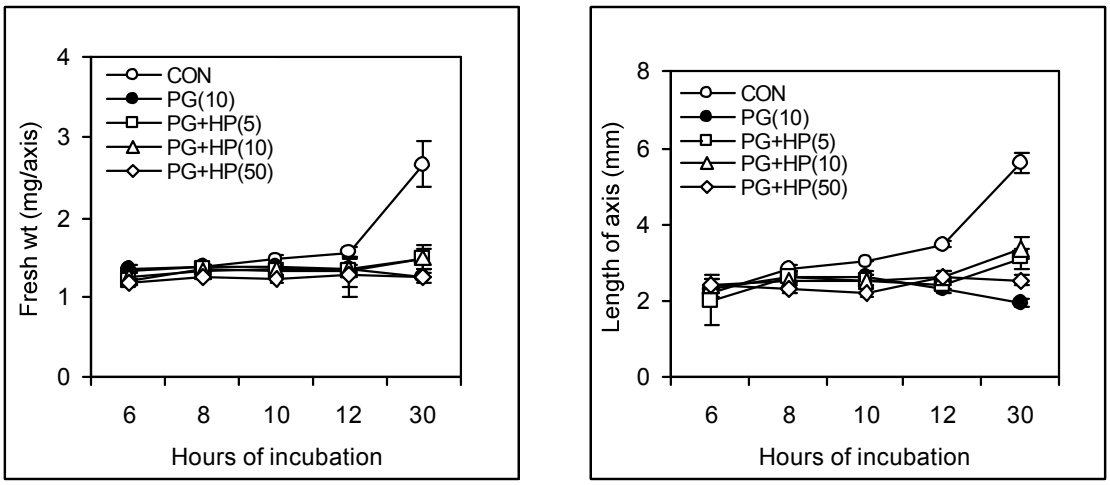


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Fig. 9: Effect of combined treatment with propyl gallate (10 mM) and hydrogen peroxide (5, 10 and 50 mM) on water uptake and growth kinetics of isolated embryonic axes of *V. radiata* seeds. SE indicated as vertical bars

increase in fresh weight al least up to 10 or 12h, respectively and then declined at 30h of incubation in PG. Almost same observation was made for changes in length of axes also under similar treatments (Fig. 7B).

Combined treatment of seeds with PG and HP (5mM, 10mM, 50mM) (Fig. 8) shows that while treatment with PG alone almost totally inhibited germination, simultaneous treatment with HP causes some germination (near 40 % at 24h and around 70% at the end of 48h). All the concentrations of HP were almost effective to the same extent in this regard. Effect of such combined treatment on fresh weight change and length of axes has been depicted in Fig. 9. Both fresh weight (Fig. 9B) and length of axes (Fig. 9A) was not much affected by combination of HP and PG as compared to PG treatment alone, except a marginal improvement in length of axes by HP (5 and 10mM) only after 30h. In a similar experiment, seeds were treated with PG in combination with Eth instead of HP and monitored for seed germination. Results (Fig. 10) revealed that, like HP, Eth also caused some germination in presence of PG, but was less effective than HP (maximum percentage at the end of 48 h was around 40%). Also, 50 μM concentration of Eth was most effective while 100 μM was least effective. The same combination was tested for any effect on fresh

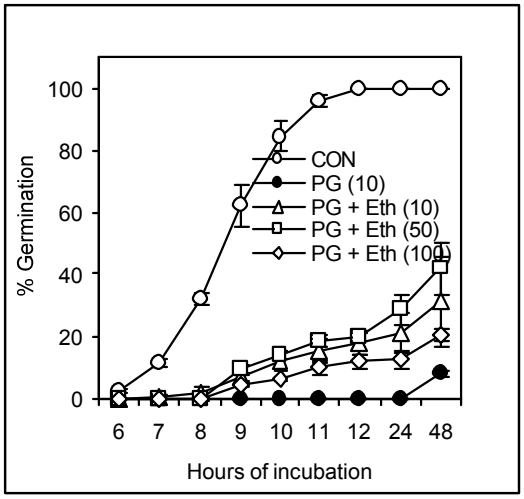

Fig. 10: Effect of combined treatment with propyl gallate (10 mM) and ethrel (10, 50 and 100 μM) on germination percentage of *V. radiata* seeds. SE indicated as vertical bars

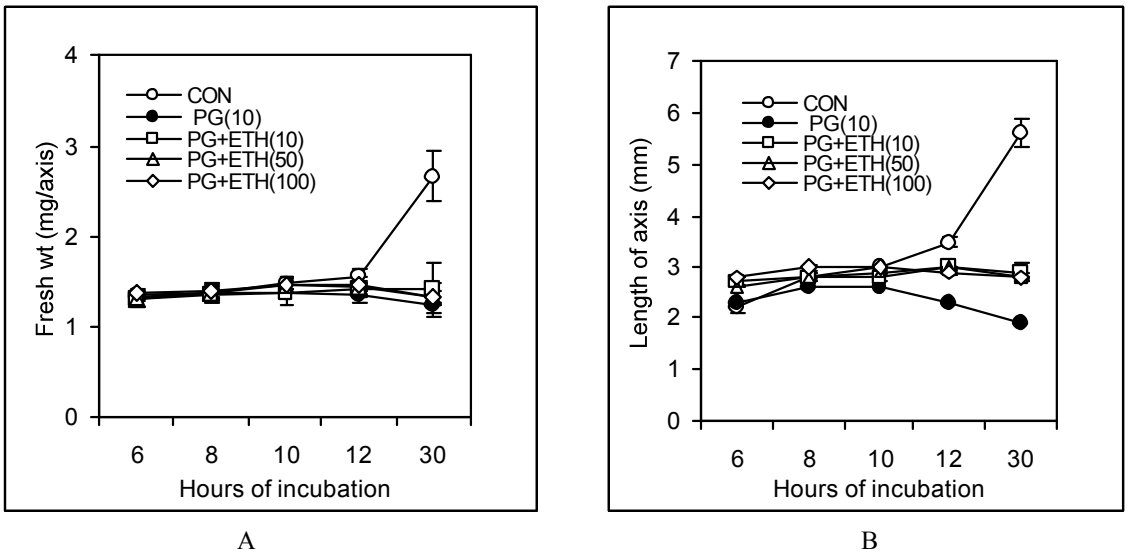

Fig. 11: Effect of combined treatment with propyl gallate (10 mM) and ethrel (10, 50 and 100 μM) on water uptake and growth kinetics of isolated embryonic axes of *V. radiata* seeds. SE indicated as vertical bars

weight and length of isolated axes (Fig. 11). This shows again that combined treatment with PG and Eth did not have any effect over PG treatment alone on both fresh weight (Fig. 11A) and length of axes (Fig. 11B). Fig 12 reveals the effect of triple combination (PG+HP+Eth) along with all double combinations (PG+HP, PG+Eth, Eth+HP) and control. While the combination of Eth and HP showed a rate of germination quite similar to control set, triple combination showed germination rate same as PG+Eth until 24h beyond which the percentage improved somewhat at 48h reaching almost to the level of what was achieved by PG+HP.

DISCUSSION

Several lines of evidence suggest that AOS including HP alleviates seed dormancy [9]. But in non-dormant seeds exogenous application of HP may have no effect, as was found in case of *V. radiata* seeds (data not shown). This may be due to generation of adequate level of HP endogenously, as reported in a number of cases [10, 11] and the presence of HP has been correlated with germination in these cases. In a reverse approach, here seeds of *V. radiata* were treated with PG, a free radical scavenger [8], to verify the role

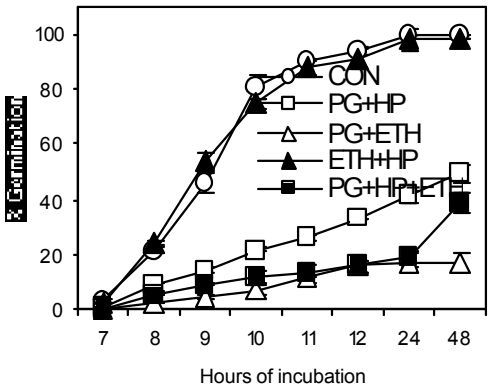

Fig. 12: Effect of different combinations of propyl gallate (10 mM), hydrogen peroxide (10m M) and ethrel (50 μM) on germination percentage of *V. radiata* seeds. SE indicated as vertical bars

of free radicals in the process of seed germination. Clearly, PG treatment inhibited germination (Fig. 1) and such inhibition was not due to osmotic stress, as similar concentrations of Suc (used as osmoticum) could not inhibit germination (Fig. 2). This was further supported by the observed water uptake and growth kinetics of isolated axes under the same treatments (Fig. 3), where again PG, but not Suc, inhibited both water uptake and growth of axes. Ogawa and Iwabuchi [5], showed a decline in seed germination rate by PG treatment. It is suggested that a temporal oxidized state of the seed embryo that is induced by HP might initiate germination and that antioxidant germination inhibitor(s) might prevent the induction of the oxidized state in seeds. However, being an antioxidant, PG was also reported to inhibit alternative respiratory pathway [12] and Eth biosynthesis [13]. Indeed, Gallardo *et al*. [14] and Nun *et al*. [15] demonstrated an inhibition of seed germination using PG and they ascribed such inhibition as due to inhibition of Eth biosynthesis and alternative oxidase, respectively. Thus, the effect of PG in these directions also cannot be ruled out at this moment, though AOS may act directly by inducing cell wall loosening process underlying cell expansion during radicle elongation [1].

In subsequent experiments on pretreatment with PG revealed that increasing duration of PG treatment inhibit germination quantitatively (Fig. 4). Similar effect was also noted in case of water uptake and growth kinetics of isolated embryonic axes (Fig. 5). Practically, effect of a treatment on germination is reflected on water uptake and growth by isolated axes, as we have shown earlier while studying the effect of cycloheximide and chilling temperature on seed germination and water uptake and growth kinetics of isolated axes of *V. radiata* [16]. Thus both water uptake and growth of axes remained inhibited through 30 h of incubation when the duration of pretreatment was prolonged for 10h or more. On the reverse, in case of pretreatment with water followed by PG treatment, increase of germination percentage (Fig. 6) and water uptake and growth by axes (Fig. 7) stopped as soon as they were transferred to PG. This suggests that a continuous supply of AOS is needed to initiate germination as well as water uptake and elongation of radicle. When PG treatment was supplemented with HP (Fig. 8), germination was recovered to a great extent (up to 70% after 48h) indicating that the presence of HP helps in germination process and PG inhibits the process by scavenging HP. However, such recovery was not found in case of water uptake and growth kinetics of isolated axes by combined treatment with PG and HP (Fig. 9).

With a possibility of interplay of HP and plant hormones like Eth, as demonstrated in several instances [1-3] and involvement of Eth in seed dormancy and germination [17], Eth was tested for possible recovery of germination of *V. radiata* seeds from PG inhibition (Fig. 10). Recovery was partial, though less effective than HP, indicating a possible cross talk between HP and Eth. However, again this was not reflected in case of water uptake and growth studies with isolated axes (Fig. 11). There are reports that HP may act upstream of Eth-related plant responses by activating ACC oxidase, thus enhancing Eth production [18] or conversely, Eth might stimulate AOS production [19]. Besides, Desikan *et al*. [20] have shown that Eth response transcription factors are up regulated by HP. Recently, Desikan *et al*. [6] showed that ethylene receptor, ETR1 mediates HP signaling in stomatal guard cells in *Arabidopsis thaliana*. Based on these findings it may be speculated that, in the present study, Eth and HP acted independently on a common element of downstream signaling leading to germination, since Eth can compensate partially the absence of AOS (in presence of PG) and further addition of HP could improve, though marginally, the recovery of germination percentage by Eth in presence of PG in the later stage (Fig. 12).

REFERENCES

1. Bailly, C., 2004. Active oxygen species and antioxidants in seed biology. Seed Science Research, 14: 93-107.
2. Vranová, E., D. Inzé and F. Van Breusegem, 2002. Signal transduction during oxidative stress. Journal of Experimental Botany, 53 (372): 1227-1236.

3. Kwak, J.M., V. Nguyen and J.I. Schroeder, 2006. The role of reactive oxygen species in hormonal responses. Plant Physiology, 141: 323-329.
4. Schopfer, P., C. Plachy and G. Frahry, 2001. Release of reactive oxygen intermediates (super oxide radicals, hydrogen peroxide and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin and abscisic acid. Plant Physiology, 125: 1591-1602.
5. Ogawa, K. and M. Iwabuchi, 2001. A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. Plant Cell Physiology, 42 (3): 286-291.
6. Desikan, R., J.T Hancock, J. Bright, J. Harrison, I. Weir, R Hooley and S.J. Neill, 2005. A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. Plant Physiology, 137: 831-834.
7. 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.
8. Nagata, T., S. Todoriki and S. Kikuchi, 2004. Radial expansion root cells and elongation of root hairs of *Arabidopsis thaliana* induced by massive doses of gamma irradiation. Plant Cell Physiology, 45 (11): 1557-1565.
9. Bogatek, R., H. Gawronska and K. Oracz, 2003. Involvement of oxidative stress and ABA in CN-mediated elimination of embryonic dormancy in apple. In The Biology of Seeds: Recent Research Advances. Nicolas, G., K.J. Bradford, D. Côme and H.W. Pritchard (Eds.). CABI Publishing, Wallingford, pp: 211-216.
10. Bailly, C., R. Bogatek-Leszczynska, D. Côme and F. Corbineau, 2002. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. Seed Science Research, 12: 47-55.
11. Morohashi, Y., 2002. Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion. Journal of Experimental Botany, 53 (374): 1643-1650.
12. Siedow, J.N. and M.E. Girvin, 1980. Alternative respiratory pathway. Its role in seed respiration and its inhibition by propyl gallate. Plant Physiology, 65: 669-674.
13. Mor, Y., H. Spiegelstein and A.H. Halevy, 1983. Inhibition of ethylene biosynthesis in carnation petals by cytokinin. Plant Physiology, 71: 541-546.
14. Gallardo, M., P. Munoz De Rueda, A. Matilla and I.M. Sanchez-Calle, 1994. The relationship between ethylene production and germination of *Cicer arietinum* seeds. Biologia Plantarum, 36 (2): 201-207.
15. Nun, N.B., D. Plakhine, D.M Joel and A.M. Mayer, 2003. Changes in the activity of the alternative oxidase in *Orobanche* seeds during conditioning and their possible physiological function. Phytochemistry, 64 (1): 235-241.
16. Kar, R.K. and R. Chakraborty, 2003. Effect of cycloheximide and chilling temperature on the control of seed germination in mungbean. Indian Journal of Plant Physiology, Special Issue, pp: 205-209.
17. Corbineau, F. and D. Côme, 1995. Control of seed germination and dormancy by the gaseous environment. In Seed Development and Germination. Kigel, J. and G. Galili. Marcel Dekker (Eds.). New York, pp: 397-427.
18. Chamnongpol, S., H. Willekens, W. Moeder, C. Langebartels, H. Sandermann, M. Van Montagu, D. Inzé and W. Van Camp, 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. In the Proceedings of the National Academy of Sciences, USA, 95, pp: 5818-5823.
19. Overmyer, K., M. Brosche and J. Kangasjarvi, 2003. Reactive oxygen species and hormonal control of cell death. Trends in Plant Science, 8: 335-342.
20. Desikan, R., S.A.H. Mackerness, J.T. Hancock and S.J. Neill, 2001. Regulation of the *Arabidopsis* transcriptome by oxidative stress. Plant Physiology, 127: 159-172.