Agric Res J **59** (6) : 1042-1046, *December* 2022
DOI No. 10.5958/2395-146X.2022.00146.6

EFFECT OF HYDROGEN PEROXIDE, BANANA PEEL EXTRACT AND SODIUM HYDROGEN CARBONATE ON SEED DORMANCY

Ankita Agrawal and Kunal Kumar*

Amity Institute of Biotechnology, Amity University, Ranchi – 834005, Jharkhand

ABSTRACT

Seed dormancy is generally an undesirable characteristic found among the seeds of agricultural crops where intact viable seeds fail to complete germination under favourable conditions. A variety of environmental and chemical stimuli along with common signal transduction chain have been considered for initiating the trigger for removal of dormancy that may differ between the seeds of different species. Experiments have been conducted to observe the effects of hydrogen peroxide (H_2O_2), oxidoreductase enzymes cocktail and sodium hydrogen carbonate on the germination of tomato, chilli, and wheat seeds. Wheat seeds could germinate faster than control seeds on treatment with Hydrogen peroxide and Oxidoreductase enzyme cocktail. On the contrary, Sodium Hydrogen Carbonate had meagre negative impact on the germination of seeds of wheat. Oxidoreductase enzymes cocktail and hydrogen peroxide could break dormancy of tomato seeds and initiated germination while control tomato seeds failed to germinate during the period of study. Likewise, control chilli seeds also failed to germinate. However, hydrogen peroxide and sodium hydrogen carbonate had positive impact on chilli seeds with onset of germination.

Keywords: Dormancy, Germination, Hydrogen peroxide, Seed, Sodium hydrogen Carbonate

Seed germination is a natural phenomenon of initiation of first developmental phase in the growth cycle of all plants. Physiologically, the process of germination starts with the imbibition of seeds and ends with the protrusion of the plumule and radicle through seed coat (Wolny, 2018). A seed starts to germinate in favourable conditions in response to environmental factors such as light, optimum temperature, balanced soil components; and the mechanisms involved together with all these responses to support germination have been well characterised. The post germinative phase includes the growth of the seedlings and maturations of the seedlings to a fully grown plant (Bentsink and Koornneef, 2008).

The intrinsic block to germination is seed dormancy. Seed dormancy is generally an undesirable characteristic found among the seeds of agricultural crops where intact viable seeds fail to complete germination under favourable conditions. Dormancy could be the result of a deficiency in some vital cellular event of germination or some dormancy-imposed event that must be negated before germination can be completed (Nakabayashi *et al.,* 2017). A variety of environmental and chemical stimuli along with common signal transduction chain have been considered for initiating the trigger for removal of dormancy that may differ between the seeds of different species. In the absence of corroborating evidence, these factors could be a base for future research. Dormant seeds of different genotypes may get triggered for germination by different chemicals and biochemicals agents (Bewley,1997).

Therefore, finding new sources and methods of breaking seed dormancy, developing novel strategies to improve the rate of germination for seeds would be beneficial to the food sector. In these contexts, we have worked on three chemical and biochemical stimuli to study their effect on the germination on one monocotyledonous and two dicotyledonous seeds.

MATERIALS AND METHODS

Collection of seeds

Seeds of tomato, chilli, and wheat were collected from the local market; and washed with distilled water and sun dried before use.

Preparation of reagents

Hydrogen peroxide (H_2O_2): 2mM hydrogen peroxide solution was prepared after diluting 30% w/v commercial hydrogen peroxide (H_2O_2) (Merck).

Source of carbon dioxide (CO_2): Dissolved carbon dioxide (CO_2) was obtained from freshly prepared 1 M solution of Sodium Hydrogen Carbonate, $NaHCO_3$(Merck) (Cavalli *et.al.,* 2012).

Crude extract of oxidoreductase enzymes cocktail: Fresh extract was prepared with 50 g of ripe banana peel ground in 50 mL distilled water. The extract was filtered, and the filtrate was used as source of the

*Corresponding author : kkumar@rnc.amity.edu
Date of receipt: 18.12.2021, Date of acceptance: 31.12.2022

oxidoreductase enzymes cocktail (Chong *et al.*, 2011).

Experimental set up

Experiments were conducted under room temperature where the variation of temperature during the day period was 25-27ºC. The night temperature dropped to 15-13 ºC. Three separate treatments with 2mM H_2O_2, 1M $NaHCO_3$ solution and oxidoreductase enzyme cocktail were given to each seed type of tomato, chilli and wheat kept on moistened cotton pads. Ten seeds on an average were taken for each individual study. Treatments with a feed size of 100µL were given every 12 h for a period of 30 days. Length of shoots and leaves was measured every 7 days to assess and compare the growth of the seedlings. A control was also taken for each case where seeds received treatment only with distilled water.

RESULTS AND DISCUSSION

The plant seeds of wheat, tomato and chilli were treated with hydrogen peroxide, Oxidoreductase enzymes cocktail and freshly prepared sodium hydrogen carbonate as a source of carbon dioxide and the germination efficiency and growth were assessed every day for a period of 30 days.

Germination was first detected in wheat seeds treated with 2mM hydrogen peroxide (H_2O_2) solution on 2nd day of treatment whereas, tomato and chilli seeds germinated on 6th and 12th day, respectively. Control of tomato and chilli seeds exhibited no germination for the complete period of the study, unlike the control of wheat seeds.

Germination in wheat was faster on treatment with cocktail of oxidoreductase enzymes. It was first detected in wheat seeds treated with cocktail of oxidoreductase enzymes on 2nd day of treatment whereas, tomato germinated on 12th day. Chilli seeds exhibited no germination with oxidoreductase enzyme treatment. Control of tomato and chilli seeds exhibited no germination for the complete period of the study, unlike the control of wheat for cocktail of oxidoreductase enzymes study (Fig. 1). The 1 M solution of sodium carbonate was found to be detrimental for the wheat seeds. Leaf width and shoot length were around 35% of that of control. Whereas sodium hydrogen carbonate exhibited no positive impact on tomato seeds. However, in chilli seeds, sodium carbonate treatment was promoting germination.

A comparative layout after 18th day of treatment demonstrated that wheat seeds exhibited faster growth with 24 cm and 25 cm shoot length and leaf length, respectively on treatment with hydrogen peroxide as compared to cocktail of oxidoreductase enzymes treatment with 21 cm and 21.5 cm shoot and leaf length, respectively. Treatment with sodium hydrogen carbonate could not promote growth and was found to be detrimental on comparison with control. A surprising observation was noted after 23rd day of treatment with H_2O_2. The shoots of wheat started withering off and the rigidity of the stems was lost. However, oxidoreductase enzyme treatment and sodium hydrogen carbonate treatment were consistent in action with negligible impact with time (Table 1).

Germination in tomato seeds was completely absent for control and sodium hydrogen carbonate treatments for the entire period of study where the variation of temperature during the day period was 25-27 ºC. The night temperature dropped to 15-13 ºC. However, under the same condition, oxidoreductase enzyme cocktail and hydrogen peroxide had positive impact on the germination rate of tomato seeds. Maximum growth with shoot length and leaf length was

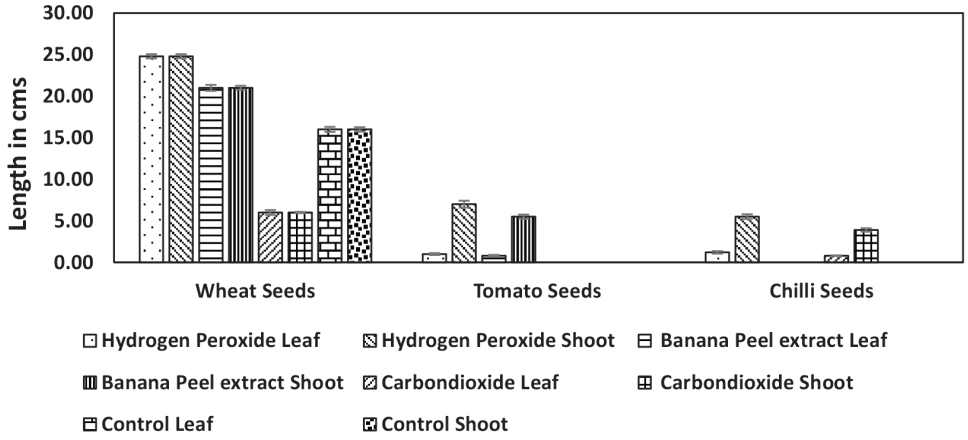

Fig. 1. Effect of hydrogen peroxide, oxidoreductase enzymes cocktail and carbon dioxide source on the germination of wheat seeds, tomato seeds, chilli seeds and expression of maximum growth in terms of shoot length and leaf length during the period of study.

Table 1. Germination and Growth assessment of wheat seeds, Tomato seeds, Chilli seeds on treatment with hydrogen peroxide, oxidoreductase enzymes cocktail, carbondioxide source on 6th, 18th , 23rd and 31st day.

		Hydrogen peroxide		Oxidoreductase enzymes cocktail		Sodium Hydrogen Carbonate (Carbon dioxide Source)		Control	
		Leaf Length	Shoot Length	Leaf Length	Shoot Length	Leaf Length	Shoot Length	Leaf Length	Shoot Length
		Measurements in Centimetres							
Wheat	6th	9.60±0.45	9.60±0.33	6.3±0.07	6.30±0.29	1.5±0.25	1.50	8.9	8.90
	18th	24.8±0.25	24.80±0.25	21±0.36	21.00±0.25	6±0.29	6.00±0.07	14±0.30	14.00±0.26
	23rd	Started withering	Started withering	21.1±0.3	21.10±0.25	6±0.2	6.00±0.21	14.06±0.25	14.06±0.26
	31st	Completely withered off	Completely withered off	21.5±0.21	21.50±0.36	6±0.4	6.00±0.45	16±0.3	16.00±0.25
Tomato	6th	Minor Result	Minor Result	No Germination	No Germination	No Germination	No Germination	No Germination	No Germination
	18th	1±0.11	7.00±0.41	Not Visible	5.00±0.25	No Germination	No Germination	No Germination	No Germination
	23rd	Started withering	Started withering	Not Measurable	5.10±0.25	No Germination	No Germination	No Germination	No Germination
	31st	Completely withered off	Completely withered off	0.8±0.11	5.50±0.25	No Germination	No Germination	No Germination	No Germination
Chilli	12th	Minor Result	Minor Result	No Germination	No Germination	No Germination	No Germination	No Germination	No Germination
	18th	1±0.07	4.10±0.25	No Germination	No Germination	Not Measurable	Not Measurable	No Germination	No Germination
	23rd	1.05±0.09	4.70±0.29	No Germination	No Germination	0.2±0.18	1.20±0.25	No Germination	No Germination
	31st	1.2±0.15	5.50±0.29	No Germination	No Germination	0.8±0.05	3.90±0.20	No Germination	No Germination

7cm and 1 cm, respectively on 18th day on treatment with hydrogen peroxide. The shoots of tomato started withering off and the rigidity of the stems was lost by the beginning of 21st day. On the contrary, tomato seeds on treatment with oxidoreductase enzyme cocktail exhibited similar growth on 31st day of treatment and the shoots maintained their rigidity throughout the period of growth (Table 1).

The maximum temperature of 27 and minimum of 13 degrees could not break the seed coat of chilli seeds during the study in case of control and in samples treated with oxidoreductases cocktail. As expected from previous trends in wheat and tomato, hydrogen peroxide promoted germination within 15 days. Unlike in tomato, chilli seeds germinated on treatment with sodium hydrogen carbonate. Maximum growth of around 1cm leaf length and 5 cm in shoot length was observed on 31st day in both the cases. Contrary to previous results, chilli shoots remained unaffected with prolonged treatment with hydrogen peroxide and rigidity of the shoots was maintained (Table 1).

The positive influence of hydrogen peroxide as evident from this study in the process of germination could be attributed to the following hypothesis. Hydrogen peroxide may bind to the receptors on the seeds which then get triggered, these receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting gibberellins (GAs). There could be another hypothesis for positive impact of hydrogen peroxide on germination. The recent research has found the expression of oxalate oxidase gene during seed germination. Oxalate oxidase (oxalate: oxygen oxidoreductase, EC 1.2.3.4) catalyzes the oxidative cleavage of oxalate to carbon dioxide with the reduction of molecular oxygen to hydrogen peroxide activity. Increased oxalate oxidase activity has also been found in response to stress and fungal infection (Lee *et al*., 2020; Jacob Kizhakedathil *et al*., 2020) in plants. This increased activity can result in sustained H_2O_2 production (Kumar and Belur, 2016), which induces the plants to produce peroxidase enzyme. Peroxidase, in the presence of H_2O_2, catalyses the oxidation of monolignols to free radicals which polymerise to give lignin (Warinowski *et al*., 2016; Tobimatsu & Schuetz, 2019). Lignification of plant cell wall is thus a response which gets initiated by expression of oxalate oxidase gene. Thus, high oxalate oxidase activity perhaps reveals the close association of the oxalate oxidase with development of the cell wall (Sathisraj and Augustin, 2012).

During the later stages of growth, hydrogen peroxide affected the rigidity of the tender stems of the seedlings, and they were found to be withering off. Previous reports suggest that accumulation of hydrogen peroxide in the plant body could be detrimental to the normal physiology. This low molecular weight compound causes cellular damage due to its potential to impart oxidative stress by interacting with most biomolecules (including nucleic acids, proteins, and lipids), Lipid peroxidation is one of the most widely documented toxic effects of H_2O_2 on cellular components and biological molecules (Wojtyla et.al., 2016).

Sodium hydrogen carbonate solution, a source of carbon dioxide had promoting effect on the germination chilli seeds which corroborates with the results of Saha *et. al* (2015), Mokashi *et al.* (2016) & Cavalli *et al.* (2012). However, sodium hydrogen carbonate treatment was slightly detrimental for wheat with restricted growth. Likewise, Fuller (2008) and Patil *et al.* (2012) found that elevated carbon dioxide levels had a negative effect on the growth of alfalfa, wheat grass and *Vigna aconitifolia*.

Banana peel extracts, a source of few oxidoreductase enzymes like polyphenol oxidase and oxalate oxidase could contribute to seed germination as evident from the work of Chong *et al.* (2011); Tadic *et al.* (2014); Lan *et al*. (1993); Dahiya *et al.* (2010). In the present study, the consistent positive impact of extracts of banana peel as a crude source of cocktail of oxidoreductase enzymes on germination of wheat and tomato seeds corroborates the previous research work mentioned above.

Thus, it is evident from the present work that chemical and biochemical stimuli can enhance the prospect of seed germination by breaking the period of dormancy. These chemical stimuli can have a positive impact even in absence of optimum temperature for germination. Hydrogen peroxide should be given in dose dependent manner only in initial few days to prevent the loss of rigidity of shoots which was otherwise observed during prolonged use in cases of wheat and tomato.

In conclusion, the assessment of growth of seeds on treatment with hydrogen peroxide, cocktail of oxidoreductase enzymes and carbon dioxide source revealed that hydrogen peroxide largely increases the germination rate in all three cases of tomato, chilli and wheat seeds as compared to cocktail of oxidoreductase enzymes and sources of carbon dioxide. Oxidoreductase enzyme cocktail promotes the germination of tomato and wheat seeds but had no impact on the germination of chilli seeds. In contrast to oxidoreductase enzyme cocktail and hydrogen peroxide, carbon dioxide retarded the growth of wheat seedlings; but had no impact on the germination of tomato seeds. However, it exhibited positive impact on the germination of chilli seeds. These results suggest that these chemicals and biochemicals agents could behave as signalling molecule in the origination of seed germination that includes specific variation at transcriptomic, hormonal,

and proteomic levels; and help in breaking down the seed coat which leads to increasing oxygen uptake and enhancing the other required metabolism.

Authors' contribution

Conceptualization and designing of the research work (KK); Execution of field/lab experiments and data collection (AA); Analysis of data and interpretation (KK); Preparation of manuscript (KK).

LITERATURE CITED

Bentsink L and Koornneef M 2008. Seed dormancy and germination. The Arabidopsis Book. **6**: e0119.

Bewley J D1997. Seed germination and dormancy. *Plant Cell* **96**:1055–66

Cavalli G, Riis T and Baattrup-Pedersen A 2012. Bicarbonate use in three aquatic plants. *Aquatic Botany* **98**: 57-60.

Chong L C, Cheng L H and Noor Aziz A A 2011. Properties of polyphenol oxidase obtained from *Musa acuminata* × *balbisiana* Colla cv. "*PisangAwak*" pulp and peel. *CyTA-J. food* **9**:25-30.

Dahiya T, Yadav S, Chauhan N, Handa P and Pundir CS 2010. Strawberry Fruit Oxalate Oxidase — Detection, Purification, Characterization and Physiological Role. *J. Plant Biochem. Biotechnol.* ***19***:247–250.

Fuller EP 2008. Do carbon dioxide levels affect the germination and growth of plants? Project summary, California state science fair. http://csef.usc.edu/History/2008/Projects/J1711.pdf

Jacob Kizhakedathil M P, Bose R and Belur P D 2020. Calcium oxalate degrading thermophilic oxalate oxidase from newly isolated *Fusarium oxysporum* RBP3. *Biocatal Agric Biotechnol* ***25***: 101583.

Kumar K and Belur P D 2016. New extracellular thermostable oxalate oxidase produced from endophytic *Ochrobactrum intermedium* CL6: Purification and biochemical characterization. *Prep Biochem Biotechnol* **46**: 734–39.

Lee Y, Choi O, Kang B, Bae J, Kim S, and Kim J 2020. Grey mould control by oxalate degradation using non-antifungal *Pseudomonas abietaniphila* strain ODB36. *Sci Rep Reports* **10**: 1605.

Mokashi K, Shetty V, Annie George S and Sibi G 2016. Sodium bicarbonate as inorganic carbon source for higher biomass and lipid production integrated carbon capture in Chlorella vulgaris, *Achiev Life Sci*. **10**:111-17.

Nakabayashi K, Graeber K, Leubner-Metzger G 2017. Genetics of Dormancy. In *Encyclopedia of Applied Plant Sciences*, Thomas B, Murray B G, and MurphyD J (eds). (Second Edition), Academic Press, pp. 504-508.

Patil N S, Apradh V T and Karadge B A 2012. Effects of alkali stress on seed germination and seedlings growth of *Vigna aconitifolia* (Jacq.) Marechal, *Pharmacognosy J* **4**:77-80,

Saha S, Chakraborty D, Sehgal V K and Pal M 2015. Rising atmospheric CO_2: Potential impacts on chickpea seed quality. *Agri Ecosystem Environ* **203:** 140–46.

Sathishraj R and Augustin A 2012. Oxalic acid and oxalate oxidase enzyme in *Costuspictus*D. Don. *Acta Physiol Plant* **34**: 657–67.

Tadic V, Marković M, Milošević S, Cingel A, Raspor M, Spasojević D, Tadic J M 2014. Effect of phenol on germination capacity and polyphenol oxidase, peroxidase, and catalase activities in Lettuce. *Arch Biol Sci* **66**:1503–14.

Tobimatsu Y and Schuetz M 2019. Lignin polymerization: how do plants manage the chemistry so well? *Curr Opin Biotechnol* **56**:75–81.

Warinowski T, Koutaniemi S, Kärkönen A, Sundberg I, Toikka M, Simola L K, Kilpeläinen I and Teeri T H 2016. Peroxidases bound to the growing lignin polymer produce natural like extracellular lignin in a cell culture of Norway spruce. *Front Plant Sci* **18**:1523.

Wojtyla Ł, Lechowska K, Kubala S and Garnczarska M 2016. Different Modes of Hydrogen Peroxide Action During Seed Germination. *Front Plant Sci* **7**:66.

Wolny E, Betekhtin A, Rojek M, Braszewska-Zalewska A, Lusinska J, Hasterok R 2018. Germination and the early stages of seedling development in brachypodiumdistachyon. *Int J Mol Sci* **19**: 2916.