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Investigation of Antioxidant Enzymes and Biochemical Changes in the Wheat Seeds (Freed) Induced by Different Pre-Sowing Treatments

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Abstract: In the present work, experiment was conducted using wheat seeds (Freed) to study the effect of different pre-sowing seed treatments on biochemical changes in the seeds. Different chemical and hormonal treatments along with non-treated controls were applied in three combinations i.e. combination A (IAA, IBA NAA and H_2O_2 100mM each), combination B (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3) and combination C (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3). Results depicted that seed treatment with different combinations increased the activity of superoxide dismutase in primed seeds. Highest superoxide dismutase activity was observed in the seed treated with the combination C. Peroxidase activity was found to be highest in hydro primed seeds. Whereas, catalase activity was increased in seeds after all applied treatment combinations with maximum increase in combination C. Results further exhibited that level of total soluble protein and protease activity were raised after all treatment combinations including simple water soaking treatment being highest in combination A. However, highest increase in amylase activity was recorded in the seeds treated with combination A.

Key words: Wheat seed · Enzymology · Priming technique

INTRODUCTION

Priming is a form of seed preparation in which seeds are pre-soaked before planting. Controlled hydration and drying in seed priming technique affect the plants by inducing more quick germination when the seeds are reimbibed. Most of plant hormones are effective in enhancing germination and seedling vigor. Hormonal priming enhanced physiological feature of wheat grass under drought and control conditions and also increased the activity of antioxidants i.e. catalyse (CAT), peroxidase (POD) and superoxide dismutase (SOD) and in turn it protect the cell against production of free radical and cellular oxidative damage [1]. Seed priming with different priming agents were more significant in increasing growth characters, yield and its components in addition to photosynthetic pigments content in the leaves, total carbohydrate percentage, as well as N, P and K contents in wheat grains as compared with other treatments or untreated plants [2].

H_2O_2 priming enhanced the formation of antioxidants in seed, which play important role in the seedlings to counteract with the oxidative damage. These changes led to the expression of stress proteins and enhanced physiological attributes against stress [3], which increased the seedling growth even under salinity. The effect of exogenous application of growth hormones on bio-productivity, growth, photosynthesis, plant water relations, various enzyme activities were different and it affects the plants that were exposed to various biotic and abiotic stresses [4].

Seed priming is an economic technique which is widely used to overcome the germination related problems or to ameliorate the stress tolerance in different crops. Several priming techniques have been reported to have a numerous beneficial effects however, priming induced changes in the seed biochemistry that are actually responsible for beneficial effects of treatments. Therefore, proposed research work was focused on investigation of biochemical changes in the seeds induced by different pre-sowing treatments with combination of growth hormones and chemicals. Special emphasis was on antioxidant enzyme modulations and bimolecular damage.

MATERIALS AND METHODS

Experiment was conducted using wheat (*Triticum aestivum* L.) genotype Fareed. Different hormonal and

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Table 1: Different pre-sowing seed treatments used in the experiments

Sr. No	Seed treatment	Treatment detail	Duration
1	Control	Nil	No treatmenat
2	Water control (Hydro priming)	Water soaking	8 h
3	Combination A	IAA, IBA NAA and $H_2O_2$100mM each)	8 h
4	Combination B	100mM BAP, kinetin, H_2O_2 and 2.8mM GA_3)	8 h
5	Combination C	100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA_3	8 h

chemical treatments were applied as pre-sowing seed treatments in three different combinations. Various treatments used in this experiment are listed in Table 1. On completion of the initial treatment, seeds were dried near to original weight with forced air under shade and used for further research activities.

In the first activity, pre-sowing seed treatments induced biochemical changes in the seeds were analyzed. Treated and non-treated seeds were homogenized in the specific extraction buffers for enzymes or extraction solutions for other biochemical parameters like soluble proteins, activities of different enzymes i.e. APX, peroxidase, catalase, superoxide dismutase, proteases, amylase, membrane lipid peroxidation (MDA), total phenolic contents and glucose were estimated in seeds.

Extraction of Antioxidant Enzymes: For extraction of enzymes, seeds (0.1 g) were ground in 50mM cold phosphate buffer (pH 7.8) and centrifuged at 15,000×*g* for 20 min at 4°C. The supernatant was separated and used for the determination of different enzyme activities.

Superoxide Dismutase (SOD): The activity of superoxide dismutase (SOD) was analyzed by calculating its ability to diminish the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Giannopolitis and Ries [5].

Catalase (CAT): For the estimation of catalase, seeds were homogenized in a standard composed of 50mM KP buffer, pH 7.1 and 1mM dithiothreitol (DTT). Activity of catalase (CAT) was measured by method of Sahu *et al*. [6] with minor modifications.

Peroxidase: For the measurement of peroxidase, seeds were homogenized in a medium composed of 50mM KP buffer (pH 7.1), 0.1mM EDTA and 1mM dithiothreitol (DTT). Activity of peroxidase (POD) was estimated using the method of Chance and Maehly [7] with some modification.

Protease: Activity of protease was measured by the method of casein digestion by using the method described by Ahmed *et al*. [8, 9]; Iqbal *et al*. [10].

Amylase: The amylase activity was determined by the modified method as reported by the Varavinit *et al*. [11].

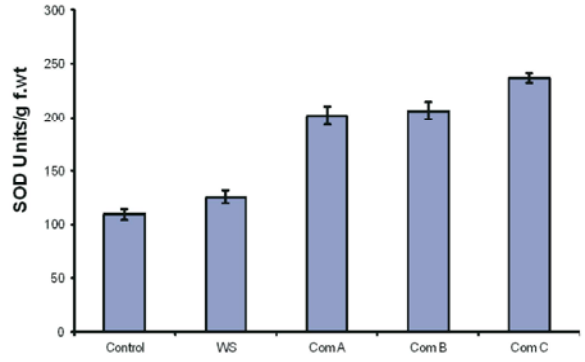
Total Phenolic Content (TPC): A micro colorimetric method was used for total phenolic assay, which utilizes Folin-Ciocalteu (F-C) reagent. For extraction of TPC, seeds (0.1 g) were ground in 85% ice-cold methanol and kept at room temperature for 48h. After 48h centrifuged at 15,000×*g* for 20 min at 4°C, the supernatant was separated and used for the determination of TPC. For estimation of TPC in seed samples 100µl of supernatant was mixed with 100µl of 10% (vol/vol) F-C reagent, vortex thoroughly and then 800µl of 700mM Na_2CO_3 was added. Samples were then incubated at room temperature for 2 hours. Blank corrected absorbance of samples was measured at 765nm by using Spectrophotometer (Hitachi, U2800).

Statistical Analysis of Data: All experiments were conducted in triplicates. The F-test was applied to find differences in variance among samples. The significance of differences between means (for stressed and control) for different parameters was measured using Student's t-Test (two tailed), at 0.01 and where applicable at 0.05 significance level.

RESULTS

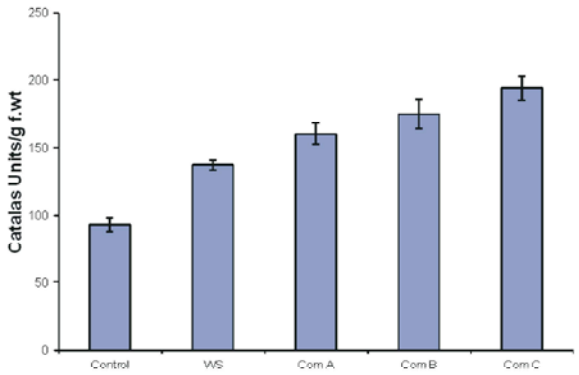
Biochemical changes in the seeds induced by different pre-sowing treatments alongwith combination of various growth hormones and chemicals were investigated. Main focus was on antioxidant enzyme modulations. Different antioxidant enzyme activities i.e. superoxide dismutase, peroxidase and catalase were measured in the primed and control seeds.

Superoxide Dismutase (SOD): Activity of superoxide dismutase was analyzed in the control and treated seeds. Super oxide dismutase activity was increased in wheat seeds, which were primed with different treatment combinations (Fig. 1). All treatment combinations raised the superoxide dismutase activity, however slight variation was observed in the effectiveness of treatment combination. Highest superoxide dismutase activity was observed in the seed treated with the combination C.



Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3)

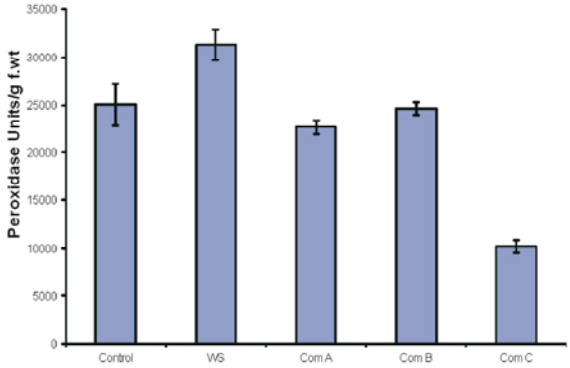
Fig. 1: Superoxide dismutase activity in wheat (*Triticum aestivum* L.) seeds after different seed priming treatments alongwith control treatment.Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3)



Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3)

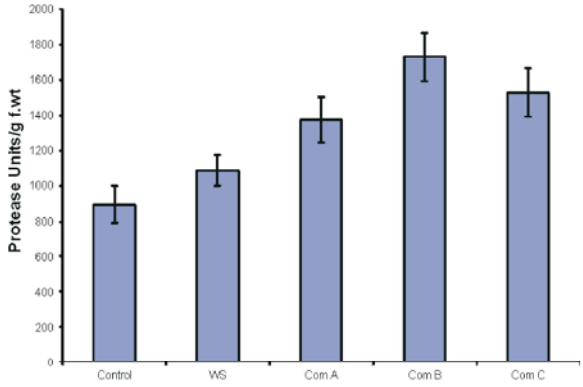
Fig. 2: Catalase activities in wheat (*Triticum aestivum* L.) seeds after different seed priming treatments along with control.

Catalase Activity: Catalase activity was increased in seeds after all applied treatment combinations. Maximum increase in the catalase activity was observed after the



Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3)

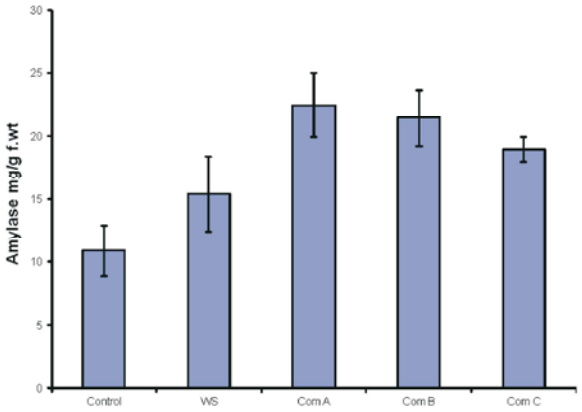
Fig. 3: Peroxidase activity in wheat (*Triticum aestivum* L.) seeds after different seed priming treatments along with control.



Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3)

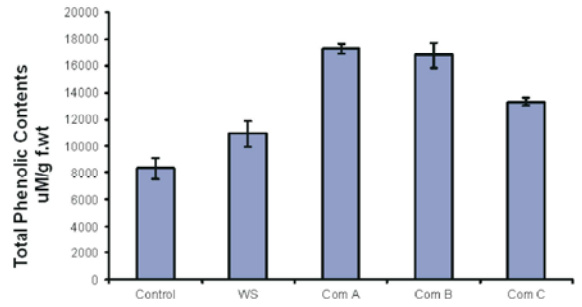
Fig. 4: Protease activity in wheat (*Triticum aestivum* L.) seeds after different seed priming treatments along with control.

Peroxidase Activity: Peroxidase activity was highest in hydroprimed seeds (Fig. 3). However, peroxidase activity was not modulated in the seeds treated with combination A and B as level was almost same as in the untreated control seeds. In contrast, peroxidase activity was decreased in the seeds treated with combination C as compared with control.



Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H_2O_2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H_2O_2 and 2.8mM GA3)

Fig. 5: Amylase activity in wheat (*Triticum aestivum* L.) seeds after different seed priming treatments along with control.



Control: Seeds without any treatment; WS: Seeds Soaked in Distilled Water; Com A: (IAA, IBA NAA and H2O2100mM each); Com B: (100mM BAP, kinetin, H2O2 and 2.8mM GA3); Com C: (100mM IAA, NAA, kinetin H2O2 and 2.8mM GA3)

Fig. 6: Total phenolic content in wheat *(Triticum aestivum* L.*)* seeds after different seed priming treatments along with control.

application of treatment combination C followed by combination B and then C. Least increase over control was observed in hydro primed or seeds soaked in the simple water. Therefore, among tested seed treatments combination C was more effective in enhancing the catalase activity in the seeds (Fig. 2).

Protease Activity: Protease activity was measured in the seeds after different treatment combinations and compared with the untreated control seed. All the tested priming treatment combinations uplifted the protease activity in the treated seeds as compared with their respective control treatment (Fig. 4). Highest increase in the protease activity as a result of priming treatment was observed in case of combination B. In this case i.e. combination B, protease level was above two folds as compared to that in untreated control seeds. Second highest level was observed in the seeds after combination C treatment followed by combination A. Protease level was also slightly raised in the seeds soaked in simple water as compared to control.

Amylase Activity: Results clearly depicted that different tested seed priming combinations uplifted the amylase activity level in the seeds (Fig. 5). Highest increase in amylase activity was seen in the seeds treated with combination A followed by combination B and C. Simple soaking of seeds in water did not promptly modulate the amylase activity in the seeds. Therefore, seed priming combination A was proved to be more effective in raising the seed amylase activity and thus enhancing the breakdown of stored starch in the seeds.

Total Phenolic Content (TPC): Changes in the level of TPC were measured in the seeds after application of different treatment combinations. All three treatment combinations enhanced the level of total phenolic content in the seeds as compared to untreated control (Figure 4.6). However, level of increase in total phenolic content in seeds was comparatively higher in case of combination A and B. in these cases, level of total phenolic content was more than double as compared with that in control seeds. Simple soaking of seeds in water also raised the total phenolic content in the seeds; however, increase was comparatively less (Fig. 6).

DISCUSSION

Superoxide dismutase is a key enzyme in cell which plays a vital role in cell against oxidative damage and severe environmental conditions. According to present observations, seed treatment with different combinations increased the activity of superoxide dismutase in primed seeds. These findings are in line with previous reports in which seed priming also increased the activity of superoxide dismutase in rice seeds [12]. It has also been reported that increased activities of antioxidant enzymes i.e. catalase (CAT), superoxide dismutase (SOD) and

peroxidase (POD) in Victoria and Victor seedlings after priming treatments [13]. Catalase is the key enzyme for removal or detoxification of excessive hydrogen peroxide in the seeds. In fact, increased level of antioxidative enzymes protects the cell against the oxidative damage by removal of free radicals or reactive oxygen species and also increased the quality of seed [14]. Amylases play a major role in breakdown of starch in cereal seeds. Present results clearly depicted that tested seed priming combinations uplifted the amylase activity in the wheat seeds. Previously, improved amylase activity in the primed rice seeds has been reported, although the extent of the change was different in seeds subjected to different priming treatments [15]. Enlarged amylase activity and sugar content were also reported in the treated rice seeds compared with the control [16]. Changes in the level of total phenolic content were measured in the seeds after application of different treatment combinations. All three treatment combinations enhanced the level of total phenolic content in the seeds as compared to untreated control (Figure 4.6). However, level of increase in total phenolic content in seeds was comparatively higher in case of combination A and B. Simple soaking of seeds in water also raised the total phenolic content in the seeds; however, increase was comparatively low.

CONCLUSIONS

In conclusion, enhanced capacity of primed seeds to scavenge free radicals by elevated antioxidants like SOD and catalase and rapid mobilization of stored carbohydrates and proteins by amylase and proteases during germination could at least partially explain the beneficial effects of priming treatments.

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