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SHORT COMMUNICATION

EFFECT OF 24-EPIBRASSINOLIDE ON ACTIVITY OF ANTIOXIDANT ENZYMES IN *BRASSICA JUNCEA* L. UNDER H_2O_2 STRESS

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Field experiment was conducted to find out the effectiveness of H_2O_2 on dynamics of growth, total proteins and antioxidant defense system of *Brassica juncea* L. growing with 24-epiBL. 15 mM H_2O_2 concentration was sub-lethal for *B. juncea* L. reducing germination rate. Shoot length was increased to significant level under 24-epiBL in H_2O_2 treated and untreated plants. H_2O_2 degraded protein content while 24-epiBL alone makes significant upgradation of total proteins. H_2O_2 played vital role in induction of abiotic stress tolerance by upregulation of antioxidant defense system. 24-epiBL further ameliorates this upgradation and makes the plants more hardy to tolerate abiotic stress.

Key words: **Antioxidant enzymes, *Brassica juncea* L., Brassinosteroids, hydrogen peroxide**

Hydrogen peroxide (H_2O_2) is the end product of photochemical reaction occurring in atmosphere which is found up to 100 μM under cold, rain, cloudy and foggy environment (Polle *et al*. 1990). Cold seasoned crops like Indian mustard (*Brassica juncea* L.) are indispensably exposed to these products while endogenously H_2O_2 is generated during normal metabolic reactions as intermediate of electron transport chain (ETC). Accumulation of H_2O_2 beyond certain limits, leads to the formation of free radicals in the form of hydroxyl ions ($OH^{\bullet}$) resulting in lipid peroxidation. To protect from this trauma, plant immune system gets activated to higher level leading to hardening of plant to face abiotic stress.

Brassinosteroids (BRs), belonging to new class of plant steroid phytohormones, demonstrated enhanced plant tolerance to a variety of abiotic stress results from regulation of antioxidant defense system at cellular level. BR being an eco-friendly chemical, has a potential application in agriculture to increase yield by regulating defense system, under field conditions (Sirhindi *et al.* 2009). The objective of present study was to examine role of H_2O_2 in induction of abiotic stress tolerance by regulating antioxidant defense system under BR treatment.

Experiment was conducted under field conditions of Plant Conservatory, Punjabi University, Patiala, India in the year 2009-10 during the winter season. Seeds of *Brassica juncea* L. cv. PBR 210 procured from Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India, were surface sterilised with 0.01 % $HgCl_2$ followed by rinsing with distilled water for 5–7 times. 16 h pre-sowing soaking treatment was given to surface sterilised seeds in different test solutions of 24-epiBL (0, 10^{-8} M), H_2O_2 (0, 15 mM) and combination of 24-epiBL and H_2O_2 (10^{-8} M + 15 mM) in triplicates. Seed emergence was noted on the 3rd day after sowing

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Table 1. Effect of 24-epibrassinolide on seed emergence on 3 DAS and shoot length, dry weight, moisture content, total protein content on 30 DAS in H_2O_2 treated and control distilled water leaf tissues of *B. juncea* L. values are the means of three replicates ± SE.

Treatments	Seed emergence (%)	Shoot length (cm)	Dry weight (%)	Moisture content (%)
Control (DW)	84.67 ± 3.52	17.6 ± 1.20	13.90 ± 0.30	86.01 ± 0.30
EBL	87.33 ± 4.05	23.0 ± 1.14*	15.00 ± 0.27	85.01 ± 0.27
15 mM H_2O_2	56.00 ± 4.00*	12.8 ± 0.86*	12.43 ± 0.12	87.56 ± 0.12
15 mM H_2O_2+EBL	73.00 ± 3.12*	16.6 ± 1.21	13.18 ± 0.15	86.81 ± 0.15

* Significant differences as compare to control (DW) using student t-test ($P \leq 0.05$)

(DAS). Youngest leaves were harvested from 30th day old plants for biomass, moisture content, enzyme activities and total protein content estimation.

Enzyme extract was prepared by homogenising 1 gm leaf tissue in 100 mM phosphate buffer (pH 7.0) supplemented with 0.1mM EDTA, and 1% (w/v) polyvinylpyrrolidone. Homogenate was centrifuged at 15,000 g for 12 minutes at 4°C in refrigerated centrifuge. Total protein was determined following the method of Lowry *et al.* (1951). Activity of superoxide dismutase (SOD, EC 1.15.1.1) was estimated following the method of Kono (1978). Catalase (CAT, EC 1.11.1.6) activity was measured according to Aebi (1983). Ascorbate peroxidase (APOX, EC 1.11.1.11) activity was measured as described by Nakano and Asada (1981). Activity of dehydroascorbate reductase (DHAR, EC 1.8.5.1) was measured by detecting formation of ascorbate from dehydroascorbate at 265 nm following the method of Dalton *et al.* (1986). Glutathione reductase activity (GR, EC 1.6.4.2) was measured according to Carlberg and Mannervik (1975). The data was analysed statistically using Student's t-test at $P \leq 0.05$.

To determine the role of BR in inducing tolerance to oxidative stress generated, we examined the change in activity of various antioxidant enzymes. Our previous results demonstrated that exogenous application of H_2O_2 under low temperature (4°C) protected the plants from oxidative stress due to abiotic stress. But the present results showed that H_2O_2 acts as a toxic molecule for a plant grown under normal temperature conditions.

It was observed that H_2O_2 decreased seed emergence as well as seedling growth in terms of shoot length. Ishibashi *et al.* (2008) showed direct correlation between germination ability and H_2O_2 scavenging substances and enzymes. Dry weight decreased in H_2O_2 treated plants on 30th day as compared to control and BR treated seedlings. Hameed *et al.* (2004) observed significant increase in 8 days old seedlings of *Triticum aestivum* while observing the influence of exogenous application of H_2O_2 on seedling growth.

The findings of the study showed that activities of enzymes such as antioxidant, enzymes like SOD, CAT, APOX, DHAR and GR increased in H_2O_2 treated plants as compared to control plants. Significant increase in enzymes activity was observed in 24-epiBL alone treated plants or plants treated with exogenous concentration of H_2O_2 and supplemented with 24-epiBL. (Fig. 1. B, C, D and E). Increase in activity of APOX in plants pretreated with BR and H_2O_2 alone and in combination was in accordance with Asada and Takahashi (1987). Activity of various enzymes involved in ADS of plant was further augmented when supplemented with BR (Kumar *et al.* 2010). Total protein content was found to be significantly increased in 24-epiBL treated plants as compared to control plants (Fig. 1. F). Similar results were reported by Upadhyaya *et al.* (2007) while analysing on detached leaves of *Oryza sativa* L. under H_2O_2 treatment, which promoted senescence and protein degradation as a consequence of H_2O_2 accumulation in detached leaves.

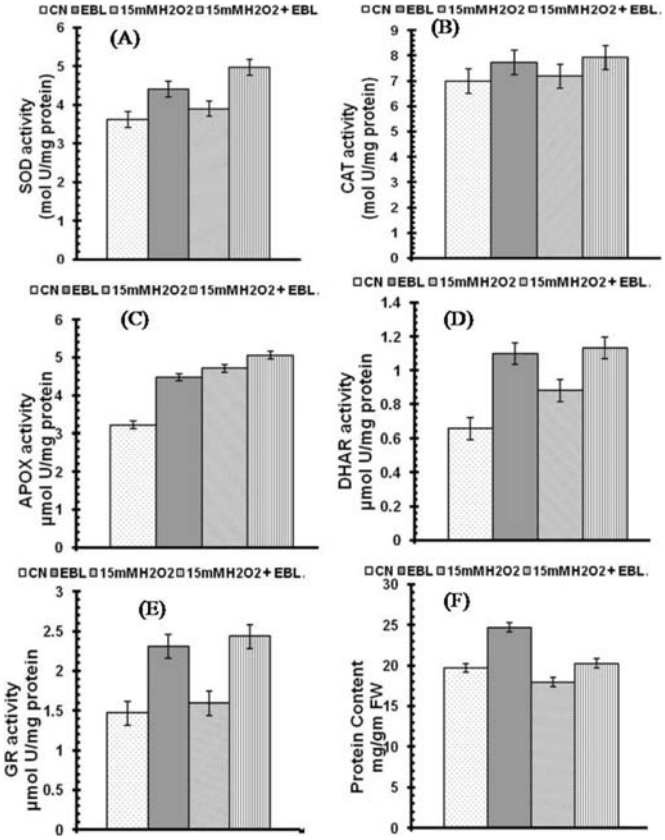

Fig. 1. Effect of 10^{-8} M 24-epibrassinolide on activity of (A) superoxide dismutase (SOD); (B) catalase (CAT); (C) ascorbate peroxidase (APOX); (D) dehydroascorbate reductase (DHAR); (E) glutathione reductase (GR) and (F) total protein content at 30 DAS in H_2O_2 treated and control distilled water leaf tissues of *B. juncea* L.

The present study concludes that H_2O_2 played a toxic role rather than acting as an anti-stress molecule in *Brassica juncea.* 24-epibrassinolide overcomes the deleterious effects of H_2O_2 on the growth parameters by improving the activity of various H_2O_2 scavenging enzymes. Exogenous H_2O_2 treatment also enhanced the scavenging enzyme activities and help in alleviating the negative effect of abiotic stress.

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