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# Influence of Exogenous Application of Hydrogen Peroxide on Root and Seedling Growth on Wheat (*Triticum aestivum* L.)

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## ABSTRACT

Effect of exogenous application of hydrogen peroxide (90 mM $H_2O_2$) was studied on initial roots and seedling growth in wheat. Fresh weight was significantly higher ($p<0.05$) in stressed seedlings (124% of the control) on 8th day. Mean weight gain per day from 5th to 8th day was significantly ($p<0.01$) higher in stressed roots and whole seedlings. Number of roots was higher in stressed seedlings on all days with a significant increase ($p<0.01$) on 8$^{th}$ day. Root length was significantly shorter ($p<0.01$) in stressed seedlings on all days indicating suppression of cell elongation probably by premature initiation of secondary wall formation. Maximum stress in terms of reduced fresh weight and length of roots ($p<0.001$) observed on 5$^{th}$ day, suggested that exogenous application of $H_2O_2$ for improvement of root growth or to control root diseases should be made after 5$^{th}$ day. The study provided evidence that exogenous application of $H_2O_2$ provided more vigorous root system in wheat.

**Key Words.** Abiotic stress; Hydrogen peroxide; Oxidative stress; Root growth; Reactive oxygen species

## INTRODUCTION

The imposition of biotic and abiotic stress conditions such as drought and salinity are known to raise concentrations of reactive oxygen species (ROS) such as hydrogen peroxide, super oxide and hydroxyl ions, resulting in oxidative damage at the cellular level (Zhang *et al.,* 2001). Exogenous hydrogen peroxide ($H_2O_2$) signals the induction of defense responses in plants against pathogen attack (Levine *et al.,* 1994; Alvarez *et al.,* 1998) abiotic (Prasad *et al.,* 1994; VanCamp *et al.,* 1998) and oxidative stresses (Morita *et al.,* 1999).

Root growth of plants growing under different type of stresses can be modulated by exogenous application of hydrogen peroxide. For example low concentrations of hydrogen peroxide when applied exogenously inhibit root development in alpine larch (Shearer, 1961). An increase concentration of $H_2O_2$ in rice roots cells, preceded root growth reduction caused by abscisic acid (ABA) (Lin & Kao, 2001). Contrary to this, low doses of hydrogen peroxide can increase in mass and length of roots (Narimanov & Korystov, 1997). In addition to this hydrogen peroxide has also been reported to stimulate germination of seeds and growth of sprouts (Narimanov & Korystov, 1997). Hydrogen peroxide can also be used for surface sterilization and disinfestations of pine (Barnett, 1976; James & Genz, 1981) and lettuce seeds (Pernezny *et al.,* 2001) to reduce root and leaf diseases caused by different soil born bacteria and fungi. However, some problems with seedling toxicity and reduced seed germination have also been reported (Edwards & Sutherland, 1979; James & Genz, 1981; Pernezny *et al.,* 2001) that warns its cautious application as seed disinfectant

The present study was, therefore, designed to characterize the influence of exogenous application of hydrogen peroxide on growth and development of wheat roots and the intact etiolated seedlings of various ages. The ultimate aim of study was to find out seedling age suitable for hydrogen peroxide induced wheat root growth enhancement and reducing soil and water borne diseases of roots. It is anticipated that any beneficial role of hydrogen peroxide on wheat root growth and physiology would be of major agricultural significance especially under water stressed environment and salinity.

## MATERIALS AND METHODS

Uniform sized seeds ($44.05 \pm 3.07$ mg) of wheat strain 1076 (*Triticum aestivum* L.) were germinated in darkness for 24 h at $25 \pm 1$°C on wet filter paper in petridishes. Germinated seeds were then covered with a lid to minimize the evaporation, and growth was continued for 24 h in darkness at $25 \pm 1$°C. To apply hydrogen peroxide treatment, water as the medium was changed with 90 mM hydrogen peroxide (Merck, Germany), and the growth of the seedlings was continued at $25 \pm 1$°C for another 6 days in darkness. Experiment was repeated thrice and for each replication at least 12 seedlings were used. The reagent solution was changed once a day for freshly prepared solutions to insure exogenous exposure of the seedlings to a uniform level of hydrogen peroxide, which is a pre-requisite, to study detailed effect of oxidative stress imposed by $H_2O_2$ on initial root growth of wheat.

**Measurement of growth.** Age of seedling was estimated in days starting from the beginning of the seed soaking time. Fresh weights of seedlings (starting from 3 to 8 days of age) were obtained immediately after taking out of petre plates. At least 36 treated and control seedlings were studied every day starting from 3rd to 8th day. After measuring total seedling fresh weight roots were separated and fresh weight of roots were taken. Root length (cm) was measured by spreading the roots on a scale calibrated in centimeters. Total number of roots for each seedling was also recorded each day to measure the rate of new root emergence. Changes in root weight, length and number were kept determined continuously at different days in both stressed and control seedlings.

**Statistical analysis.** All experiments were repeated three times, every time with three replications (12 seedlings per replication). Similar results and identical trends were obtained each time. The data being presented here is for one experiment replicated three times with 12 seedlings per replication for each day. The descriptive statistics including mean, standard deviation, median and sample variance were applied to analyze and organize the data. The significance of differences between means (for stressed & control seedlings) for different parameters was measured using Student's t-Test (two tailed) assuming unequal variances at 0.01 and where applicable at 0.05 significance level (add reference).

## RESULTS

**Fresh weight of seedlings.** Fresh weight of both control and stressed seedlings increased up to 4th day (Fig. 1) and then decreased. The age (day) at which seedling fresh weight was minimum differed in control and stressed seedlings. It was minimum (55.3mg) on 5th day in stressed seedlings and (117.8 mg) on 6th day in control seedlings. After 5th and 6th day there was a steady increase in fresh weight in both stressed and control seedlings respectively. Mean weight gain/day from 5th to 8th day in stressed seedlings (31.4 mg/day) was significantly higher ($p<0.01$) compared to that measured from 6th to 8th day in control (26.1 mg/day). Statistically, fresh weight of stressed seedlings (Fig. 1) was significantly lower ($p<0.01$) than control on 3rd to 7th day however the difference became non significant ($p>0.01$) on 8th day where percent fresh weight of stressed seedlings was 87.9 % of control. Maximum and highly significant difference ($P<0.001$) in fresh weight of stressed and control seedlings were observed on 5th day, here stressed seedlings fresh weight was just 44.404 % as that of control.

**Fig. 1. Mean fresh weight of control (*hatched columns*) and stressed (*white columns*) seedlings on various days**

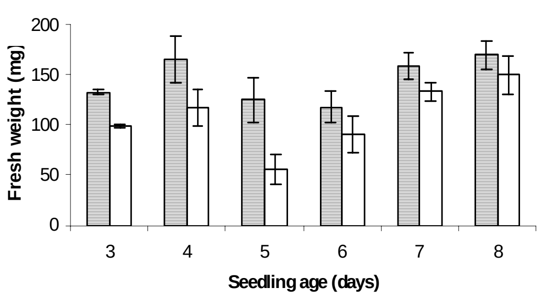
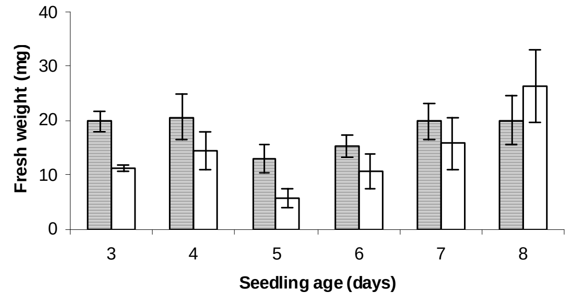



**Fig. 2. Mean fresh weight of roots in control (*hatched columns*) and stressed (*white columns*) seedlings on various days**




**Fresh weight of roots.** Fresh weight of roots in control and stressed seedlings increased slightly up to 4th day (Fig. 2) and then decreased significantly to a minimum of 5.7±1.8 mg on 5th day that was 44.1 % of control. Fresh weight of roots growing under stress was significantly ($p<0.01$) low (Fig. 2) compared to control on 3rd to 7th ($p<0.05$) day with the lowest ($P<0.01$) on 5th day. On 8th day however, mean fresh weight of roots in stressed seedlings increased significantly (24% of control). After 5th day there was a steady increase in fresh weight of roots in both stressed and control seedlings. Mean weight gain per day from 5th to 8th day was significantly higher ($p<0.01$) in stressed seedlings (6.9 mg/day) compared to control (2.5/day).

**Root length.** Mean root length increased significantly but only in control seedlings between 3rd and 4th day. After that a significant ($p< 0.01$) shrinkage was observed on 5th day in

**Fig. 3. Comparison of seedling age and root length in control (*hatched columns*) and stressed (*white columns*) seedlings**

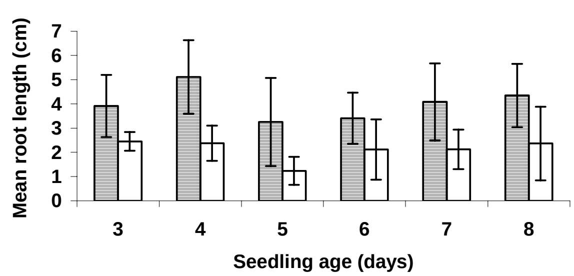
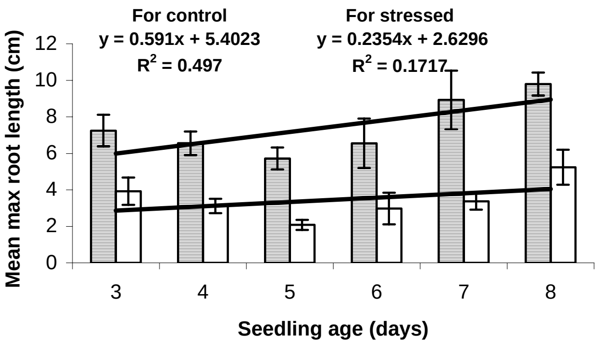


**Fig. 4. Comparison of seedling age and mean maximum (primary) root length in control (hatched columns) and stressed (white columns) seedlings**



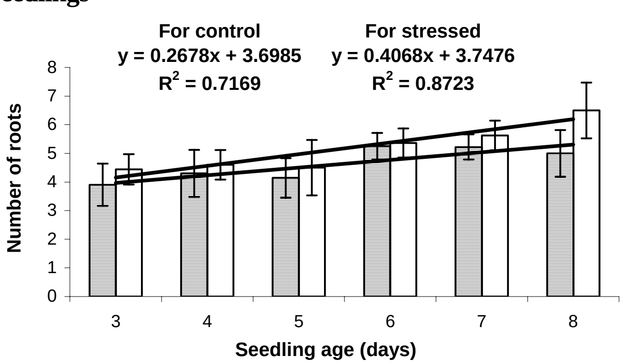

both stressed and control seedlings (Fig. 3), which resulted into a decrease in root length.

After 5th day, an increase in mean root length was observed in control seedlings while in stressed seedlings no considerable change occurred between 6th and 7th day. Mean increase in root length/day from 5th to 8th day was almost similar in control (0.39 cm/day) and stressed seedlings (0.37 cm/day in). In general mean root length was significantly lower ($p<0.01$) in stressed seedlings compared to control (Fig. 3) on all days.

Primary root length (cm) was significantly lower ($p<0.01$) in stressed seedlings on all days with highly significant ($P<0.001$) difference on 5th, 7th and 8th day (Fig. 4). Shrinkage in primary root length was observed from 3rd to 5th day in control as well as stressed seedlings with maximum shrinkage observed on 5th day. However the rate of shrinkage in maximum root length per day from 3rd to 5th day was almost similar stressed and control. After 5th day primary root length started increased gradually in control and stressed seedlings with a mean increase of 1.357cm/day in control and 1.053 cm/day in stressed seedlings. Root elongation process was more rapid in control ($R^2$ 0.497) then in stressed ($R^2$ 0.172) seedlings.

**Root number.** Number of roots in control and stressed seedlings increased with increasing age (Fig. 5) but rate of increase was more rapid in stressed seedlings ($R^2$ 0.873) compared to that in control ($R^2$ 0.717). Contrary to other parameters such as root weight and length, root number was higher in stressed seedlings on all days in general and on 7th and 8th day old seedlings in particular. However difference in root number of stressed and control seedlings became significant ($P<0.01$) on 8th day) (123.077% of control).

**Fig. 5. Comparison between seedling age and root no in control (hatched columns) and stressed (white columns) seedlings**




## DISCUSSION

The influence of oxidative stress induced by hydrogen peroxide on growth and development of roots in wheat during early ontogenesis has not yet been studied nevertheless it has been documented (Anonymous, 2002) recently that oxidative stress produce at cellar level by low dose irradiation of seeds can stimulates seed germination and other stages of plant development. For example, oxidative stress induced by ionizing radiation and hydrogen peroxide can stimulates growth of sprouts and roots in barley, wheat, pea, maize and melon (Anonymous, 2002). Irradiation of seeds can also stimulate growth of roots in terms of increase in mass and length (Narimanov & Korystov, 1997). In present study, it was observed significantly higher weight gain per day in stressed seedlings (Fig. 1) and roots (Fig. 2) compared to that in control after 5th day. This indicated that oxidative stress induced by $H_2O_2$ could accelerate seedling and specifically root growth in wheat. The process of weight gain started one day earlier in stressed seedlings (Fig. 1) and 24% more fresh weight of stressed roots on 8th day (Fig. 2) provides not only the further evidences for growth enhancing effect of $H_2O_2$ but at the same time it also signify the importance of exogenous application of $H_2O_2$ for wheat root growth enhancement and prevention from diseases (Barnett, 1976; James & Genz 1981; Pernezny *et al.*, 2001). This also suggest that exogenous application of $H_2O_2$ should be made after 5th day of seedling age especially in wheat, thus bypassing the period in which $H_2O_2$ treatment may have growth suppressive effect.

Significant reduction in root length under stressed condition (Fig. 3 & 4) indicates suppression of root elongation process i.e. cell elongation and division. In the present study however, reduction in root length may have occurred due to inhibition of cell elongation process alone and not due to cell division, as there was a significant increase in root number and weight, which is possible only if active cell division in taking place. Hydrogen peroxide therefore enhances cell division either as primary or secondary effect to counter balance the inhibition process of cell elongation. As reported earlier (Potikha *et al.*, 1999), exogenous application of $H_2O_2$ prematurely promoted the secondary wall formation; therefore inhibition of root cell elongation may be a consequence of premature secondary wall formation in root cells thus blocking the further cell elongation.

Oxidative stress induce by irradiation of seeds with 20 cGy, has been reported (Narimanov & Korystov, 1997) to significantly increase the number of lateral roots as compared to control melon seedlings. In the present study we have also observed higher number of roots (Fig. 5) in stressed seedlings on all days with a significant increase on 8th day, which increases root weight of stressed seedlings up to 24% on the same day. Thus $H_2O_2$ induced oxidative stress appeared to have the ability of enhancing the process of new root emergence, which can be attributed to plant defense response to abolish the effect of stress (reduction in root length & weight) thereby, helping the plant to establish properly for attaining proper growth under stress condition. Moreover a significantly low root length (Fig. 3 & 4) and high root number (Fig. 5) in stressed seedlings indicates that primary root growth is halted while secondary root emergence and growth is promoted under $H_2O_2$ induced oxidative stress. This observation can be supported indirectly by the study of Ren *et al.* (2000), who observed an increased level of $H_2O_2$ in wheat root cells, which enhanced the root growth under drought stress. Exogenous application of $H_2O_2$ to wheat seedlings can thus result in to a significant increase in fresh weight and number of root by promoting new roots emergence that may provide more vigorous root system to wheat plant. Furthermore, it has been reported very recently in a field trial that early vigorous root growth was major factor for higher nitrogen up take in wheat (Liao *et al*., 2004). More vigorous root growth by exogenous hydrogen peroxide consequently will cause higher nitrogen uptake ensuing better growth and yield of wheat plant.

Collectively root growth enhancement by exogenous application of $H_2O_2$ may be of tremendous agricultural importance especially in water deficient and saline area where crops cannot survive due to poorly developed root system hence well-developed root system can improve seedling survival rate. In addition vigorous root growth by applying exogenous hydrogen peroxide can be used to increase nitrogen uptake resulting in better growth and yield.

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