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Influence of Hydrogen Peroxide on Initial Leaf and Coleoptile Growth in Etiolated Wheat (*Triticum aestivum* L) Seedlings

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Abstract: Influence of oxidative stress induced by exogenous application of hydrogen peroxide on initial leaf and coleoptile growth in etiolated wheat seedlings was studied. Leaf and coleoptile growth parameters (mean fresh weight and length) showed a transient stress effect during very initial age, becoming non-significant ($P>0.05$) in later age (8th day). Mean leaf fresh weight increased steadily after 5th day in both stressed and control seedlings, however the magnitude was lower in stressed seedlings and the difference was non-significant ($p>0.05$). Mean coleoptile fresh weight was significantly ($p<0.01$) lower in stressed seedlings on all days except on 6th and 8th day where the difference was non-significant ($p>0.05$). Mean leaf and coleoptile length were significantly ($p<0.01$) lower in stressed seedlings during 5 to 7th day, however the difference was non-significant ($p>0.05$) on 8th day. Stress effect was more pronounced on 5th day in case of leaf and coleoptile length and coleoptile fresh weight. Collectively oxidative stress induced by exogenous application of hydrogen peroxide induces a transient and finally a non-significant effect on initial leaf and coleoptile growth, which indicated the adaptation of seedlings to applied stress.

Key words: Abiotic stress, H_2O_2, oxidative stress, reactive oxygen species, *Triticum aestivum* L

INTRODUCTION

Hydrogen peroxide (H_2O_2) is a key player in oxidative stress signaling leading to the induction of defence response (Morita *et al.*, 1999; VanCamp *et al.*, 1998). The chilling (Prasad *et al.*, 1994), heat (Dat *et al.*, 1998), oxidative stress (Morita *et al.*, 1999), pathogens (Levine *et al.*, 1994 and Alvarez *et al.*, 1998), drought and salt stress can give rise to excess concentrations of hydrogen peroxide, resulting in oxidative damage at the cellular level (Zhang *et al.*, 2001). For example heat stress can affect coleoptile length and eventually the germinability in wheat (Sharma and Tandon, 1995) as some times seedlings did not emerge when their coleoptile stopped growing and the first true leaf developed below the soil surface (Guedira *et al.*, 1997). Excess hydrogen peroxide can also reduce root growth and development in alpine larch (Shearer, 1961) and rice (Lin and Kao, 2001).

Contrary to above exogenous application of hydrogen peroxide has been reported to stimulate seed germination and growth by increasing mass and length of sprouts and roots (Narimanov and Korystov, 1997). Hydrogen peroxide has also been effectively used for surface sterilization, disinfestations and as a management to reduce many bacterial and fungal root and leaf diseases in Pine (Barnett, 1976; James and Genz, 1981) and Lettuce (Pernezny *et al.*, 2001) along with problems of seedling toxicity and reduced seed germination (Edwards and Sutherland, 1979; James and Genz, 1981; Pernezny *et al.*, 2001).

The growth of the first leaf could be considered as an indicator of the success of crop management strategies at the sowing and early growth stages (Boubakeur *et al.*, 1999). Studying the influence of hydrogen peroxide on initial leaf and coleoptile during early growth stages using few-days-old seedlings may provide reliable predictions, which could be of tremendous agricultural importance for better crop production. Moreover this approach is much simple, less time consuming and labor inexpensive.

In the present studies we have analyzed the influence of hydrogen peroxide on initial leaf and coleoptile growth during early ontogenesis, in intact etiolated wheat seedlings of various ages.

MATERIALS AND METHODS

Uniform sized seeds (44.05±3.07 mg) of wheat line1076 (*Triticum* aestivum L.) (Developed by Plant Molecular Breeding Group (PMBG) of NIAB, Faisalabad, Pakistan) were germinated in darkness for 24 hours at 25±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±1 °C. To apply hydrogen peroxide treatment, water as the medium was changed with 88 mM hydrogen peroxide (Merck, Germany) and the growth of the seedlings was continued at 25±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 (88 mM hydrogen peroxide) was changed once a day for freshly prepared solutions to ensure 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 leaf and coleoptile of wheat.

Measurement of growth: Seedling age was estimated in days starting from the beginning of the seed soaking time. Growth changes in initial leaf and coleoptile in at least 36 treated and control seedlings were studied every day starting from 3rd to 8th day by measuring changes in seedling fresh weight and length. Leaf and coleoptile elongation rate (increase in length per day) were also calculated and compared in stressed and control seedlings. For these measurements initial leaf and coleoptile from each seedling were weighed immediately. Coleoptile and leaf lengths were measured from the point where the seedling ruptured the seed coat to the tip. Length was measured by spreading the leaf and coleoptile on a scale calibrated in centimeters.

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 4 seedlings per replication for each day. The descriptive statistics were applied to analyze and organize the resulting data. The f-test was applied to find differences in variance among samples. The significance of differences between means (for control and H_2O_2 treated) 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. All the statistical calculations were performed by using computer software Microsoft Excel 2000.

RESULTS

Leaf fresh weight: Mean leaf fresh weight (mg) increased gradually (Fig. 1a) in both control and stressed seedlings. Magnitude of mean leaf fresh weight was lower in stressed seedlings after 3rd day however the difference was non significant ($p > 0.05$). Rate of increase in mean leaf fresh weight was slow during 3rd to 5th day in both type of seedlings followed by more rapid increase between 6th to 8th day of germination. Minimum and maximum values for leaf fresh weight were also observed on similar days in both types of seedlings. Collectively hydrogen peroxide treatment induced a non-significant effect on leaf growth in etiolated wheat seedlings during early ontogenesis.

Leaf length: Mean leaf length (cm) was significantly ($p < 0.01$) lower in stressed seedlings (Fig. 1b) as compared with control but only on 5 to 7th day, however the deference was non-significant ($P > 0.01$) on 8th day. Mean leaf length increased steadily (R^2 0.970) in control seedlings from 3rd to 8th day with least increase on 5th day. In stressed seedlings leaf elongation rate was slightly lower than control (R^2 0.970) as evident from regression coefficient value (R^2 0.866). Mean leaf elongation rate (increased in length per day) in stressed seedlings was 0.086 cm for 4 to 5th day which indicated that no increase in leaf length take place between these day. In other word there was a non-significant ($P > 0.05$) deference in leaf length on 4 and 5th day in stressed seedlings.

Max leaf length increased from 3rd to 4th day and then decreased on 5th day in both control and stressed seedlings (Fig. 1c). After 5th day max leaf length increased gradually till 8th day in control seedlings. In stressed seedlings max leaf length increased on 6th day followed by a decrease on 7th day and then again increased rapidly on 8th day. Comparing mean maximum leaf length in both control and stressed seedlings, it was almost equal on 3rd day but lower in stressed seedlings on 4th, 5th (maximum 44% inhibition) 6th and 7th day (41% inhibition) and again almost equal in both types of seedlings on 8th day.

Fresh weight of coleoptiles: Fresh weight (mg) of coleoptile in control and stressed seedlings increased from 3rd to 4th day (Fig. 2a), followed by a decrease to a minimum 12.7 mg on 5th day in stressed seedlings and on 6th day (21.5 mg) in control seedlings. After 6th day in control seedlings, coleoptile fresh weight first increased (27.5 mg) on 7th day and then decreased on 8th day to 22.0 mg, similar to that in stressed seedlings (22.2 mg) on same day. In stressed seedlings after 5th day, coleoptile fresh weight increased (17.6 mg) on 6th day to a value previously on 3rd day and was similar to that in control on 6th day. Then coleoptile fresh weight remained almost same between 6 and 7th day (17.7 mg) and increased on 8th to 22.2 mg.

Coleoptile fresh weight was significantly lower ($p < 0.01$) in stressed seedlings (Fig. 2a) on 3rd ($p < 0.01$), 4th ($p < 0.01$) 5th, ($p < 0.01$) and 7th ($p < 0.05$) day. Mean

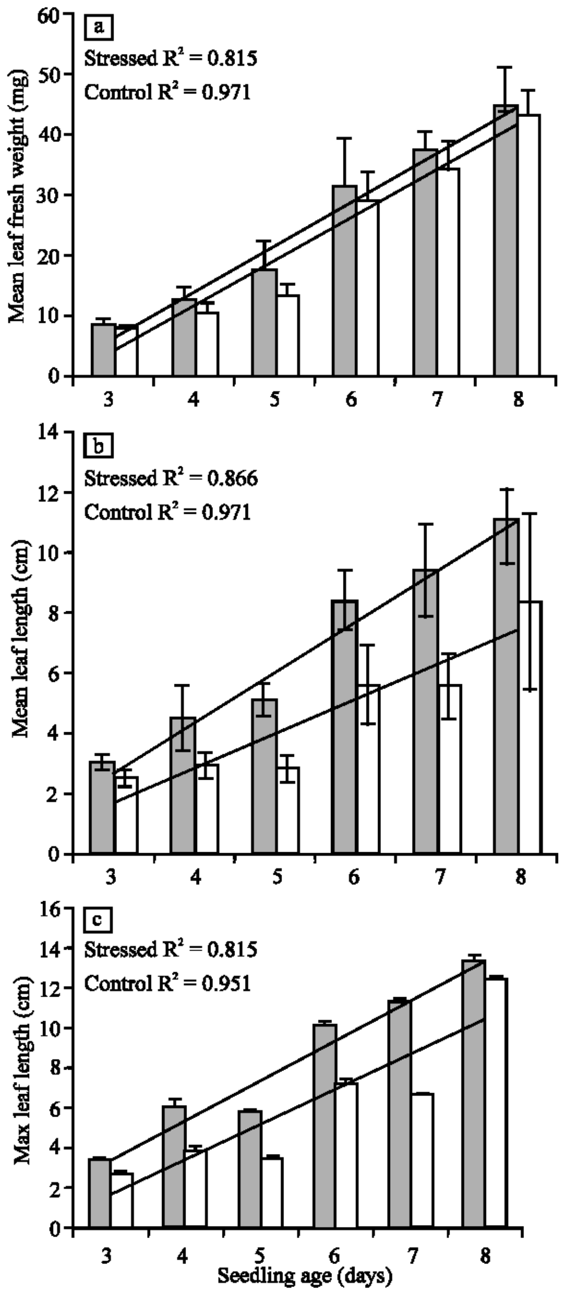

Fig. 1: Comparison of leaf growth rates in seedlings grown in absence (control) (gray columns) or presence (white columns) of exogenous hydrogen peroxide. Seedling age (x-axes) is expressed in days. a. Comparison of seedling age and mean leaf fresh weight. b. Comparison of mean leaf length and seedling age. c. Comparison of seedling age and maximum leaf length

coleoptile fresh weight was same in control and stressed seedlings on 6th and 8th day with a non-significant

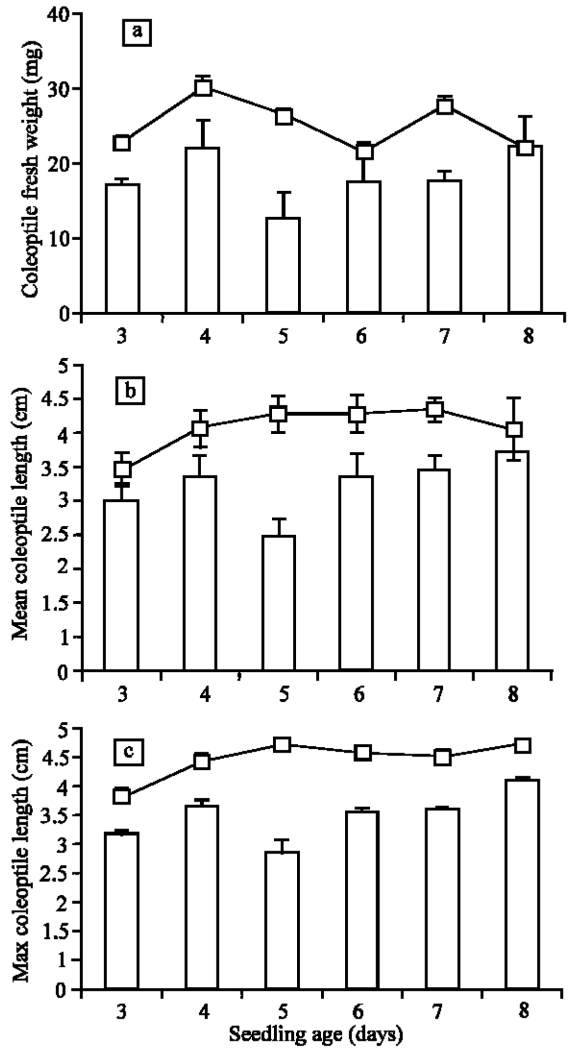

Fig. 2: Comparison of coleoptile growth rates in seedlings grown in absence (control) (line) or presence (columns) of exogenous hydrogen peroxide. Seedling age (x-axes) is expressed in days. a. Comparison of seedling age and mean coleoptile fresh weight. b. Comparison of mean coleoptile length and seedling age. c. Comparison of seedling age and maximum coleoptile length

($p>0.05$) difference. Maximum and highly significant ($P<0.001$ and 52% inhibition) difference in coleoptile fresh weight was observed on 5th day.

Coleoptile length: Mean coleoptile length (cm) showed a parabolic curve like trend in control seedlings, a consistent but slow increase from 3rd to a maximum 3.822 cm on 7th day followed by a decrease on 8th day (Fig. 2b). In H_2O_2 treated seedlings, on the other hand, mean coleoptile length increased from 2.5 cm on 3rd day to 2.8

cm on 4th day. On 5th day there was a slight and transient reduction in mean coleoptile length (Fig. 2b) most probably due to extensive water loss and cell shrinkage. On 6th day, mean coleoptile length increased again to 2.9 cm almost same as on 4th day. Here after mean coleoptile length increased slowly to a maximum 3.2 cm on 8th day. Mean coleoptile elongation rate between 6th and 7th day was negligible bother 0.06 cm in control and stressed seedlings. This designates that mean coleoptile length remained same between 6 and 7th day.

Mean coleoptile length was significantly ($p<0.01$) lower in stressed seedlings during 5th to 7th day, however the deference was non-significant ($p>0.05$) on 8th day. Maximum and highly significant ($P<0.001$) difference was observed on 5th day.

In control seedlings max coleoptile length increased up to 5th day (4.2 cm) and then there was no net increase in length but a slight decrease on 6 and 7th (0.2 cm) day (Fig. 2c). In stressed seedlings max coleoptile length increased up to 4th day (3.2 cm), then decreased hasty ($p<0.01$) on 5th day with a maximum 47% inhibition and then there was a gradual increase.

DISCUSSION

The growth of the first leaf could be considered as an indicator of the success of crop management strategies at the sowing and early growth stages (Boubakeur *et al*., 1999). Therefore studying the influence of hydrogen peroxide on initial leaf and coleoptile during early growth stages using few-days-old seedlings may provide reliable predictions about its possible impact on crop after its field application.

Our studies indicated that exogenous application of hydrogen peroxide induces age dependent cyclic changes in leaf length. Effect of H_2O_2 treatment on maximum leaf length appeared after one day and here after with cyclic increase and decrease stress effect start diminishing. A non-significant difference in mean leaf and coleoptile length on 8th day indicates that exogenous application of hydrogen peroxide during initial growth of seedlings induces a transient effect on leaf and coleoptile elongation process and effect reduces to a significant extant within next few days. The possible explanation for this behavior is two fold i.e. either seedlings had passed away the growth period in which they may had adverse effect of treatment or they got adapted to stress via inducing their defense response. The first possibility is explained by the fact that induction of anti growth activity like apoptosis due to exogenous application of hydrogen peroxide has been reported during this period in both leaf and coleoptile (Zamyatnina *et al*., 2002). Second possibility that seedlings got adapted to stress with passage of time is also sported by the fact as it has been reported that Exogenous hydrogen peroxide 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). Therefore hydrogen peroxide is a key player in oxidative stress signaling and leading to the induction of defence response, which enabled the seedlings to adopt the stress.

As there was transient leaf and coleoptile growth suppression during initial days, exogenous application of H_2O_2 for any beneficial use i.e. root growth improvement and prevention from disease, should be made after 8th day of seedling life. Bay passing the period of growth suppression by H_2O_2 and applying it at least after 8th day of germination, this treatment may have a pronounced growth stimulating effect on sprout as already reported in barley, wheat, pea, maize and melon after low dose oxidative stress (Anonymous, 2002).

Oxidative stress at cellular level in abiotic stresses, for example heat, can affect coleoptile length as well as number of primary roots and eventually the germinability, in wheat (Sharma and Tandon, 1995). Under stressed condition, proper coleoptile growth is very important, as some times seedlings did not emerge when their coleoptile stopped growing and the first true leaf developed below the soil surface (Guedira *et al.*, 1997). A final non-significant change in coleoptile weight and length therefore roll out such possibility as a result of hydrogen peroxide treatment.

Furthermore hydrogen peroxide has also been used for surface sterilization and disinfestations of pine (Barnett, 1976; James and 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 and Sutherland, 1979; James and Genz, 1981; Pernezny *et al*., 2001) that warns its cautious application as seed disinfectant. Collectively, as evident from a final non-significant effect on leaf and coleoptile growth, no seedling toxicity and reduced seeding growth due to exogenous application of H_2O_2 was observed. Therefore potential use of hydrogen peroxide for surface sterilization and disinfestations of wheat seeds to reduce root and leaf diseases caused by different soil born bacteria and fungi, which could be of tremendous agricultural importance for better crop production may be investigated.

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