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Glutathione Metabolism in Four Indian Wheat Cultivars Exposed to Long-term Sulfur Dioxide

By

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Summary

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The toxicity induced by H_2O_2 due to the exposure of 58 µg m^{-3} SO_2 for 6 h day^{-1} for 18, 20 and 32 days was tolerated by cv. Lok 1, cv. Sujatha and cv. WH 147. Further exposure resulted in accumulation of damaging H_2O_2 levels (critical) and visible injury symptoms, while cv. Raj 1555 tolerated the SO_2-induced stress even when exposed for 45 days. This was related to their ability to enhance glutathione reductase (GR) activity and maintain high GSH/GSSG ratio. Our results indicated that plants do not accumulate damaging levels of H_2O_2 as long as they maintain high GSH/GSSG ratio via enhanced GR activities. Possible ways of H_2O_2 oxidation has been discussed.

Introduction

It is widely accepted that gaseous SO_2 turns to SO_3^{2-} and HSO_3^- with in leaf tissue and only few percent of these species are incorporated into the sulfur metabolites in plants (HALLGREN 1978). Most of the SO_3^{2-} and HSO_3^- ions are photooxidized to less toxic SO_4^{2-} in the chloroplast and this photooxidation is accompanied by propagation of superoxide anion (ASADA & KISO 1973). Superoxide anion, in chloroplasts, is catalyzed by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). H_2O_2 is potentially toxic. Low concentrations can inactivate the Calvin cycle enzymes substantially. Furthermore, H_2O_2 can react with superoxide to form highly reactive and toxic hydroxyl radicals (HALLIWELL & FOYER 1978). It is, therefore, essential for plants to have an effective means of detoxifying H_2O_2.

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Ascorbate peroxidase (AP) catalyses the peroxidation of ascorbate (ASA) to dehydroascorbate (DHA) and DHA reductase utilizes reduced glutathione (GSH) to regenerate ASA (JABLONSKY & ANDERSON 1981). Oxidized glutathione (GSSG) is, in turn, reduced by NADPH dependent glutathione reductase (GR) (FOYER & HALLIWELL 1976). Thus, GSH appears to play an important role in metabolizing H_2O_2 to H_2O by providing sufficient ASA levels.

The differential response of plants to SO_2 has been attributed to their ability to modify antioxidants like glutathione and ascorbate. However, studies which support the protective nature of antioxidants have been obtained from short-term studies involving high concentrations. Till, to date, to our knowledge, we are devoid of information regarding the protective nature of antioxidants under long-term SO_2 exposure. In the present paper we have attempted at investigating the impact of long-term SO_2 exposure on the glutathione metabolism.

Materials and Methods

Seeds of certain local cultivars of wheat (*Triticum aestivum* L. cv. Lok 1, cv. Sujatha, cv. WH 147 and cv. *Triticum durum* Desf. cv. Raj 1555) were obtained from Regional Wheat Research Institute, ICAR, Indore, India and were grown in 1 x 1 m plots containing black cotton soil. Upon emergence, the seedlings were thinned with adequate addition of fertilizers (N P K) and the plots were irrigated with rain water on alternate days. The farming conditions employed in the present study are similar to those of local farming practices.

The plants were allowed to grow for 29 days. On day 30, the plants were exposed to SO_2 in 1 x 1 x 1 m polyethene open top chambers with internally distributed teflon pipe system for uniform pollutant distribution. The plants were fumigated with SO_2 for 6 h day^{-1} for 45 days. Desired SO_2 concentration was generated by bubbling air through 1 % aqueous sodium metabisulphite and the available concentration at plant canopy height was monitored by Toxic Gas Monitor 555, CEA Inst., USA at regular intervals (58 ± 8 µg m^{-3}). Control plants were placed in similar chambers with no addition of SO_2 to the background levels (5.4 ± 1.4 µg m^{-3}). The climatic conditions during the experimental period were as follows (mean values) : temperature, 35/18° C (day/night); relative humidity, 42 - 58 %; and PAR (natural) ranging between 0 - 1236 µmol m^{-2} s^{-1} (9 to 10 h photoperiod).

The flag leaves were collected periodically at around 10:00 hours and were analyzed for foliar H_2O_2, GSH/GSSG ratio and GR activity following ASADA 1984, GRIFFITH 1980 and HALLIWELL & FOYER 1978, respectively. The whole experiment was a randomized block design with two replicates each yielding at least 3 leaf samples and the results were statistically analyzed by ANOVA.

Results and Discussion

H_2O_2 accumulated significantly in the flag leaves of cv. Lok 1, cv. Sujatha, cv. WH 147 and cv. Raj 1555 when exposed to SO_2 for 4, 10, 20 and 31 days, respectively, compared to that of unexposed plants (Fig. 1). On further exposure to SO_2, H_2O_2 levels increased significantly in cv. Lok 1, cv. Sujatha and cv. WH 147 while there was no significant enhancement in cv. Raj 1555 till end of the experiment (Fig. 1). SO_2 exposure for 4 days resulted in enhanced GSH/GSSG ratio (enhanced GSH content, individual data not presented) and GR activity in all the

four cultivars, however, with maximum changes in cv. Raj 1555 followed by that of cv. WH 147, cv. Sujatha and cv. Lok 1 when compared to that of unexposed plants (Fig. 2, 3). Further exposure of SO_2 resulted in declined GSH/GSSG ratio (enhanced GSSG content) and GR activity in cv. Lok 1, cv. Sujatha and cv. WH 147 when compared to that of unexposed plants, while the ratio and GR activity of cv. Raj 1555 increased or remained constant till the end of fumigation. Visible injury symptoms appeared on cv. Lok 1, cv. Sujatha and cv. WH 147 during 18, 20 and 32 d of SO_2 exposure while cv. Raj 1555 exhibited no visible injury symptoms even when exposed for 45 days (represented by * in Fig. 1).

The toxicity of SO_2 is generally believed to increase with increase in oxygen free radical formation (MEHLHORN & al. 1987). Plants are believed to posses certain scavengers like glutathione to counteract the oxygen free radical toxicity and the ability of a plant to detoxify oxygen free radicals is considered to be a tolerance mechanism (GUPTA & al. 1991, RAO 1992). Though the role of total glutathione has been investigated, to some extent, in a number of plant stresses which act, at least in part, via oxygen species (PRICE & al. 1990), the relative importance of its distribution between GSH and GSSG has not been extensively investigated. Glutathione, apart from scavenging the oxygen free radicals, also participate in sulfur metabolism and gene expression (ALSCHER 1989) and hence, the ratio of GSH/GSSG shall be an important aspect to understand the protective nature of glutathione.

Enhanced H_2O_2, GSH/GSSG ratio and GR activity was the general response observed in plants exposed to SO_2, however, dependent on exposure period. SO_2 exposure for 18 and 20 d enhanced GR activity in cv. Lok 1 and cv. Sujatha, respectively, and thereby restored sufficient GSH levels (high GSH/GSSG ratio) to metabolize H_2O_2. On further exposure, GR activity of both the cultivars declined with a concomitant decline in GSH/GSSG ratio which, in turn, resulted in accumulation of H_2O_2 resulting in visible injury symptoms. Similarly, cv. WH 147 exposed to SO_2 for 32 d maintained sufficient GSH levels to metabolize H_2O_2 and further exposure resulted in declined GR activities accumulating H_2O_2 (critical levels) and resulting in visible injury symptoms. But, cv. Raj 1555 even when exposed to SO_2 for 45 d maintained enhanced GR activities and sufficient GSH levels to metabolize H_2O_2 which, in turn, exhibited no visible injury symptoms. The above results permit us to conclude that cv. Lok 1, cv. Sujatha, cv. WH 147 and cv. Raj 1555 posses the ability to tolerate SO_2 induced stress for 18, 20, 32 and 45 days, respectively. Basing on the H_2O_2 scavenging system (critical levels of H_2O_2), the species studied are arranged in the decreasing order of tolerance as follows:

cv. Raj 1555 > cv. WH 147 > cv. Sujatha > cv. Lok 1.

From the above results, it is clear that as long as plants posses the ability to enhance GR activity and thus restore sufficient GSH levels, low concentrations of SO_2 do not result in accumulation of critical H_2O_2 levels and visible injury symptoms.

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References

ALSCHER R.G. 1989. - Physiol. Plant. 77: 457-464.

ASADA K. 1984. - In: PACKER L. (ed.). Methods in Enzymology, Vol. 105, Academic Press, New York, pp. 422-429.

— & KISO K. 1973. - Eur. J. Biochem. 33: 253-257.

FOYER C. H. & HALLIWELL B. 1976. - Planta 133: 21-25.

GRIFFITH O. W. 1980. - Anal. Biochem. 106: 207-212.

GUPTA A. S., ALSCHER R. G. & MCCUNE D. 1991. - Plant Physiol. 96: 650-655.

HALLGREN J. E. 1978. - In: NRIAGU O. (ed.) Sulphur in the Environment, John Willey & Sons, New York, pp. 163-209.

HALLIWELL B. & FOYER C. H. 1978. - Planta 139: 9-17.

JABLONSKI P. P. & ANDERSON J. W. 1981. - Plant Physiol. 67: 1239-1244.

MEHLHORN H., COTTAM D. A., LUCAS P. W. & WELLBURN A. R. 1987. - Free Radical Res. Comm. 3: 193-197.

PRICE A., LUCAS P. W. & LEA P. J. 1990. - J. Exp. Bot. 41: 1309-1317.

RAO M. V. 1992. - J. Plant Physiol. (in press).

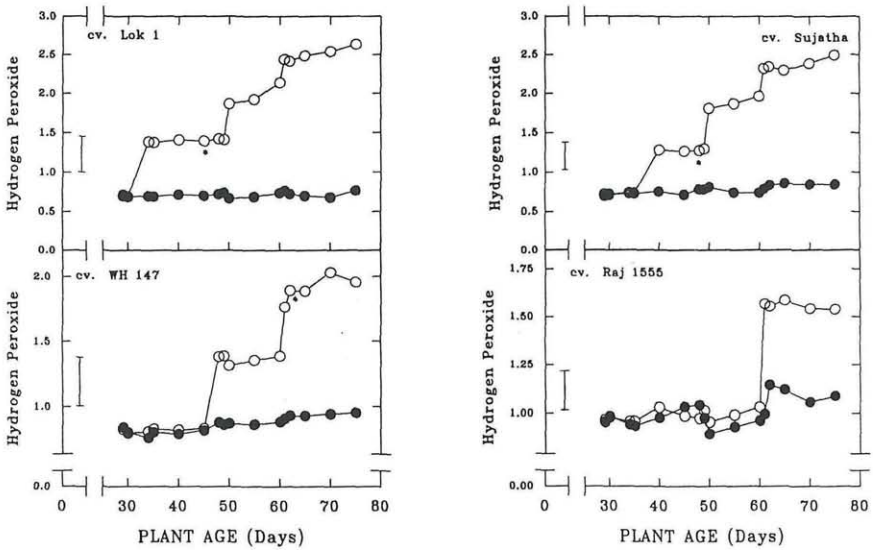

Fig. 1. The impact of 58 µg m^{-3} SO_2 for 6 h day^{-1} for 45 days on the foliar hydrogen peroxide (H_2O_2) (µmol g fresh $weight^{-1}$) of four different Indian wheat cultivars. Vertical bars indicate LSD ($P < 0.05$). • Unexposed; o SO_2 exposed. * Indicate appearance of visible injury symptoms.

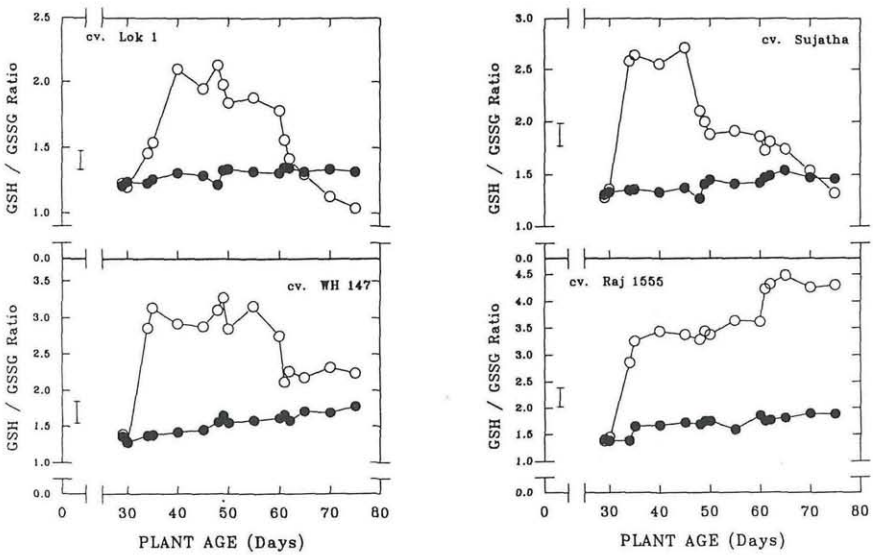

Fig. 2. The impact of 58 µg m^{-3} SO_2 for 6 h day^{-1} for 45 days on the foliar GSH/GSSG ratio of four different Indian wheat cultivars. For legends see Fig. 1.

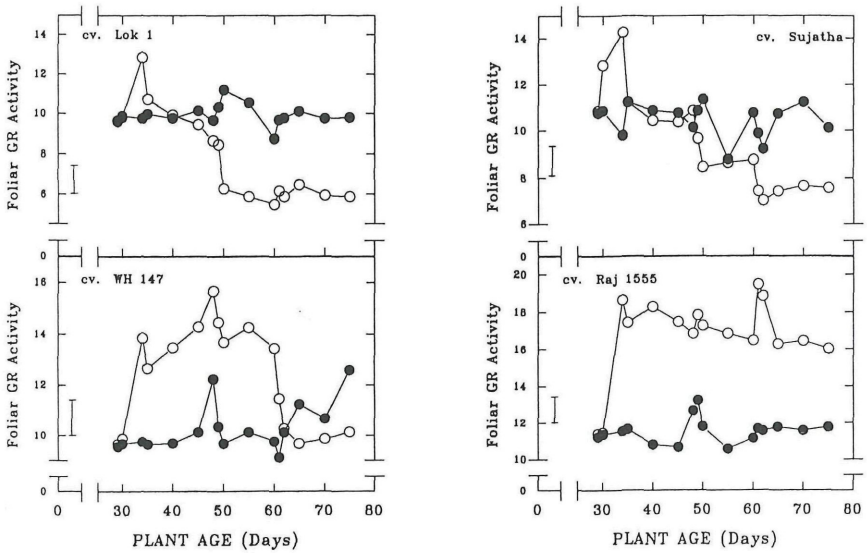

Fig. 3. The impact of 58 µg m^{-3} SO_2 for 6 h day^{-1} for 45 days on the foliar glutathione reductase (GR) activity (nmoles NADPH oxidized mg $protein^{-1}$ min^{-1}) of four different Indian wheat cultivars. For legends see Fig. 1.