EFFECTS ON PROLINE, PHENOLICS AND ANTHOCYANIN CONTENT OF *RAPHANUS SATIVUS* L. CV. PUSA CHETKI UNDER SO_2 POLLUTION STRESS

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Abstract.

For examining the content of proline, phenolics and anthocyanin in radish (*Raphanus sativus* L. cv. Pusa chetki), field experiments were conducted under artificial exposure of 1306 μm^{-3} SO_2 in closed polythene chambers for 2 h at alternate days. On prolonged exposure, significant increase in proline, phenolics and anthocyanin content was observed. SO_2 gas is absorbed in mesophyll through stomata and alters the metabolic processes of plants and cause abiotic stress. Proline has been shown to protect plants against free radical induced damage which accumulation is linked to quenching of single oxygen. Total phenolics typically have high oxygen radical absorbance capacity (ORAC) value, which helps in amelioration of SO_2 phytotoxicity. Anthocyanins have been suggested to act as potent antioxidants. So, proline, phenolics and anthocyanins can play important role against abiotic stress.

Keywords- SO_2 pollution; proline; phenolics; anthocyanines, stress, antioxidants.

I. Introduction

Sulphur dioxide (SO_2) is one of the major phytotoxic pollutants and emission level of SO_2 is increasing rapidly due to industrialization and urbanization. After absorption, SO_2 dissolves in the aqueous phase of the cell wall to form bisulphite (HSO_3^-) or sulphite (SO_3^{2-}), which then undergoes enzymatic conversion to SO_4^{2-} (Jeyakumar *et al*, 2003). SO_2 dissolves in extra cellular fluid of plants and reacts with plant materials to produce ionic species and free radicals, which are generally more reactive than sulphur dioxide (Hoffman and Jacob, 1984). This dissolved sulphur dioxide is potentially capable of behaving as an oxidant and reductant depending upon redox potential of the system. As a result of reaction of these ionic species with lipid and proteins in cell walls and membranes, chain reactions are initiated and giving rise to more free radicals such as superoxides, single oxygen, hydroxyl ion (OH^-) *etc*. So, the level of ascorbic acid, β-carotene and phenolic compounds increase which provide protection against sulphur dioxide phytotoxicity by removing these free radicals (Mandal and Mukharji, 1998; Jeyakumar *et al*., 2003). The effects of SO_2 pollution have been extensively studied in several crop plants but a little work has been done on amelioration of SO_2-induced phytotoxic effects in crop plants. The present study will help in identifying natural chemicals for amelioration of SO_2-inuced phytotoxicity.

II. Materials and Methods

The present study was conducted at Agricultural Research Farm, C.C.R.(P.G.) College, Muzaffarnagar. The seeds of *Raphanus sativus* L. cv. Pusa chetki were sown in research plots with line to line distance of 30 cm and plant to plant to plant distance of 10 cm. The fumigation chamber was made up of transparent polythene (1m x 1m x 1m dimension) supported on iron frame. A rubber tube was fixed to each chamber for entry of SO_2 gas. Small fan was used to circulate the air to reduce leaf boundary layer resistance. SO_2 was produced by passing a continuous current of air through aqueous sodium metabisulphite ($Na_2S_2O_5$) solution, which is ionized under pressure to produce SO_2 (Agrawal *et al*., 1982). SO_2 was passed through anhydrous calcium chloride for absorbing moisture from the gas. Gas was introduced within fumigation chamber along with additional flow of air through the perforated alkathene tubes for uniform distribution of gas within chamber. The plants were exposed to 1306 $\mu g m^{-3}$ concentration of SO_2 on alternate days for two hours till maturation in four beds. A control was run in identical condition but without any SO_2 fumigation.

Four destructive harvests of some plants were made at 15 days interval so as to analyze the plants with respect to biochemical analysis of proline (Bates *et al*., 1973), phenolics (Sadasivam and Manickam,1992) and anthocyanin (Mancinelli *et al.*, 1975). The data were statistically analyzed applying *t*-test

III. Results

In comparison to control, significant increase was observed in content of proline, phenolics and anthocyanin. Total foliar proline content was increased significantly in SO_2 treated plants comparison to control. For example, in 15-days old plants, percent increase in proline contents with respect to control was 10.3percent, while in case of 60-days old plants, increase in foliar proline content was 46.7 percent (Table-1). Total foliar phenolic content was also increased significantly in SO_2 treated plants. For example, in 15-days old plants, percent increase in phenolic contents of SO_2 treated plants in comparison to control was 18.1, while in case of 60-days old plants, increase in foliar phenolic content was 54.3, percent (Table-2). Significant increase in foliar anthocyanin content in SO_2 treated plants in comparison to control was observed. For example, the percent increase of anthocyanin content in 15-days old plants was 24.0 percent against control, while in 60-days old plants, increase in foliar anthocyanin levels was 65.7 percent (Table-3).

Table-1. Effect of SO_2 pollution on content of proline (µmoles/gram fresh weight) of leaf extract in *Raphanus sativus* L. cv. Pusa chetki.

Plant age (Days)	Control Plant	SO_2treated plants
15	4.64 ± 0.92	5.12 ± 0.68**
30	6.98 ± 0.94	9.07 ± 0.72**
45	8.69 ± 0.47	12.10 ± 0.54**
60	10.32 ± 0.65	15.14 ± 0.56**
Values are in mean ± SD; Significance of difference from control.; *P < 0.05; **P < 0.01 and † non significant		

Table-2. Effect of SO_2 pollution on content of total phenolics (mg/gram fresh weight) of leaf extract in *Raphanus sativus* L. cv. Pusa chetki.

Plant age (Days)	Control Plant	SO_2treated plants
15	0.11 ± 0.12	0.13 ± 0.05**
30	0.24 ± 0.14	0.33 ± 0.06**
45	0.38 ± 0.19	0.52 ± 0.09**
60	0.46 ± 0.08	0.71 ± 0.14**
Values are in mean ± SD; Significance of difference from control.; *P < 0.05; **P < 0.01 and † non significant		

Table-3. Effect of SO_2 pollution on content of total anthocyanin (mg/gram fresh weight) of leaf extract in *Raphanus sativus* L. cv. Pusa chetki.

Plant age (Days)	Control Plant	SO_2treated plants
15	0.050 ± 0.02	0.062 ± 0.07**
30	0.131 ± 0.04	0.189 ± 0.06**
45	0.164 ± 0.09	0.268 ± 0.08**
60	0.216 ± 0.08	0.358 ± 0.11**
Values are in mean ± SD; Significance of difference from control.; *P < 0.05; **P < 0.01 and † non significant		

IV.

Discussion

The SO_2 gas is absorbed into mesophyll of leaves through the stomata, and toxicity of SO_2 is largely due to reducing properties of gas. SO_2 gas combines with water in intercellular spaces to form sulphurous acid (H_2SO_3), which dissociates into H^+ and HSO_3^- ions. Thus the foliar injury in sulphur dioxide treated plants is caused by accumulation of sulphites in the mesophyll tissues of leaves and inside the leaf the SO_2 or its breakdown products react with cellular components, mainly cellular membranes causing injury or death to tissues (and eventually leads to interveinal necrosis (Rao *et al.*, 1985). Mature leaves were more susceptible to sulphur dioxide injury. This may be due to increased intercellular spaces in mature leaves which facilitate rapid gas flow (Kumar and Singh, 1986).

Contents of total proline and phenolics in leaves of chilly were increased significantly in SO_2 exposed plants. Similar results have been observed by other researchers (Matysik, *et al.* 2002; Jeyakumar *et al*. 2003 and Rai and Agrawal, 2008).

Proline takes participation in a lot of reactions of plant metabolism such as activation of respiration, regulation of acceptance of O_2, contributes to synthesis of chlorophyll and supplies amino groups for the synthesis for some amino acids. It is well known that proline accumulates in plants during adaptation to various types of environmental stress, such as draught, salinity, nutrient deficiency, high temperature and exposure to different types of pollutants including SO_2(Oncel *et al.*, 2000). There are three possible causes of the free proline accumulation under stress: first, stimulation of proline synthesis from glutamic acid (Boggess *et al*,, 1976), which has been found to be dependent on the abscissic acid concentration (Stewart, 1980); second, inhibition of proline oxidation to other soluble compounds; and, third, inhibition of protein synthesis (Stewart, 1973).

Hanson *et al.* (1977) considered proline accumulation to be a symptom of damage. However, many researchers have ascribed to proline a positive role associated with some sort of adaptive response. According to Stewart and Lee (1974), proline is a substance inducing osmotic adjustment. Other researchers have suggested that proline is a source of energy, carbon and nitrogen for the recovering tissues. Kurkdjian and Guern (1989) suggested that proline may be involved in alleviating cytosolic acids associated with several stresses. The removal of excess H^+ occurring as a result of proline synthesis may have a positive effect on reduction of SO_2 induced damage. From the results obtained, it is suggested that proline can protect cells and tissues against damage induced by SO_2.

Phenolics are diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin), abundant in plant tissues (Grace and Logan, 2000). Polyphenols possess ideal structural chemistry for free radical scavenging activity and they have been shown to be more effective antioxidants *in vitro*. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and also from their ability to chelate transition metal ions (Rice-Evans *et al.*, 1997). Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes (Arora *et al*., 2000). These changes can hamper the diffusion of free radicals and restrict peroxidative reactions. Phenolic compounds also involved in the hydrogen peroxide scavenging cascade in plant cells (Takahama and Oniki, 1997). Anthocyanins, a glycosilated form of anthocyanidins, are a group of flavonoids mostly responsible for the colour of fruits from red through purple to blue. Anthocyanins have been suggested to act as potent antioxidants. The potent antioxidant activities of anthocyanins are related to their unique structures. The O^+ (oxonium ion) in the C-ring and their capacities to facilitate stable radical products after interrupting chain reactions (Van Acker *et al.*, 1996; Larson, 1997). By forming complexes with transition metals, anthocyanins have been demonstrated to prevent the conversion of H_2O_2 and O_2 to destructive OH radicals through Haber-Weiss-Fenton reactions (Van Acker *et al.*, 1996) .

V. Conclusion

Thus, it may be concluded that exposure of *Raphanus sativus* L. cv. Pusa chetki plants to 1306 μgm^{-3} SO_2 caused various physiological and metabolic changes leading to the development of injury symptoms in leaves. To reduce SO_2 stress plant produces more amount of proline and phenolic compounds. This study can also useful in identifying the chemical to mitigate SO_2 stress. During stress condition plant produces more secondary metabolites. So, high content of secondary metabolite can be produced by giving stress to plants.

VI. References

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