Doctoral Thesis

Effects of Polycyclic Aromatic Hydrocarbons (PAHs) and Acid Mist on Plants and Their Mitigation

(Summary)

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Plants are subjected to many challenges including infestation by pathogens, insect pests, drought, extreme low and high temperature and environmental pollutants. These challenges are mainly categorized under biotic and abiotic stresses. Polycyclic aromatic hydrocarbons (PAHs) being oxidative stress inducers are a large group of organic compounds and are classified as carcinogens and mutagens. They are generally formed as a result of pyrolysis of organic substances or incomplete combustion of oil, gas, wood, coal and other organic materials. PAHs are emitted to the environment through two main sources, (1) anthropogenic sources (i.e., > 90%) and (2) natural sources and after depositing on the plant leaves, they cause stress in the plants through generation of reactive oxygen species (ROS).

Chapter I presents general introduction of PAHs and nitro-PAHs, their environmental fate, deposition and toxicity on humans and plants, generation of ROS, induction of stress in the plants and mitigating their negative effects using ROS scavengers. It also discusses the main objectives of our studies throughout the experiment. They were; characterization of stress responses of marigold and tomato plants to PAHs and nitro-PAHs, evaluation of the role of ROS in the effects of PAHs and nitro-PAHs on the plants, evaluation of the effects of low pH (3.0) on PAHs stress induction capacity, and evaluation of ROS scavenging activities of mannitol and catechin in combination with PAHs.

Chapter II describes a study carried out to characterize marigold stress response to PAHs (oxidative stress inducers) with and without sulfuric acid (S.Acid; pH 3) (acid-stress inducer) and to evaluate ROS scavenging activity of mannitol (Mann). Marigold (*Calendula officinalis*) seedlings were grown in a greenhouse and

fumigated with fluoranthene (FLU), phenanthrene (PHE), Mann, and S.Acid individually and in various combinations for 40 days. Various physiological and biochemical parameters among others were analyzed using standard methods. The results revealed that fumigation of FLU induced oxidative stress to the plants via ROS generation leading to negative effects on photosynthesis at near saturating irradiance (A_{max}), stomatal conductance (G_s), internal carbon dioxide concentration (C_i), leaf water relations and chlorophyll pigments. Significant per cent inhibition of A_{max} (54%), G_s (86%) and C_i (32%), as well as per cent reductions in chlorophyll a (Chl.a) (33%), Chl.b (34%), and total chlorophyll (Tot.Chl) (48%) contents were recorded in FLU fumigated treatment in comparison to control. Combination of Mann with FLU scavenged the generated ROS and substantially lowered the oxidative stress on the plants hence all the measured parameters were not significantly different from control. PHE fumigation had varied effects on marigold plants and was not as deleterious as FLU. Combined fumigation of S.Acid with both the PAHs had significant negative effect on leaf water relations, and positive effect on fresh and turgid weight of the plants but had no effect on the other measured parameters. The lowest proline contents and highest catalase and ascorbate peroxidase activities in FLU fumigated plants further confirmed that oxidative stress was imposed *via* the generation of ROS. From the results, it is evident that Mann could be an efficient scavenger of ROS-generated by FLU in the marigold plants.

Chapter III describes a study conducted to characterize the toxic effects of 1-Nitropyrene (1NP) (oxidative stress inducer) on tomato (*Lycopersicon esculentum*) plants, and to evaluate reactive oxygen species (ROS) scavenging

activity of mannitol (Mann) and catechin (CTH). Tomato seedlings were pot-grown in a greenhouse in Hiroshima University during May-August 2017. They were fumigated alone and in various combinations of 1NP, Mann and CTH for around 10 weeks (3 days a week) from mid-June to late August. Various physiological and biochemical parameters among others were analyzed using standard methods. The results revealed that 1NP induced oxidative stress to the plants through generation of ROS which ultimately had negative effects on certain measured parameters. The values of SPAD and chlorophyll fluorescence (ChlF) were significantly lowered 7 and 10 weeks after fumigation (WAF) compared to control, while this effect was not shown at 2 and 4 WAF. Chlorophyll a (Chl. a), total chlorophyll (Tot. Chl) and carotenoid (Carot) contents were significantly declined with 32.6%, 18.9% and 59.2%, respectively. Leaf water relations were significantly imbalanced, and catalase (CAT) and ascorbate peroxidase (APX) enzymes activities were significantly enhanced in comparison to control. Root fresh weight was significantly lowered while no significant effect was recorded in root dry weight as well as shoot fresh and dry weights. In one hand, the negative effects of 1NP was not shown in all the measurements while Mann was combined, and was not significantly different from control throughout the results which thus shows scavenging activity of Mann. On the other hand, CTH also scavenged the negative effects of 1NP on some measured parameters but was not as effective as Mann. From the findings, it was concluded that 1NP was responsible for generation of ROS which ultimately lead to the induction of oxidative stress in tomato plants. Mann effectively scavenged ROS-generated by 1NP, whereas CTH could only do so with lower efficiency.

Chapter IV gives general discussion and conclusions of the studies along with recommendations and future line of work. The significance of the current work was foliar fumigation of PAHs and nitro-PAHs on the plants. Plants were subjected to PAHs previously but most of the studies were on plants exposure to PAHs through soil contamination. It was a novel study and as per our knowledge the first attempt to fumigate nitro-PAH (1NP) on the foliar parts of the plants. We recommend Mann to be widely used for the protection of higher plants from PAHs and nitro-PAHs generated stress in the urban areas. The findings of this study is not only important in the field of environmental dynamics but also in the plant protection as well as human health risk reduction.