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Effect of Indole Acetic Acid on Wheat (*Triticum aestivum* L.) Irrigated with Sewage Water

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Summary

A field experiment was conducted to study the accumulation of heavy metals by winter wheat (*Triticum aestivum* L.) variety PBW-343 grown under sewage water (collected from Harun Nagla channel of Bareilly city, U.P., India.) of two different concentrations (50% and 100%). Effects of indole-3- acetic acid (IAA) of two different concentrations (10ppm and 20ppm) were applied after 25, 50, 75 and 100days of seed germination. The quantities of heavy metals like Pb, Cr, Zn, Cu and Fe were determined at physiological maturity in wheat plant. Indole-3- acetic acid treatment alleviated the adverse effect of toxic heavy metals contents of sewage water due to production of enzymatic and non-enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), carotenoids, and proline respectively, and enhanced the plant growth and yield of wheat. The 100% concentration of sewage water with 20ppm of Indole-3- acetic acid showed decrease in uptake of heavy metals like Cr, Pb whereas an increase in Zn, Cu, Fe uptake was noticed with 10ppm of Indole-3- acetic acid and 100% concentration of sewage water by wheat grains.

Key Words: Sewage water (SW), Heavy metals, Indole-3- acetic acid (IAA), Antioxidants, Wheat

Introduction

Problem of sewage water is common both in growing population, urbanization and industrialization; the quantity of sewage water is increasing day by day. Farmers in peri-urban areas utilize sewage water not only as a source of water but also for supplementing nutrients. Thus, higher yields are often reported with the application of sewage water than of the fresh waters [1]. Sewage water is a heterogenous mixture of organic and inorganic components, which has plant nutrients as well as elements not essential and toxic for plant growth. Excessive accumulation of heavy metals such as Pb, Cd, Cr and Ni in the soil due to sewage water treatment and the resultant phytotoxicity is reported [2, 3]. Therefore, plant must adapt certain mechanism to scavenge these heavy metal stresses.

Tolerance to stress in plants has been reported to be associated with an increase in antioxidant activity. Heavy metals like Cr, Cd and Pb ions induced inactivation of mitochondrial electron transport and super oxide generation has been demonstrated in higher plants [4]. High concentrations of ROS such as singlet oxygen, hydroxyl ions and hydrogen peroxide at cellular levels cause oxidative stress and this explain most of visual heavy metals (Cr, Pb) toxicity symptoms observed at whole plant level [5]. ROS may play two very different roles: exacerbating damage or signaling the activation of defense responses. Such a dual function has recently been demonstrated during several abiotic stress responses [6]. The synchronous action of various antioxidant enzymes viz. SOD, CAT with the thiol regulated enzymes DHAR and GR of the ascorbate glutathione pathway is a predominant mechanism of ROS quenching under heavy metal stress [7].

Plant growth regulator IAA are active members of the signal cascade involved in the induction of plant stress responses [8]. Exogenous application of IAA modulates the expression of antioxidant enzymes and increase tolerance to oxidative stress [9]. Similarly, Birecka and Galston [10] reported alteration in activity and peroxidase isoenzyme pattern by hormones like IAA in pea. Kamboj and Kler [11] have reported increased wheat grain yield on application of IAA. The present work has been carried out to study the effect of IAA on wheat plant irrigated with sewage water.

Material and Methods

Wheat (*Triticum aestivum* L., cv. PBW 343) was obtained from the National seed corporation, New Delhi. Before use, seeds were surface sterilized with 30% sodium hypochlorite for 10 min, thoroughly washed with redistilled water and placed on moist filter paper for germination, which were grown and irrigated with sewage water collected from Harun Nagla channel, Pilibhit bypass road, Bareilly city, U.P., India. Experiment was performed in three replicates. Sewage water of 50% and 100% concentrations were used for irrigation. Two different concentrations (10 ppm and 20 ppm) of IAA were sprayed after 25, 50, 75 and 100 days of plant growth. Plants were harvested at physiological maturity for several biochemical parameters like carotenoid, proline, SOD, CAT and GR.

Carotenoid was measured using the process of Duxbury & Yentsch [12]. Proline concentration was determined using the method of Bates et. al [13]. Known amount of fresh leaves were homogenized in 10ml of 30% aqueous sulphosalicylic acid. The homogenate was centrifuged at 9000 rpm for 15 minutes. A 2ml of supernatant was mixed with an equal volume of acetic acid & acid ninhydrin and incubated for one hour at 100^0C. The reaction was terminated in an ice bath and extracted with 4ml of toluene which was aspirated after 20 s and absorbance was read at 520nm.

SOD activity was assayed by using the photochemical NBT reduction method as described by Giannopolitis and Reis [14]. Reaction mixture (3ml) contain 50mM Potassium phosphate buffer (pH 7.8), 1.3 μM Riboflavin, 0.1 mM EDTA, 13 mM Methionine, 63 μM NBT, 0.05M Sodium carbonate (pH 10.2) and enzyme extract (100 μL). The reaction mixture was illuminated for 20 minutes at light intensity of 4000 lux. The photoreduction of NBT (formation of purple formazone) was measured at 560nm.

CAT activity was determined in terms of decrease in absorbance due to decomposition of H_2O_2 at 240nm using an extinction coefficient of 39.4 $mM^{-1}cm^{-1}$ by Aebi [15]. GR activity was assayed by measuring the decrease in absorbance due to oxidation of NADPH at 340nm using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$ as described by Schaedle and Bassham [16].

Determinations of metals in sewage water as well as plant materials were measured as per APHA [17]. Oven dried plant materials were wet digested with Nitric acid: Percholric acid (3:1, v/v) mixture which was further analysed by Perkin- Elmer (Analyst model 300) atomic absorption spectrophotometer. Data was subjected to analysis of variance (ANOVA) and Microsoft excel for standard error.

Results and Discussion

To cope the oxidative stress damage, wheat plants have developed antioxidant defense system which comprises both enzymatic and non-enzymatic antioxidants [18, 19]. Non-enzymatic antioxidants viz. carotenoid and proline were recorded higher in 100% SW as compared to 50% SW irrigated wheat plants (figure 1). A significant decrease was noticed in carotenoid and proline content with 100% SW + 20ppm IAA and 50% SW + 20ppm IAA as compared to 100% SW +10ppm IAA and 50% SW + 10ppm IAA. Antioxidant defense system is important for scavenging of ROS, based on the fact that level of one or more antioxidants increase in wheat plants during stress and this increased level is related to increased stress tolerance [19,20,21].

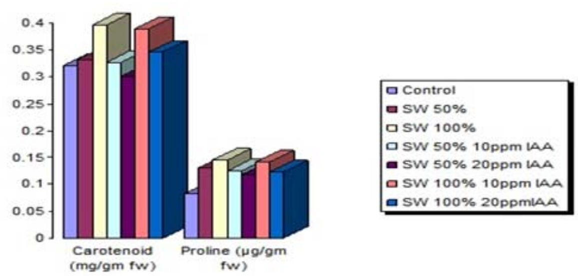

Fig.1: Effect of different concentrations of IAA in combination with different concentration of SW on Carotenoid (mg/gm fw) and Proline (μg/gm fw) in wheat plant. Values are means of ± SE (n = 3)

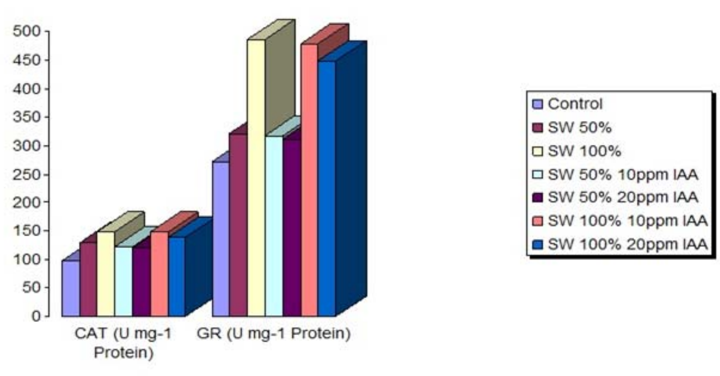

Fig.2: Effect of different concentrations of IAA in combination with different concentration of SW on CAT (U mg^{-1} Protein) & GR (U mg^{-1} Protein) in wheat plant. Values are means of ± SE (n = 3)

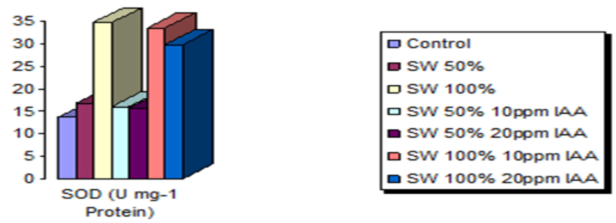

Fig.3: Effect of different concentrations of IAA in combination with different concentration of SW irrigation on SOD (U mg^{-1} Protein) in wheat plant. Values are means of ± SE (n = 3)

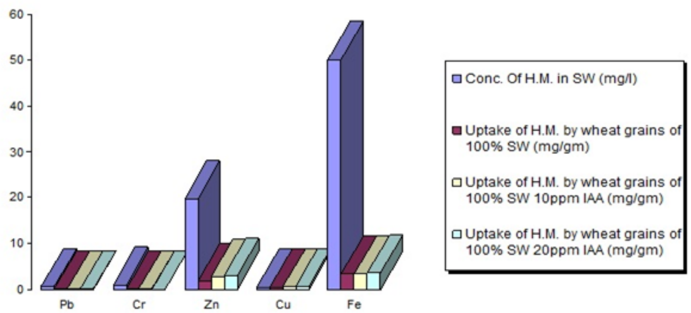

Fig.4: Absorption of Heavy metals by wheat plant (grains) after irrigation with 100% SW along with different concentrations of IAA. Values are means of ± SE (n = 3)

At physiological maturity both the 100 % and 50% SW with 20 and 10ppm IAA treatments caused a significant decrease in enzymatic antioxidants viz. SOD, CAT and GR as compared to 100% and 50% SW irrigation (figure 2 & 3). The 100% SW with 20ppm IAA and 50% SW with 20ppm IAA decreased CAT and GR activity as compared to 100% SW with 10ppm IAA, 50% SW with 10ppm IAA, 100% and 50% SW irrigation. Decreased activity suggested possible delay in removing of H_2O_2 which in turn enhanced the free radical (H_2O_2) mediated lipid peroxidation. Increase in SOD, CAT and GR was noted in 100% and 50% SW respectively as compared to control due to oxidative stress caused by toxic heavy metal present in SW. ROS like singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) can attack lipids, proteins and nucleic acids and cause their oxidation. The increased activities of these antioxidants might be a part of adaptive strategy of wheat plant irrigated with different concentrations of SW. Yannarelli et. al. [22] showed that inhibition in CAT activity could be correlated with excessive production of ROS that induces modifications in proteins such as fragmentation, increased susceptibility to proteolysis and cross-linking reactions. Same pattern was observed for GR which decreased at 100% SW with 20ppm IAA and 50% SW with 20ppm IAA as compared to 100% SW and 50% SW irrigated wheat plant. Such type of trend might have suggested dis-functioning of ascorbate-glutathione cycle, which may also be accounted for increased level of H_2O_2. Increase in H_2O_2 causes decrease in GR and DHAR activities as suggested by Yannarelli et. al. [22].

SW comprises of plant nutrients as well as elements not essential and toxic for plant growth. Zn, Cu and Fe are generally beneficial for the plant growth. Due to application of IAA in combination with 100% and 50% SW, uptake of Zn, Cu and Fe by wheat grains found to be increased. IAA induced the uptake mechanism in wheat grains with 100% SW + 20ppm IAA more as compared to 100% SW concentration (Figure 4). Ye et. al. [23] showed that application of sewage sludge can effectively improve growth of *Sesbania* species, this may be due to enrichment of nutrients e.g., C, N, P & K and reduction of metal toxicity whereas Pb and Cr showed adverse result as compared to Zn, Cu and Fe uptake by wheat grains. Toxicity due to Pb and Cr resulted in increased antioxidant defense system both enzymatic and non-enzymatic. Uptake of Pb and Cr by wheat grains irrigated with 100% SW + 20ppm IAA was decreased as compared to 100% SW + 10ppm IAA and 100% SW concentration. Singh et. al. [21] postulated that the accumulation of Cr and Fe was found lowest in fruits of tomato.

Conclusion

Two different concentrations of SW(50% and 100%) in combination with 20 and 10ppm IAA on wheat plant resulted into decreased activity of non-enzymatic antioxidants like proline and carotenoid as compared to 50% and 100% SW irrigated wheat plant while reverse trend were observed in 50% SW and 100% SW irrigated wheat plant as compared to control. Antioxidant enzyme activity was significantly induced when the plant were exposed to toxic heavy metal stress. Accumulation of beneficial metal (Zn, Cu) by wheat grains irrigated with 100%SW with 20ppm IAA increased due to exogenous application of IAA whereas accumulation of Pb and Cr by wheat grains reduced due to antioxidant defense mechanism.

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