Study of indole acetic acid and antioxidant defense system of wheat grown under sewage water

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ABSTRACT

Effect of 10 and 20 ppm of indole acetic acid (IAA) with 50 and 100% concentrations of sewage water (SW) collected from Sanjay Nagar channel of Bareilly city, U.P. India on wheat cultivars (PBW 343) was investigated in soil. There were seven treatments including control, each with three replicates. Uptake of heavy metals such as Pb, Zn, Cu and Fe by wheat grains was determined at physiological maturity. Levels of enzymatic (SOD, CAT & GR), non-enzymatic (Carotenoid & Proline) activities and heavy metals were significantly (p<0.01 or p<0.001) higher in 100% as compared to 50% SW treatments. However, significant (p<0.001) increase in SOD, CAT, GR, Carotenoid and Proline was observed in wheat plant irrigated with SW+20 ppm IAA followed by SW+10 ppm IAA and SW as compared to control. Further, uptake of Pb by wheat grains found to decrease significantly (p<0.05) in 20 ppm as compared to 10 ppm IAA with SW. Uptake of Zn, Cu and Fe was also found to decrease significantly (p<0.01 or p<0.001) in SW with IAA as compared to uptake by wheat grains irrigated with SW only.

Keywords: Sewage water, Heavy metals, Antioxidants, IAA.

1. Introduction

The clean and safe environment is the basic requirement of human existence. Today, due to constraint in availability of fresh water for irrigation, waste water especially sewage water is being used for irrigation of agricultural fields. Various studies confirm that treated sewage waste water can be useful as an additional water resource for irrigation (Palese et al. 2009; Mehrdadi et al. 2007). In India, sewage water generation is 29000 million litres per day against the existing treatment capacity of 6000 million litres per day (Central Pollution Control Board, 2004). The use of treated sewage in irrigation was emphasized in the water (Prevention and Control of Pollution) Act, 1974. Due to lack of facilities; untreated sewage water is being used by farmers to satisfy crop water needs.

Application of sewage water improved the physico – chemical properties and nutrient status of the soil and increases crop production as it supplies N, P, K and also valuable micronutrients than wheat crop requires (Panicker 1995). On the other hand, the use of sewage water in agriculture is associated with health risks because of presence of pathogenic micro-organisms (Toze 2006), metallic contaminants like Cu, Ni, Cd, Cr, Zn (Misra and Mani 1991) and polychlorinated substances (Bansal 1998).
In the plants experiencing the heavy metals, the formation of reactive oxygen species increased which overwhelm the defensive capacity of the plants resulting in oxidative damage to biomolecules like lipids, protein and nucleic acids and retard the plant growth (Davies 1987; Dionisio-sese and Tobita 1998). The elevated concentrations of heavy metals may damage plant tissues, in the stimulation of free radical production by imposing oxidative stress (Foyer et. al 1997).

The level of ROS in plants is controlled by synchronous action of enzymatic and non-enzymatic antioxidants. Among enzymatic antioxidants, superoxide dismutase (SOD), Catalase (CAT) and Glutathione reductase (GR) are important scavengers of ROS (Bowler et al., 1992; Noctor and Foyer 1998; Salin 1998; Mittler 2002). Non-enzymatic antioxidants include Ascorbate, Carotenoid and Proline which are also important and discharge wide array of functions (Lamoureux and Rusness 1993; Buettner and Jurkiewiez 1996). Thus, an understanding of synchronous action of enzymatic and non-enzymatic antioxidants in economic crops is a relevant topic of investigation for identification of key components involved in mitigation of oxidative stress and in manipulation of tolerance in plants under metal stress.

Beside nutrients, plant hormones are other organic substances which regulate plant growth and development. Plant hormones may be part of a signal transduction pathway, or their presence may stimulate reactions that are signals and/or causative agents for stress responses. Indole Acetic Acid (IAA) is a type of auxin that stimulates growth through cell elongation and lateral root formation which probably support more absorption of minerals (Egamberdieva 2009). Recently, few of several investigators reported that exogenously applied IAA at concentrations between 0.01 ppm and 100 ppm can promote growth as well as alleviate toxic effects of abiotic stresses in different plant species (Chakrabarti and Mukherji 2003; Ali et al. 2007; Egamberdieva 2009). In contrast, other investigators reported that exogenously applied IAA at the concentrations ranging from (250 to 1000 µM)/10 ppm to 100 ppm induces greater generation of ROS and causes oxidative damage with concomitant induction or suppression of antioxidant system in plants and animals (de Melo et al.,2004 ; Wang et al..2007). Thus, results regarding the effect of exogenously applied IAA are contradictory and need further investigations to know more about the action of exogenously applied IAA in plants.

In the present study, an effort has been made to know the mechanism of wheat plant responses to IAA under metal toxicity in relation to the metabolism against oxidative damage caused by heavy metals i.e. Pb, Cr, Cu, Zn and Fe. To elucidate possible mechanism of interaction between heavy metals and IAA, parameters like enzymatic, non-enzymatic antioxidants and uptake of heavy metals by wheat grains were investigated.

2. 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 distilled water and placed on moist filter paper for germination (24 h), after that seeds were evenly sown in the field (0.5 cm depth) and irrigated with sewage water collected from Sanjay Nagar channel, Bareilly, U.P., India. 50% and 100% concentrations of Sewage water (SW) was used along with exogenous application of 10 ppm and 20 ppm IAA after 25, 50, 75 and 100 days of sowing.
2.1 Determination of antioxidant enzymes activities

Fresh leaf samples (1 g) were homogenized in 10 ml of chilled 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinylpyrolidone in mortar and pestle under cool condition. The homogenate was centrifuged at 20,000 rpm for 10 min at 4°C and supernatant was used as enzyme. All enzymatic measurements were carried out at 25°C by using Rayleigh UV/VIS spectrophotometer (Model 2601).

Super oxide dismutase (SOD) activity was assayed by Giannopolitis and Reis (1977). Reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.8), 1.3 µM riboflavin, 0.1 mM EDTA, 13 mM methionine, 63 µM NBT, 0.05 M sodium carbonate (pH 10.2) and enzyme extract (100 µl). The reaction mixture was illuminated for 20 min at light intensity of 4000 lux. The photoreduction of NBT (formation of purple formazone) was measured at 560 nm. One unit of SOD activity is defined as the amount of enzyme, which is required to cause 50% inhibition in the reduction of NBT.

Catalase (CAT) activity was determined in terms of decrease in absorbance due to decomposition of H2O2 at 240 nm using an extinction coefficient of 39.4 mM^-1 cm^-1 (Aebi 1984). 2 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 10 mM H2O2 and enzyme extract (200 µl). One unit of enzyme activity is defined as 1 n mol H2O2 decomposed mg^-1 protein min^-1.

Glutathione reductase (GR) activity was assayed by measuring the decrease in absorbance due to oxidation of NADPH at 340 nm using an extinction coefficient of 6.2 mM^-1 cm^-1 (Schäfele and Bassham, 1977). Reaction mixture (2 ml) contained 50 mM potassium phosphate buffer (pH 7.8) containing 2 mM EDTA, 0.15 mM NADPH, 0.5 mM oxidized glutathione (GSSG) and enzyme extract (200 µl). One unit of enzyme activity is defined as 1 n mol NADPH oxidized mg^-1 protein min^-1.

2.2 Determination of non-enzymatic activities

Carotenoid content was determined by extracting fresh leaves (100 mg) with 80% chilled acetone and centrifuging at 10,000 rpm as per method of Duxbury and Yentsch, 1956. Proline concentration was determined using the method of Bates et. al.(1973). Known amount of fresh leaves were homogenized in 10 ml of 30% aqueous sulphosalicylic acid. The homogenate was centrifuged at 9000 rpm for 15 min. 2 ml of supernatant was mixed with an equal volume of acetic acid and acid ninhydrin and incubated for one hour at 100°C. 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 520 nm.

2.3 Determination of heavy metals

Determination of heavy metals in sewage water was measured as per APHA (1998). Approximately 4 gm of grains were digested with 10 ml Nitric acid and 0.5 ml of concentrated Percholric acid and Sulphuric acid (1:9, v/v) mixture (Adrian, 1971) which was further analyzed by Perkin- Elmer (Analyst model 300) atomic absorption spectrophotometer. Total five heavy metals were estimated viz. Lead (Pb), Chromium (Cr), Zinc (Zn), Copper (Cu) and Iron (Fe) in SW.
2.4 Statistical analysis

Data were summarized as Mean of replicates ± SD. Treatments were compared by two way analysis of variance (ANOVA) and the significance of mean difference within and between the treatments was done by Duncan’s multiple range test (DMRT).

3. Results and discussion

Water is fundamental requirement for life on earth and its scarcity results in drastic reduction in growth. Heavy metal contamination of soil and sewage water is one of the important factors i.e. affecting physiological and biological processes of organisms including plants. Effect on enzymatic antioxidants viz. SOD, CAT and GR of wheat when treated with different concentrations of SW and IAA (Control, T1: SW, T2: SW + 10 ppm IAA and T3: SW + 20 ppm IAA) is summarized in Figure 1. At physiological maturity both the 100% and 50% SW with 20 and 10 ppm IAA treatments caused the significant (p<0.01 or p<0.001) decrease in enzymatic antioxidants i.e. SOD, CAT and GR as compared to 100% and 50% SW irrigation respectively. Excess of heavy metal is reported to affect physiological processes, plant growth and development (Shi et. al. 2006; Shi and Zhu, 2008). Increase in SOD (units mg \(^{-1}\) protein) was found in 50% (15.301) and 100% SW (27.804) as compared to control (13.619). However, decrease in SOD content was evident in 50% (13.398) and 100% (22.102) SW with 20 ppm IAA as compared to 50% (14.616) and 100% (24.871) SW with 10 ppm IAA.

Results clearly depicts that SW with 20 ppm IAA lowered the SOD content as compared to SW only. Likewise activity of CAT (units mg \(^{-1}\) protein min \(^{-1}\)) was also noticed i.e. decrease in CAT was found in SW with 20 ppm IAA as compared to 10 ppm IAA. However, significant (p<0.001) increase in CAT was observed in 100% SW as compared to 50% SW for both the concentrations of IAA. Similar findings was also observed in maize shoots where SOD and CAT activities with Pb+IAA treatments were generally lower than the Pb or IAA only treatments presumably because Pb+IAA caused heavier oxidative stress and inhibited the antioxidant defense mechanism (Wang et. al. 2007). Decrease in SOD, CAT and GR was also observed in wheat plant irrigated with 100% SW with 20 ppm IAA compared to 100% SW with 10 ppm IAA and 100% SW of Harun Nagla channel, Bareilly, U.P. (Kumar et. al. 2011).

Significant (p<0.001) increase in GR (units mg \(^{-1}\) protein min \(^{-1}\)) was evident in 100% SW (358.652), 100% SW 10 ppm IAA (343.507) and 100% SW 20 ppm IAA (326.736) as compared to 50% SW (307.421), 50% SW 10 ppm IAA (298.813) and 50% SW 20 ppm IAA (276.157) respectively. However, gradual decrease in GR was noticed in SW with 20 ppm IAA as compared to SW with 10 ppm IAA and SW only at both the concentrations of SW suggesting that exogenous application of IAA caused more oxidative stress to the wheat grains stressed with heavy metals which resulted in increased production of ROS thus inhibiting the antioxidant defense mechanism. Significant (p<0.05 or p<0.001) decrease in activities suggested that there might be possible delay in removing of H\(_2\)O\(_2\) which in turn enhanced the free radical (H\(_2\)O\(_2\)) mediated lipid peroxidation.

Yannarelli et. al. (2006) showed that the inhibition in enzymatic activities could be correlated with excessive production of ROS that induces modifications in proteins such as fragmentation, increased susceptibility to proteolysis and cross-linking reactions. Many reports indicated that the SW stress reduces the plant growth and yield (Ashraf et.al. 1992; Elhafid et. al. 1998; Azhar et. al. 2005). Significant (p<0.01 or p<0.001) increase in SOD,
CAT and GR was observed in 100% SW and 50% SW respectively as compared to control and SW with IAA, due to oxidative stress caused by toxic heavy metals present in SW. Significant (p<0.05 or p<0.01) increase in these activities might be a part of adaptive strategy of wheat plant against toxicity due to heavy metals present in SW.

**Figure 1:** Effect on enzymatic activities i.e. CAT, SOD and GR of wheat irrigated with different concentrations of SW with IAA (Control, T1= Irrigation with SW, T2=SW with 10 ppm IAA, T3=SW with 20 ppm IAA).
Effect on non-enzymatic antioxidants viz. Carotenoid (mg g\(^{-1}\) fw) and Proline (µg g\(^{-1}\) fw) of wheat when treated with different concentrations of SW and IAA (Control, T1=Irrigation with SW, T2=SW with 10 ppm IAA, T3=SW with 20 ppm IAA) is depicted in Figure 2. Treatment with different concentrations of SW in combination with IAA resulted into significant (p<0.05 or p<0.001) decrease in non-enzymatic antioxidants such as carotenoid and proline as compared to 50% and 100% SW while increase in these activities was observed in 50% and 100% SW concentrations as compared to control. Significant (p<0.001 or p<0.01) decrease in carotenoid was observed in 100% SW with 20 ppm IAA (0.330) followed by 100% SW with 10 ppm IAA (0.344) as compared to 100% SW only (0.361). Increase in carotenoid was found for all the treatments as compared to control (0.321).

Figure 2: Effect on non-enzymatic activities i.e. Carotenoid and Proline in wheat irrigated with different concentrations of SW with IAA (Control, T1=Irrigation with SW, T2=SW with 10 ppm IAA, T3=SW with 20 ppm IAA).
Similar pattern was also noticed for 50% SW with IAA and 50% SW only. Similar trend was also followed by proline content of the wheat irrigated with SW & IAA and SW alone. Gradual increase in proline was evident in 100% SW alone and also with IAA as compared to respective treatments of 50% SW. Increase in carotenoid and proline content may thus be attributed to the plant defense strategy to overcome the heavy metal stress (Singh and Agarwal, 2010; Chandra et al. 2009).

Similar findings were reported in *A.esculentus* where carotenoid and proline increased significantly with increasing sludge amount (Singh and Agarwal, 2009). In contrast, treatment of 20 ppm IAA with 100% and 50% SW concentrations regulated H₂O₂ level that might participated in signalling processes associated with regulation of antioxidant defense system to protect wheat plants under heavy metal stress.

The study of heavy metal accumulation from SW by the crops is important as this would affect human and animal health directly through the food chain. The present results suggest a general decrease of metal uptake by wheat grains irrigated with SW with and without IAA (Figure 3). SW was estimated for Pb, Cr, Cu, Zn and Fe. Cr was not detected in the sewage channel used for the study. Slight decrease in uptake of Pb by wheat grains was observed in 100% SW with 20 ppm IAA (0.069 mg g⁻¹ dw) as compared to 100% SW with 10 ppm IAA (0.071 mg g⁻¹ dw) and 100% SW (0.085 mg g⁻¹ dw) only. This is in agreement with the findings that concentration of the metal is generally much higher in roots and leaves and the smallest in flower buds and fruit (Smical et. al. 2008). Sometimes roots can act as barriers to the transfer of toxic metals through soil-plant system (Kumar et. al. 2009).

Likewise uptake of Pb, significant (p<0.001) increase in uptake of Zn, Cu and Fe was evident in 100% SW with IAA and 100% SW only as compared to 50% SW with IAA and 50% SW only. Gradual decrease in uptake of Zn, Cu and Fe was observed in 100% SW with 20 ppm IAA followed by 100% SW with 10 ppm IAA as compared to 100% SW only. Similar pattern was also observed for 50% SW / 50% SW IAA. A similar finding was also observed in maize roots and shoots the levels of mineral nutrients (K, Ca, Mg, Fe, Mn, Cu & Zn) with Pb+IAA treatments were generally lower than Pb or IAA only treatments (Wang et. al. 2007). Results clearly depict that nutritive metal uptake by wheat grains tend to decrease with increase in IAA application. However, increase in uptake of nutritive metals (Zn, Cu & Fe) by wheat grains was observed with increase in IAA concentrations with SW (Kumar et. al. 2011) due to IAA application which decreased the effect of oxidative stress due to heavy metals (Pb & Cr). Pb causes the imbalance of minerals K, Ca, Mg, Mn, Zn, Cu and Fe within the tissues by physically blocking the access of these ions to the absorption sites of roots (Godbold and Kettner, 1991). On the other hand, a number of studies have shown that plant hormones in general and auxins in particular, can significantly affect the uptake and further transport of nutrients within plants by regulating the sink action of developing tissues (San-Francisco et. al. 2005).
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![Iron (mg g⁻¹ dry wt.)](image)

ns- p>0.05, **-p<0.01, ***- p<0.001

**Figure 3**: Effect on uptake of heavy metals (Pb, Zn, Cu & Fe) by wheat grains irrigated with different concentrations of SW with IAA {T1= Heavy metals present in SW (mg l⁻¹), T2= Uptake of heavy metals by wheat grains (mg g⁻¹ dw) irrigated with SW, T3= Uptake of heavy metals by wheat grains (mg g⁻¹ dw) irrigated with SW alongwith 10 ppm IAA, T4= Uptake of heavy metals by wheat grains (mg g⁻¹ dw) irrigated with SW alongwith 20 ppm IAA}.

4. Conclusion

Results of the study undertaken indicated that wheat plants showed differential responses to application of IAA due to stress caused by heavy metals present in sewage water. Application of 20 ppm IAA and 10 ppm IAA together with 50% and 100% SW resulted in lesser uptake of Pb by wheat grains as compared to SW only. SW with IAA application resulted, decrease in antioxidant activities as compared to SW without IAA. It seems that IAA application tends to nullify the metal stress to wheat plant up to some extent.

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5. References


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