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RESEARCH ARTICLE

TOTAL CHLOROPHYLL AND TOTAL PROTEIN CONTENT IN WHEAT (TRITICUM AESTIVUM) GROWN UNDER ARSENIC STRESS

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ABSTRACT

Heavy metals are widely distributed in soils, plants and animals in most of their tissues. Heavy metals are included in the main category of environmental pollutants as they can remain in the environment for long periods; their accumulation is potentially hazardous to plants and animals may adversely affect soil productivity. Wheat was grown with Arsenic (As) stress which affects various metabolic activities of wheat at different concentrations. Effect of Arsenic was observed on the pigment content and the result showed that the maximum toxicity was observed at As 150 mg/kg. The reduction of chlorophyll *a* and *b* contents of leaves was detected with enhanced Arsenic accumulation in leaves. The protein content was lower in wheat at higher concentration of arsenic that is more toxic to wheat. We studied the toxicity of arsenic on total chlorophyll content and total protein in two varieties (WH-711, C-306) of wheat. Arsenic enters the plant system, may enter the food chain and cause harm to humans and animals. By observing these different parameters of the two varieties of wheat we concluded that the variety WH-711 is more sensitive to heavy metals than C-306.

INTRODUCTION

Wheat is one of the most ancient domesticated crops, with archaeological evidence of the cultivation of various species in the Fertile Crescent dating back to 9600 B.It has also been noticed that wheat grass juice may have antibacterial chlorophyll that limits the growth of many types of germs not by directly killing them but by providing them environment, which interferes with their growth especially against anaerobic bacteria that do not require oxygen. Wheat protein can be classified into four group on the basis of either solubility or their extraction in a series i.e. albumin which is water soluble, globulin which is salt soluble (diluted salt) gliadin soluble in 70% ethyl alcohol and glutenin which is soluble in diluted acid or sodium hyderoxide. World wheat production doubled during the 25 years period to 1984-85 (Briggle and Curtis, 1987).Biological stress is not easily defined, but it implies adverse effects on organisms. Plant stress can be divided into two primary categories. Biotic stress is a physical (e.g.- light , temperature) or chemical (salts, pollutants) that the environmalists may impose on a plant. Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage. This could be due to long term use of phosphatic fertilizers, sewage sludge application, and dust from smelters industrial waste and bad watering practices in agricultural land (Bell *et al*, 2001). Excess use of arsenic as pesticides has significantly contributed to the elevation of arsenic concentration in soils Arsenic is a semi-metallic element found in soils, groundwater, surface water, air and some foods. Arsenic occurs naturally in the earth's crust, with higher concentrations in some geographic areas and in some type of rocks and mineral. Plants tolerate such high levels of toxic elements by two principal strategies, both involving detoxification by combining the toxic element with an organic molecule. Arsenic threshold of toxicity is mostly determined by environmental pollution following industrial & agricultural activities. Moreover, tillage and crop rotation practices similarly have a greater impact upon the As content of food than does the concentration of As in soils. High levels of arsenic in soils have been phytotoxic in plants which leads to decrease the plant growth and fruit yield (Carbonell-Barrachina *et al* 1995), discoloured and stunted roots; withered and yellow leave reduction in chlorophyll and protein contents, and in photosynthesis capacity (Marsin *et al* 1993).

METHODOLOGY

Chlorophyll content in wheat

Amount of leaf sample used for extraction depended upon the availability and other requirements which ranged from 100-500 mg. The chlorophylls and carotenoids were extracted by the method of Arnon (1949) and Holden (1965). For extraction, 50 mg of plant sample was homogenized with about 10 ml of ice cold 80% acetone (AR Grade). A pinch of $CaCO_3$ was added to avoid the destruction of chlorophylls and other pigments. Extraction has to be carried out under dim light to avoid photoxidation of the pigments. It was centrifuged in a Remi

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centrifuge at low speed (5000 rpm) for about 20 min. Ice cold pastle and mortar were used for grinding the samples. After centrifugation cell wall debris were settled down and was discarded. Only supernatant was taken out and raised to a specific volume of 10 ml with ice cold 80% acetone. Absorbance was recorded soon after extraction was over with the help of UV-Vis spectrophotometer at wavelengths of 663, 645, 510 and 480 nm. The amount of total chlorophylls and carotenoids were estimated by the formula of Arnon (1949).

Protein content in wheat

The soluble protein in various fruits was determined by the method of Lowery *et al* 1951 using Bovin Serum Albumin as standard protein. This method is based on the protein reacts with folin ciocalteau's reagent to give a colored complex. The color so formed was due to the reaction of alkaline copper with the protein and reduction of Phosphomolybdate by tyrosine and tryptophan residues present in protein. Extraction method: The sample of wheat was weighed 500mg and was grinned separately with pestle and mortar in 10 ml of buffer. Centrifuged and used the supernatant for protein estimation.

Number and weight of grain per spike

Number of grain per spike is calculated after harvesting in triplicate in both the varieties.Weighing is done by electric weighing balance.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) to determine the statistical significance of the differences between means of treatments.

RESULT

To estimate the chlorophyll and carotenoids content in wheat

Arsenic treatment affected the chlorophyll amount of leaves. The reduction of chlorophyll *a* and *b* contents of leaves was detected with enhanced arsenic accumulation in leaves. Light-induced chlorophyll accumulation was inhibited by increasing arsenic concentration in the soil. Under arsenic stress, the chlorophyll *b* content of leaves was more affected than the chlorophyll a content. Total chlorophyll content of the leaves decreased significantly with increasing arsenic concentration as represented in Table No.1. Total chlorophyll content decreased approximately by 40% at the arsenic concentration 100mg/kg. The arsenic concentration of 150mg/kg caused the maximum decrease of Chl amounts.

The highest carotenoid content was measured in control plants and it decreased with increasing arsenic concentration.

Figure1 Wheat (C-306) grown in control

Figure2 Wheat (WH-711) grown in control

To estimate the total protein in wheat unde arsenic stress-

The result shown in table the protein content is reduced in both the two varieties of wheat when arsenic conc. increased. Protein in control was 12.4 mg/gm.fr.wt which is reduced to 10.84 mg/gm.fw under stress of conc. 50 mg/kg in C-306 and in WH-711 protein was 11.75mg/ gm.fr.wt and reduced to 6.50mg/ gm.fr.wt at 150 mg arsenic concentration.

The inhibition of excess arsenic in amino acids and proteins might be due to binding of metals with the sulphydryl group of protein and causing deleterious effect in the normal protein formation

Table.1 Total chlorophyll and carotenoid content of C306 and WH711 under Arsenic stress

Metal applied (mg/kg)	Chlorophylla (mg/gmfr.wt)		Chlorophyll b (mg/gmfr.wt)		Total chlorophyll (mg/gmfr.wt)		Carotenoids (mg/gmfr.wt)	
	C-306	WH-711	C-306	WH-711	C-306	WH-711	C-306	WH-711
Control	2.10±0.4	2.08±0.5	0.09	0.09	2.19	2.17	0.529	0.520
As 50	2.01±0.7	1.99±0.5	0.081	0.066	2.091	2.056	0.498	0.488
As 100	1.75±0.8	1.69±0.3	0.060	0.054	1.81	1.74	0.454	0.445
As 150	0.52±0.5	0.47±0.2	0.040	0.036	0.56	0.506	0.427	0.407

Table2 Total protein content in wheat variety C-306 and WH-711 under Arsenic stress

Metal conc. in mg/kg soil	Total protein content (mg/ gm.fr.wt) C-306	Total protein content(mg/ gm.fr.wt) WH-711
Control	12.4	11.7
As 50	10.89	10.12
As 100	8.5	7.9
As 150	7.2	6.5

To estimate Weight & number of Grain per Spike in wheat unde arsenic stress-

Table 3 Showing Weight & number of Grain per Spike of two Wheat cultivars.

Metal conc.mg/kg	Weight of Grain per Spike (gm)		Number of Grains per spike	
	C-306	WH-711	C-306	WH-711
Control	1.56	1.76	33	43
As50	1.37	1.35	28	37
As100	1.21	1.19	23	30.4
As150	1.01	1.56	21	26

Figure3 Wheat (C-306) grown in Arsenic concentration 50mg/kg of soil

Fig 4 Wheat (WH-711) grown in Arsenic concentration 50mg/kg of soil

Figure-5 Wheat (C-306) grown in Arsenic concentration 100mg/kg of soil

Fig6 Wheat (WH-711) grown in Arsenic concentration 100mg/kg of soil

Figure-7 Wheat (C-306) grown in Arsenic concentration 150mg/kg of soil

Fig.-8 Wheat (WH-711) grown in Arsenic concentration 150mg/kg of soil

DISCUSSION AND CONCLUSION

Arsenic is a nonessential element and its uptake by plants is an inadvertent event driven by transporters for essential/beneficial elements. The plant material which was included in our study was wheat variety C-306 and WH-711 which are mainly grown in northern India. The seeds were sown in earthen pots containing equal quantities (8 Kg) of loamy sand soil. Metal treatment of arsenic was prepared using sodium arsenate solution with concentration of 50, 100 and 150 mg/Kg of soil. The extent of plant injury by elevated concentration is specific and strongly depends on the environmental conditions and on the availability of heavy metals Photosynthesis is the major pathway of all the green plants and some other micro organism (Algae, etc) present on earth, the green pigment responsible for photosynthesis is chlorophyll present in chloroplast. Chlorophyll content decreased by high concentration of As treatment, as shown in **Figures 1-8** and **Table1.** Chlorophyll content (a+b) in leaves of wheat seedling decreased significantly with increase in Arsenic concentration. Maximum reduction in chlorophyll content was noted in 150 mg Kg^{-1} arsenic treated plants because heavy metal entered in the leaves and accumulated excessively in some parts and combined with –SH base of protein or substituted for Fe^{2+}, Zn^{2+}, Mg^{2+} and so on, and then destroyed the structure and function of chloroplast. Protein content in arsenic treated seedling decreases in a dose dependent manners showing maximum reduction of 150 than 50 and 100 mg/Kg As in treated seeds more % of protein was reduce in WH-711 at 150mg/kg. The reduction in the amount of protein could be due to increase in protein denaturation Arsenic had an adverse effect on spike number per plant. Grain number and weight of grain were reduced under As treatments. From ou observation of different parameters of the two varieties of wheat we concluded that the variety WH-711 is more sensitive to heavy metals than C-306.But overall arsenic is toxic to wheat and reduce the productivity to alter various metabolic pathways of growth and metabolism.

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