EFFECT OF ARSENIC ON THE GROWTH AND PHYSIOLOGY OF *TRITICUM AESTIVUM* L. SEEDLINGS.

A Dissertation submitted to the Central University of Punjab

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BY

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September, 2012

CERTIFICATE

I declare that the dissertation entitled “EFFECT OF ARSENIC ON THE GROWTH AND PHYSIOLOGY OF *TRITICUM AESTIVUM* L. SEEDLINGS” has been prepared by me under the guidance of Dr. Sunil Mittal, Assistant Professor, Centre for Environmental Science and Technology, School of Environment and Earth Sciences, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

Effect of Arsenic on the Growth and Physiology of *Triticum aestivum* L. seedlings.

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The effects of Arsenic (As) were investigated on four varieties of *T. aestivum viz.* PBW 343, PBW 550, PBW 621 and HD 2967 to elucidate the toxicity of As on morphological, physiological, biochemical and antioxidant processes of plant in early stage. The results showed that seed germination, root length, shoot length and biomass decreased gradually with increase in concentrations of As (125-750µM) and this decrease was biologically significant. The photosynthetic and respiratory activity of the test plants was also investigated. The decrease in chlorophyll and carotenoids content with increase in As content indicates negative effect of As on the photosynthetic pigments. However, the total cellular respiration increased with increase in As concentration and this probably may be due to the enhancement in respiratory enzymes which leads to high production of energy by the respective cells. The contents of water soluble carbohydrates and proteins increased with increasing As concentrations while their hydrolyzing enzymes i.e. *α* amylase and protease were decreasing with increase in As concentration. The activity of antioxidant enzymes, superoxide dismutase and peroxidase followed the increasing trend indicating the induction of oxidative stress under high concentration of As. Further, among the various varieties HD 2967 was the most tolerant variety and PBW 550 was most affected variety.

(Shilpa Nag) (Dr. Sunil Mittal)

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(Shilpa Nag)

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LIST OF ABBREVIATIONS

Sr. No.	Full form	Abbreviation
1	Adenosine diphosphate	ADP
2	Adenosine triphosphate	ATP
3	Aluminium-Arsenic	Al-As
4	Ammonical Copper Arsenate	ACA
5	Arsenic	As
6	Arsenate	As(V)
7	Arsenite	As(III)
8	Calcium-Arsenic	Ca-As
9	Chromated Copper Arsenate	CCA
10	Dimethylarsinic acid	DMA
11	Environmental protection act	EPA
12	Fluor Chrome Arsenic Phenol	FCAP
13	Iron-Arsenic	Fe-As
14	Monomethylarsinic acid	MMA
15	Peroxidase	POD
16	Phytochelatins	PCs
17	Reactive oxygen species	ROS
18	Superoxide dismutase	SOD
19	Tricarboxylic acid cycle	TCA
20	Trimethylarsine oxide	TMAsO
21	World Health Organization	WHO

CHAPTER - 1

INTRODUCTION

1.1 ARSENIC AND ITS SPECIATION:

Arsenic (As) belongs to Group 15 of the periodic table and is naturally present in the environment. It has atomic number 33 and atomic weight 74.92. It is a redox sensitive metalloid and is ubiquitous in nature. It exists both in organic and inorganic forms and the common states of Arsenic are -3 {arsenide (AsIII-)}, +3 {arsenites As(III)}, and +5 {arsenates As(V)}. Both organic and inorganic forms of As are colourless to white in appearance. The toxicological and physiological effects of arsenic depend on its speciation (Mandal and Suzuki, 2002). As(III) is more toxic, more mobile and more soluble than As(V) (Cullen and Kenneth, 1989). As(V) is most stable form of As and is dominant under aerobic conditions. It has strong affinity to combine with clay, manganese, carbon, hydrogen, iron etc. The another form arsenite, As(III) exists in reducing environment. In nature, it gets converted into monomethylarsinic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAsO) by the action of microorganisms present in soil (Cullen and Kenneth, 1989). Interaction of As with soil depends on factors like pH, redox potential, organic matter in soil and type of the soil. Under acidic soil, the arsenate exists in close association with aluminium and iron while in calcareous and alkaline soil, it is widely present as calcium arsenate (Li, 1982; Fordyce *et al*., 1995).

1.2 ARSENIC CONTAMINATION IN ENVIRONMENT:

1.2.1 As from Natural sources:

In nature As exist in more than 200 different mineral forms. These include 60% arsenates, 20% sulphides and sulfosalts and 20% arsenides, arsenites, silicates and oxides (Onishi, 1969; Cullen and Kenneth, 1989; Matschullat, 2000). As in nature is mainly sourced from weathering of sedimentary and igneous rocks and the average concentration of As in these rocks is 2mg/kg (Gulledge and O'Connor, 1973). Higher concentration of As is found in argillaceous, phosphorites, iron deposits, manganese nodules etc. (Mandal and Suzuki, 2002). As also escapes from weathered rocks as salts of arsenous and arsenic acids (Irgolic *et al*., 1995). As is commonly found in the form of ores like arsenopyrite, galena, chalcopyrite,

iron pyrite and sphalerite (Goldschmidt, 1954). As occurs in soil in higher amount than the rocks. Sandy soils have less As than the alluvial and organic soils (Peterson *et al*., 1981; Kabata-Pendias and Pendias, 1984).

As is naturally present in water, but usually its concentration is very low. As per EPA (Environmental Protection Agency) and WHO (World Health Organization) the maximum permissible limit of As in drinking water is 10µg/L (WHO, 2001). Sea water normally contains 0.001- 0.008mg/L of As and the major chemical form in which arsenic appears to be thermodynamically stable is arsenate ion (Johnson, 1972).

Microorganisms play a vital role in degradation of As. Arsenic fungi *Penicillium brevicaule*, and *Scopulariopsis* are some common species which converts As into more volatile and toxic arsine gas (Mcbride and Wolfe, 1971). Methylation is the process of conversion of As to its derivatives like MMA, DMA, TMAsO, etc. (Thom and Raper, 1932). In atmosphere the arsine gas adsorbs on particulate matter and then changes to other compounds of arsenate and arsenite (Davidson *et al*., 1985).

Arsenic is commonly found in marine animals like coelenterates, crustaceans and molluscs averaging from 0.005 to 0.3mg/kg (Bowen, 1966). As is also present in shellfish and fresh water fish having concentration 100µg/g and 0.54µg/g respectively (Whitacre and Pearse, 1972). Other marine organisms also possess different compounds of As like arsenocholine and arsenobetaine which do not readily gets chemically degraded (Lauwerys *et al*., 1979).

1.2.2 Anthropogenic sources of As:

Overexploitation of natural resources is the main reason for As toxicity in the soil, air and water. In 1970’s, As consumption has been reported nearly 80% in agriculture sector mainly because of its use in pesticides like lead arsenate, copper acetoarsenate, copper acetoarsenite, monosodium methanearsonate, disodium methanearsonate etc. (USEPA, 2001). Arsenic acid was also used as a desiccant in the cotton cultivation. The common inorganic arsenic form of sodium arsenite was extensively used as weedicide (USEPA, 2001). Fluor–Chrome–Arsenic–Phenol (FCAP) was the first chemical used for wood preservation in early 1918 in developed countries (USDA, 1974). Later many other combinations of As

like Ammonical Copper Arsenate (ACA), Chromated Copper Arsenate (CCA), Osmosalts, Zinc Arsenate, Chromium Arsenate etc. were applied on woods (Lansche, 1965).

Currently, As is widely used in number of industries like embalming fluids, paint pigments, metal alloys, batteries etc. and through their discharge enters the water resources and soil. Smelters, thermal power plants, use of fossil fuel are the potential sources of As contamination in the air (Sadler *et al*., 1994).

1.3 ARSENIC TOXICITY:

As is highly harmful and is categorized as Group A carcinogen and No.1 hazardous substance (USEPA, 2001; ASTDR, 2007). The exposure level that produces carcinogenic effects is uncertain, but it is probably above 100 $\mu g/m^3$ for a brief exposure (ATSDR, 2007).

1.3.1 Toxicity in Animals:

Animals exposed to different forms of As showed histopathological changes in various tissues. Liver has been reported the most affected part (Rozenshtein, 1970). As can pass through placenta and thus is highly harmful to the embryo as it may be deadly to the growth of the foetus (ASTDR, 2007). As toxicity results in abnormalities in immune system, circulatory system, nervous system, kidney, heart and prolonged exposure leads to cancers of skin, lung etc. (Piver, 1983). Human exposure to As is either by drinking As contaminated water or through consumption of As contaminated food (Ruby *et al*., 1996; Zhu *et al*., 2008). Half-life of inorganic arsenic in humans is 10 hours (Rossman *et al*., 2007). Arsenic toxicity leads to irritation in nasal tract, bronchitis, laryngitis, rhinitis, tracheae, pharyngitis and chest sounds etc. (Gerhardsson *et al*., 1988; Nagvi *et al*., 1994; Mazumder *et al*., 1997; Milton *et al*., 2001).

Bare foot disease is common in As affected areas. Loss of circulation in the hands and feet, gangrene formation, myocardial infarction, arterial thickening and hypertension are reported in people (Lagerkvist *et al*., 1988; Nagvi *et al*., 1994; Chiou *et al*., 1998; Rahman *et al*., 1999). Acute As toxicity may lead to keratosis, hyperkeratosis of palms and soles, melanosis, leucomelanosis, basal cell carcinoma or squamous cell carcinoma and transverse white indentations called ‘Mees’ or ‘Aldrich-Mees’ lines appear on fingernails (Lagerkvist *et al*., 1988).

As induces chromosomal aberrations, promotes DNA damage and inhibits the repair mechanism in the cells (Leonard and Lauwerys, 1980; Li and Rossman, 1991). The long term As toxicity is known to have carcinogenic and mutagenic potential (IARC, 2004; Stone, 2008; Zhu *et al*., 2008; Chauhan *et al*., 2009).

1.3.2. As UPTAKE IN PLANTS:

Arsenic is a known toxic metalloid and its presence in the soil hampers the growth of the plant. Uptake and translocation of As is similar to phosphate uptake by the plant (Meharg *et al.*, 1994). At low soil pH, phosphate is taken up as H_2PO_4 and at high pH as HPO_4. Similarly at low pH, arsenate is taken up as H_2AsO_4 and at high pH as $HAsO_4$ (Schachtman *et al.,* 1998). Accumulation of As in plant system follows the trend as: Roots> stems> leaves/seeds (Meharg *et al.*, 1994).

1.3.2.1 Factors Affecting Arsenic Uptake in plants:

Uptake of As and its accumulation in plants is influenced by the nature of soil, pH, redox potential, presence of ions like phosphate and exposure time (Khattak *et al.,* 1991; Jiang and Singh, 1994; Matschullat, 2000).

- **pH:**

  pH of the soil plays an important role in the toxicity of the As (Akins and Lewis, 1976; Adriano, 2001).

Table 1: Dominant forms of As present in soil at different pH:

pH	Dominant form	Reference
4	Iron-Arsenic (Fe-As)	Akins and Lewis, 1976
6	Aluminium-Arsenic (Al-As)	Akins and Lewis, 1976
8	Calcium-Arsenic (Ca-As)	Fayiga *et al.,* 2007

- **Phosphate:**

  Arsenate is an analogue of phosphate (Williams *et al.,* 2003; Mahimairaja *et al.,* 2005) and competes with it for uptake by the plant (Asher and Reay, 1979; Pickering *et al.,* 2000; Esteban *et al.,* 2003).

- **Reduction potential:**

  Redox potential or the reduction potential plays an important role in As uptake. The plant absorbs As(V) more readily than the As(III) due to its analogous nature with the phosphate. Under natural conditions the Arsenate is present as negatively charged species $H_2AsO_4^-$ while Arsenite is uncharged H_3AsO_3 and is highly unstable. The presence of oxides like FeO and Fe_2O_3 transforms the As(III) into As(V) which is then taken up by the plant (Fordyce *et al.,*1995; Thornton,1996).

- **Humus:**

  Humus plays an important role in fertility of soil. Its presence have positive effect on the As(III) and As(V) adsorption in the soil. The presence of large amount of organic acid helps in retention of arsenic in the soil (Lund and Fobian, 1991).

- **Microorganisms:**

  Presence of microorganisms (symbiotic bacteria and arbuscular mycorrhizal fungi) in the soil plays an important role in As uptake. The plants resistance gets amplified, an increase in nutrient storage and tolerance to salinity were observed (Gyaneshwar *et al.,* 2002).

1.3.2.2 As Hyper accumulator plants:

As accumulation in plant parts helps in sequestration of As from the environment (Rathinasabapathi, 2006). Hyperaccumulating plants possess different detoxification mechanisms like compartmentalization, chelation, biotransformations, efflux of As via roots, methylation (As reduced to less toxic forms) and cellular repair (Salt *et al.,* 1998). Various plants are used as hyper accumulators like Chinese brake fern (*Pteris vittata* L.) which can tolerate upto 1500mg/kg soil arsenic concentration and accumulates upto 2.3% As in its biomass (Ma *et al.,* 2001).

Table 2: List of Arsenic hyper accumulator plants:

Sr.No.	As accumulation in Plants	Reference
1	*Isatis capadocica* and *Hesperin persica*	Karimi *et al.,* 2010
2	*Spirodela polyrhiza*	Rahman *et al.,* 2007
3	*V*etiveria *zizanioides* and *V. nemoralis*	Srisatit *et al.,* 2003
4	*Pityrogramma calomelanos*	Francesconi *et al.,* 2002
5	*P. cretica*, *P. longifolia*, *P. umbrosa* and *P. argyraea*	Zhao *et al.,* 2002
6	*Pteris vittata*	Ma *et al.,* 2001
7	*Hylocomium splendens*	Richardson, 2001
8	*Oryza sativa*	Onken and Hossner, 1995
9	*Spartina alterniflora*.	Sanders and Osman, 1985
10	*Andropogon scoparius*	Rocovich and West, 1975

1.3.2.3 Effect of As in plants:

The lower concentration of As is known to stimulate growth in plants. However, high concentration of arsenate negatively affects the plants in terms of stress to plants, inhibition of growth, physiological disorders, decrease in biomass and yield of the crops (Yun *et al*., 2008; Panaullah *et al*., 2009).

High concentration of As leads to reduction in germination rate in *Lathyrus sativus, Zea mays, Triticum aestivum* and *Oryza sativa* (Abedin *et al.,* 2002; Liu *et al.,* 2005; Chun-xi *et al.,* 2007; Mallick *et al.,* 2011; Talukdar, 2011). Arsenic shows negative effects in plants by affecting transpiration rate, uptake and transport of water, and micronutrients (Liu *et al.,* 2005).

Plants grown in the presence of As shows injury symptoms. It has been reported that leaves show necrotic spots, chlorosis, distorted growth, etiolation, discolouration of the leaf ends mostly violet coloured, browning of the roots, cell

plasmolysis, wilting and resulting in defoliation of the leaves when exposed to high concentrations of As (Thompson and Batjer, 1950; Rumburg and Engel, 1960; Kabata-Pendias and Pendias, 1992).

Toxicity of As in plants affects the root length, shoot length and chlorophyll content (Kabata-Pendias and Pendias, 1992). Elevated level of As results in elevation of SOD and POD activity of the cell in the plants (Chun-xi *et al.,* 2007; Singh *et al.,* 2007).

1.4 ARSENIC IN MALWA REGION OF PUNJAB:

As contamination is found in many parts of world including India (Nickson *et al*., 1998; Chowdhury *et al.*, 2008). States like West Bengal, Bihar, Assam, Chhattisgarh, eastern part of Uttar Pradesh, Chandigarh and south west part of Punjab, Himachal Pradesh, Haryana etc. are known to have high concentrations of As in ground water and soil (Chakraborti *et al*., 2003; Patel *et al*., 2005; Ahamed *et al*., 2006). The groundwater of southwestern parts of Punjab (Malwa region) has also been reported to have high concentration of As in groundwater (Dutta and Kaul, 1976).

According to studies conducted by Hundal and Kumar (2007) arsenic bearing rocks in the earth crust are responsible for naturally high content of As in water and soil in southern part of Punjab.

The As concentration ranges from 1.09 to 2.48mg/kg in Bathinda and average As content in Mansa soil was 1.67mg/kg (Singh *et al.,* 2010). The average content of arsenic was 11.13µg/l in water samples collected from Bathinda while average content of As in water samples of Mansa was 10.10µg/L (Singh *et al.*, 2010).

1.5 WHY WHEAT WAS CHOSEN FOR THE STUDY:

Wheat, (*Triticum aestivum* L.) is one of the major crops of Punjab. It is widely grown in Bathinda district. It belongs to family Poaceae and is commonly known as bread wheat. This is grown alternately with rice crop which require excessive irrigation for which groundwater is used. But the presence of As and other heavy metals has been reported in the groundwater of this region. Studies revealed that flooding of rice paddies creates the reducing environment which increases arsenite concentration in soil solution (Abedin *et al.,* 2002). After rice, wheat is cultivated in the same fields having high arsenite content. There may be possibility

that the arsenite present in soil solution or in the water used for irrigation will be taken by the wheat plants.

The current study is carried on the HD 2967, PBW 343, PBW 550 and PBW 621 varieties of wheat, which are grown in the south western parts of Punjab. This will provide the information on the potential risks of As to the wheat i.e. the alteration in growth pattern, effect on macromolecules, antioxidant enzymes of the monocot crop. This will help us to know about the fate of crops grown under As contaminated areas. Further, it will also help to find that out of these four varieties which one is the most resistant variety and can be grown in this region.

CHAPTER - 2

REVIEW OF LITERATURE

2.1 ARSENIC AND PLANT

Arsenic is present in more than 245 minerals (Mandal and Suzuki, 2002). Under natural conditions, As is insoluble and thus scarcely available to plants (Tu *et al.,* 2004). Kabata-Pendias and Pendias (1984) observed that average concentration of As in plant is nearly 3.6mg/kg. Studies of Meharg and Hartley-Whitaker (2002) revealed that once As enters the plant system, it gets converted into other organic and inorganic form, as a result the plants which are sensitive to As toxicity suffer adverse effects. As is present in different forms and thus its toxicity level is also different. As toxicity decreases in the following order: arsenite > arsenate > organic As compounds (Adriano, 2001).

Plants possess different mechanisms to counter balance the toxic effects of As. Generally, it was observed that plant exhibit suppression of phosphate uptake which results in reduction of As uptake by the plant and in this manner plant saves itself from toxicity effects (Meharg *et al.*, 1994). However, the amount of As translocated in such plants is accumulated in different regions of the cell by the action of phytochelatins (PCs) (Vatamaniuk *et al.,* 2000).

2.2 As ACCUMULATION IN PLANTS

The studies by different researchers have indicated that As gets accumulated in different plant parts. However, the main site of As accumulation in number of plants is roots. Hoffmann and Schenk (2011) studied accumulation of As in *Oryza*

sativa and observed that roots accumulated 20 to 100 times more than shoots. Similarly, the effect of Arsenate was studied on the *Zea mays* and it was reported that with increase in As concentration, increasing trend of accumulation was observed. The uptake was more in leaves and roots till first three days. But after three days, there was no significant effect (Mallick *et al.,* 2011).

Cucuminis sativus grown under hydroponic system suggested hyperaccumulation of As in shoots instead of roots (Hong *et al.,* 2011). High amount of Arsenate toxicity was studied in *Lathyrus* and *Trigonella,* the amount of As accumulation was more in roots of the two plants than in the shoots. Further both plants have difference in uptake level, the plant *Lathyrus* showed more accumulation than the *Trigonella* (Talukdar, 2011).

Many researchers while working on different plants has reported more accumulation in roots than the shoots (Pickering *et al.,* 2000; Singh *et al.,* 2007; Lin *et al.,* 2008; Zhang *et al.,* 2009).

Chaturvedi (2006a) working on different genotypes of rice grown under different As concentration, showed more uptake of As by the genotype CN 1035-60. At highest concentration, As accumulation was more in the roots of the CN 1035-60 rice genotype, thus it was deduced that this genotype is more tolerant to the As concentration and is good accumulator of As and thus can be grown in As contaminated soil.

Fern *Pteris vittata, Pteris ensiformis and Nephrolepis exaltata* were grown under different concentration of As, and it was observed that As got accumulated in all parts of the ferns. Highest level of accumulation was reported in fronds than rhizome and roots. *P. vittata* accumulated maximum as than *P. ensiformis* and *N. exaltata* thus it can be deduced that *P. vittata* is most tolerant fern species which can be used as capable phytoremediator (Srivastava *et al.,* 2005).

2.3 EFFECT OF As ON GROWTH, MORPHOLOGY AND YIELD

As toxicity has significant effect on the morphology of the plant. Visual symptoms of phytotoxicity include leaf wilting, retardation of root and shoot growth (Liebig, 1965). Rice seedlings when exposed to higher concentrations showed chlorosis and twisting in young leaves and necrosis in older leaves (Shaibur and Kawai, 2011).

Discoloration of root and necrosis of leaf tips and margins, indicate inhibition of root and water uptake which ultimately results in death of plant from wilting (Woolson *et al*., 1971). Shaibur and Kawai (2010) reported that increase in As concentration results in decrease in leaf blade, leaf number, leaf area and leaf height of *Brassica rapa.*

Carbonell-Barrachina *et al.* (1997) observed that presence of As in the soil affects the metabolism of the plant system. It was reported that low concentration of As may show enhancing effect on the growth of root and shoot. But as the concentration of As is elevated, the root and shoot growth, vitality index, germination energy, germination index, germination percentage, biomass of plant are inhibited (Chun-xi *et al.,* 2007).

Disturbance of plant mineral nutrition is the main cause for decrease in yield. Exposure to arsenate contamination results in stress and may inhibit the growth of the root and can cause cell death (Carbonell-Barrachina *et al.,* 1997). The presence of arsenic leads to inhibition of plant growth as it causes water deficiency and thus alters the nutrient balance (Shaibur *et al*., 2006).

As hampers the growth and physiology of the plants (Meharg *et al*., 1994). As in excess amount is phytotoxic (Woolson *et al*., 1971). Coniferous plants grown under As concentration reported abscission of needles, inhibition of roots and retarded growth (Ormrod, 1978). At high As concentration, the plant showed reduction of chlorophyll content, protein content, root nodulation, total biomass and stunted growth (Mallick *et al*., 2011).

2.4 EFFECT OF As ON PHYSIOLOGY

It has been reported that when rice was treated with different concentrations of arsenite, the rate of photosynthesis gets reduced along with the rate of transpiration indicating less water uptake by the plant (Hoffmann and Schenk, 2011). Initially, As(V) causes decline in total chlorophyll content and carotenoids in *Zea mays* (Mallick *et al.,* 2011). Moreno-Jiménez *et al.* (2009) reported that photosynthetic pigments chlorophyll a and chlorophyll b were affected by the application of arsenic in the plant *Pistacia lentiscus* and *Tamarix gallica.*

As(III) treatment showed decrease in chlorophyll content with lesser effect on the carotenoids content which resulted in reduction of photosynthetic activity of *Zea*

mays (Stoeva *et al.,* 2003). Toxic concentration of arsenate in red clover results in reduction of chlorophyll and carotenoids, thus drop in rate of photosynthesis (Mascher *et al.,* 2002). Chlorophyll content in maize has been reported under high arsenic concentration and it was observed that inhibition of chlorophyll was more pronounced in the higher concentration of arsenic than the low level of arsenic (Jain and Gadre, 1997).

Chun-xi *et al.* (2007) indicated that under low concentration of arsenic, the chlorophyll a and b increases but with increase in As concentration growth of wheat seedlings decreased. Shao *et al.* (2011) observed increase in percent cellular respiration in wheat plants grown under As concentrations. Also same trends were followed in case of mung beans (Singh *et al.,* 2007).

2.5 EFFECT OF As ON MACROMOLECULES AND THEIR HYDROLYZING ENZYMES

Under stress condition the metabolism of plant is affected, more soluble sugars gets accumulated in *Zea mays* and *Oryza sativa* (Dubey and Singh, 1999). As concentration not only affects the physiological activity of the plant but also affect its biochemical activity. Mallick *et al.* (2011) observed that in *Zea mays* excess amount of arsenate leads to changes in carbohydrate content but no significant change was reported in protein content. But in study conducted by Stoeva *et al.* (2003), protein degradation was also observed in *Zea mays* at higher Arsenite concentration.

Chun-xi *et al.* (2007) reported that carbohydrate and protein content in wheat seedlings increased with increase in As concentration. This might be due to decrease in activity of amylase and protease enzymes in response to As toxicity. In germinating seeds of *Zea mays*, *α*-amylase and *β*-amylase both act as starch degrading enzymes and convert polysaccharide starch into monosaccharides (Dubey and Singh, 1999). Liu *et al.* (2005) had reported some similar observations where the total amylolytic activity of the different wheat varieties decreased at high concentrations of As. Similarly, Jha and Dubey (2005) also reported reduction in *α*-amylase and *β*-amylase activity of rice seedlings grown under As_2O_3.

2.6 EFFECT OF As ON ANTIOXIDANT ENZYMES

Superoxide dismutase (SOD) protects the cell from oxidation due to reactive oxygen species (ROS) which interferes with the cellular metabolism (Mckerise and Lesham, 1994; Smirnoff, 1993). Excess Arsenic has enhancing effect on the SOD activity in many plants. Mallick *et al.* (2011) reported increase in SOD activity in *Zea mays* under As environment. Similar results had been reported by Singh *et al.* (2007) in their study in *Phaseolus aureus* seedlings. High concentration of Arsenic shows enhancing effect on SOD activity in the wheat seedlings (Chun-xi *et al.,* 2007). Enhancing effect was observed in *Brassica juncea* under elevated As concentration (Srivastava *et al.,* 2010).

Further, Srivastava *et al.* (2005) studied As toxicity in ferns *Pteris vittata, P. ensiformis and Nephrolepis exaltata* and it was observed that SOD activity followed the increasing trend. The activity was maximum in fronds than rhizome and roots of *P. vittata* which was followed by *P. ensiformis* and *N. exaltata*.

Peroxidase enzyme plays a major role in antioxidant defense against the metal toxicity, the POD activity was studied in leaves and the roots of *Vicia faba*. In case of leaves the POD activity was drastically reduced at higher concentration of As (Lin *et al.,* 2008).

Table 3: Review of the research work done in previous years:

S.No.	Plant	Results	Reference
1	*Oryza sativa*	Reduction in growth and fresh matter were reported with increase in As concentration.	Hoffmann and Schenk, 2011
2	*Cucuminis sativus*	The root length was much affected at high As concentration which further showed the reduction in growth of the plant. The accumulation of As was more in shoots than the roots.	Hong *et al.,* 2011
3	*Zea mays*	Elevated level of As is harmful to the plants. The fresh and dry weight was drastically reduced with high concentration and duration of exposure i.e. treatment days.	Mallick *et al.,* 2011
4	*Triticum aestivum*	More sensitivity was observed in roots at high As concentration. The biomass was also decreasing with increase in As concentration.	Shao *et al.,* 2011
5	*Oryza sativa*	Length of shoot and dry weight decreased more than root, which indicates that shoots are more affected by arsenite.	Shaibur and Kawai, 2011
6	*Lathyrus sativus* and *Trigonella foenum-graecum.*	At high concentrations, the dry weight and length were reduced more in roots than the shoots. Accumulation of arsenate was more in roots.	Talukdar, 2011

S.No.	Plant	Results	Reference
7	*Pistacia lentiscus* and *Tamarix gallica*	Inhibition of the root length was observed while shoots were less affected..	Moreno-Jiménez *et al.,* 2009
8	*Pteris ensiformis*	More accumulation of As was observed in roots than the upper parts. This leads to decrease in biomass and reduction in growth of the plant. The fronds showed necrotic symptoms as arsenic concentration was increasing.	Singh *et al*., 2009
9	*Triticum aestivum.*	Total biomass showed significant decrease with high concentration of arsenate.	Zhang *et al.,* 2009
10	*Amaranthus retroflexus*	Inhibition of root length, shoot length and biomass was observed.	Choudhury *et al.,* 2008
11	*Oryza sativa*	Growth and yield was affected by high concentration of As in the water. The biomass was also less as the As affects the basic metabolism of the plant.	Das *et al.,* 2008
12	*Vicia faba*	Growth was reduced under the elevated concentration of the arsenic. At lower concentration the roots were thicker and shorter than the control while there was less inhibition of shoots.	Lin *et al.,* 2008

S.No.	Plant	Results	Reference
13	*Triticum aestivum L.*	Seed germination was highly affected. The plants showed stunted growth and twisting in the leaves.	Chun-xi *et al.,* 2007
14	*Triticum aestivum L.*	Toxic concentration of arsenite and arsenate showed decrease in root and shoot length. More toxicity was observed in arsenite treated wheat plants than the arsenate treated.	Liu and Zhang, 2007
15	*Phaseolus aureus* (Mung beans)	Root length decreased more under high As concentration while the shoots were less affected.	Singh *et al.,* 2007
16	*Oryza sativa*	Arsenic toxicity was analysed in genotypes of Rice Mahsuri and CN1035-60. More effect was observed in root length than the shoot. CN 1035-60 genotype was more susceptible to the arsenic toxicity.	Chaturvedi, 2006a
17	*Brassica juncea*	Growth of the plant was affected as the As concentration was increased, inhibition was more in roots than shoots.	Chaturvedi, 2006b
18	*Oryza sativa*	Reduction in shoot dry weight, shoot length was reported.	Shaibur *et al*., 2006
19	*Oryza sativa*	Reduction in grain yield due to elevation in As concentration.	Islam *et al.,* 2004

S.No.	Plant	Results	Reference
20	*Zea mays*	Reduction in growth and yield of the plant was observed as the As concentration was increased.	Stoeva *et al.,* 2003
21	*Oryza sativa*	Leaves showed curling and twisting, reduction in root length, shoot length and biomass under high As concentration.	Abedin *et al.,* 2002
22	*Trifolium pretense* (red clover)	Arsenate toxicity showed significant effect on the growth of shoots.	Mascher *et al.,* 2002
23	Cereals and Beans	As concentration showed negative impact on plants growth and yield. The germination was also affected.	Nickson *et al.,* 1998
24	*Lycopersicum*	High concentration of As led to decrease in growth and fruit yield.	Carbonell-Barrachina *et al.,* 1997
25	*Hordeum vulgare* (Barley) and *Lolium perenne* (Ryegrass)	Reduction in yield and growth was reported at higher concentration of As.	Jiang and Singh, 1994

2.7 MECHANISM OF As ACTION IN PLANTS

The plants which are grown in soils with high arsenate concentration take up arsenate readily because it is chemically very much similar to the phosphate and it enters root system with the same mechanism as of Phosphate (Meharg *et al*., 1994; Asher and Reay, 1979). The uptake of As is either by the mode of mycorrhizal fungi present in the close vicinity of the plant or through the mode of roots (Sharples *et al*., 2000; Abedin *et al*., 2002). Translocation of Arsenate from roots to shoots occurs through the mode of xylem (Patra and Bhowmik, 2004).

The phytochelatins (PCs) present in the cytoplasm helps in arsenate binding to the cell wall. As get stored in shoot vesicles as AsIII-tris glutathione complex and in root vesicles as AsIII-tristhiolate complex. Due to its analogous nature with phosphate, As(V) readily enters the plasma membrane (Meharg and Jardine, 2003; Patra and Bhowmik, 2004). In the cytoplasm of the cell, the arsenate gets reduced into Arsenite and other organic forms like MMA, DMA, trimethylarsine oxide (TMAsO), tetramethylarsonium ions by either enzymatically (arsenate reductase) or non enzymatically (glutaredoxin) (Meharg and Hartley-Whitaker, 2002).

As toxicity in cell results in loss of fumaric acid which is an essential component of TCA cycle. As the As(III) and As(V) enters the cell, activation of enzyme fumarase takes place. Further, fumarase convert fumaric acid to L-malate (Ullrich-Eberius *et al.*, 1990). Accumulation of As(III) and As(V) in the plant leads to oxidative stress and generation of ROS (Reactive Oxygen Species) and thus results in synthesis of enzymes like catalase, superoxide dismutase, and glutathione-S-transferase, ascorbate and glutathione (Dat *et al.*, 2000).

Inside the cytoplasm, As(V) competes with phosphate in ATP and abolishes the coupled phosphorylation of ADP which results in disruption of cell mechanism (Meharg and Hartley-Whitaker, 2002). Consequently, Adenosine Triphosphate is not formed and this results in disintegration of membrane due to which there is loss of turgor and finally plant shows abnormal effects like wilting and stunted growth (Mallick *et al*., 2011; Woolson *et al*., 1971; Liebig, 1965).

Leonard and Lauwerys (1980) reported that As(III) interferes with protein functioning by combining with sulfhydryl groups. Presence of arsenate in the plant

system results in abnormal functioning in the cells metabolism, the enzyme like glutamate dehydrogenase, keto glutarate oxidase, and d-amino levulinic acid nitrate reductase (Lieberman and Biale, 1956; Das and Roy, 1962, Mishra and Srivastava, 1983). Chauhan *et al*. (2009) reported that the rate of respiration decreases as the As(III) forms pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase by binding to thiols present in the cell.

CHAPTER - 3

OBJECTIVE

The objective of our study was to evaluate the effects of As on early growth and physiology of selected varieties of *T. aestivum.*

In order to accomplish this objective, the following work was carried out.

1. To study the effect of Arsenic on seedling growth of test varieties *of T. aestivum* seedlings in terms of
   - Percent germination
   - Seedling length
   - Root length
   - Shoot length
   - Biomass Dry weight

2. To study the effect of Arsenic on physiology of test varieties *of T. aestivum* seedlings in terms of
   - Chlorophyll a
   - Chlorophyll b
   - Carotenoids
   - Percent cellular respiration

3. To study the effect of Arsenic on macromolecules and their hydrolyzing enzymes of test varieties *of T. aestivum* seedlings in terms of
   - Carbohydrate content
   - Amylase activity
   - Protein content
   - Protease activity

4. To study the effect of Arsenic on antioxidant properties of test varieties *of T. aestivum* seedlings in terms of
   - Superoxide dismutase activity
   - Peroxidase activity

CHAPTER - 4

MATERIALS AND METHODS

4.1 MATERIALS:

4.1.1 Collection of seeds: The seeds of *Triticum aestivum* L. were purchased from Punjab Agricultural University, Ludhiana. Four varieties of *Triticum aestivum* L. *viz*. PBW 343, PBW 550, PBW 621 and HD 2967 were used for the study. The seeds were kept under dry and hygienic conditions.

4.2 INSTRUMENTS:

4.2.1 Electronic Weighing Balance

Sartorius Model TE64 with least measurement 0.1mg was used for weighing of salts to prepare solutions.

4.2.2 pH meter

The pH meter with glass electrode (KCl filled, range 0-14) was used to measure the pH of the solutions. Calibration of the pH meter was done at pH 4.0, 7.0 and 9.2 with buffer solutions.

4.2.3 Centrifuge

Spinwin MC-02 (RPM of 13500, Digital Timer) was used for the centrifugation of plant homogenate.

4.2.4 Water-bath

JSGW Rectangular water Bath with a maximum temperature of 110°C was used for heating.

4.2.5 Ultra Violet and Visible (UV-VIS) Spectrophotometer

Double beam UV-VIS spectrophotometer of Systronics model 2202 was used to analyse the enzymatic activities, total protein content, carbohydrate content, chlorophyll content, respiration ability based on different wavelengths.

4.3 EXPERIMENTAL SET-UP:

- Uniform sized seeds were collected.
- Surface sterilization was done with 5% Sodium hypochlorite.
- 4 different concentrations of arsenic were prepared as solution of Sodium Arsenate *viz*. 125µM, 250µM, 500µM, 750µM in distilled water.
- The pre-sterilized germinating trays were lined with thin layer of sterilized cotton and filter paper. 40 seeds were placed in each tray. The different trays were treated with 15ml of different concentration of sodium arsenate as prepared above.
- Triplicates were taken for each concentration of sodium arsenate and seeds grown with distilled water served as control.
- The germinating trays were kept in the laboratory for 5 days at well aerated place, at 25ºC temperature under dry and hygienic conditions.
- The seedlings were terminated on 6^{th} day and various parameters were studied.

4.4 METHODS:

4.4.1 Germination Studies:

Uniform and healthy seeds of *Triticum aestivum* L. were soaked in 0, 125, 250, 500 and 750µM concentration of sodium arsenate for 18 hours. After 18 hours, the seeds were placed in the trays lined with thin layer of sterilized cotton and filter paper. To each tray, 40 seeds were placed. The tray watered with distilled water served as control. Further, different trays were watered with 15 ml of 125µM, 250µM, 500µM and 750µM sodium arsenate. On 6^{th} day, the number of germinated seeds was counted and the results were expressed as percentage over control.

The seedlings were harvested on 6^{th} day and the surface of the plants was blotted with tissue paper. Shoot height and root length of plants was measured using metric scale in centimeter. Biomass was estimated of freshly germinated seedlings and after drying at 60 ± 2°C for 48 h in an air-circulated oven.

$$\textbf{Germination percentage} = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds taken for germination}} * 100$$

4.4.2 Chlorophyll content (Lichtenthaler and Welburn, 1983)

Reagent: Acetone 80%.

Procedure:

100mg of test plant material was taken in pre-chilled pestle mortar. Plant material was homogenized by adding 5ml, 80% acetone into it and then taking the paste into test tube. Further test tubes were centrifuged at 1000 rpm for 5 minutes. The chlorophyll and carotenoids were thus recovered in 80% acetone and were measured at wavelength of 646, 663 and 470nm using 80% acetone as blank.

Calculation:

The calculation for both chlorophyll a and chlorophyll b was done on dry weight basis.

$$\text{Chlorophyll a} = (12.21 * A_{663}) – (2.81 * A_{646}) = X$$

$$\text{Chlorophyll b} = (20.31 * A_{646}) – (5.03 * A_{663}) = Y$$

$$\text{Carotenoids} = [1000\ A_{470} - 3.27(\text{Chl a}) – 104(\text{Chl b})] / 229$$

Where, $A_{646,}$ A_{663} and A_{470} represent extinction values at 646nm, 663nm and 470nm, respectively.

4.4.3 Estimation of percent cellular respiratory ability (Steponkus and Lanphear, 1967)

Reagents:

a) Reagent A: Phosphate buffer solution, 0.1M, pH 7.4.
b) Reagent B: Triphenyl tetrazolium chloride (2-3%) (TTC) solution: 0.6w/v was prepared by dissolving 600mg of TTC in 100ml of Phosphate buffer 0.1M.

Procedure:

To test tubes containing 1.5ml of freshly prepared (0.6% w/v) TTC solution, 50mg of tissue from fully expanded leaves of test plants were dipped. The test tubes were incubated at room temperature for 18 hours in dark. The colour of the plant tissue in test tube turned red. After 18 hours, TTC solution was drained off from the test tubes. The remaining leaf tissues in test tubes were gently washed 2-3 times with distilled water to completely remove TTC solution. After washing, the red color of leaf tissues was extracted in test tubes containing absolute alcohol

(5ml). The test tube content was boiled for 20 minutes on water bath. The extinction value was read at 530nm and expressed in terms of dry weight equivalent. Dry weight equivalents of each sample were determined by keeping 50mg of fresh leaves in an oven at 80°C for 24 hours. The cellular respiratory ability was expressed as a percent with respect to control.

4.4.4 Carbohydrate estimation (Loewus, 1952)

Reagents:

a) Anthrone reagent: Dissolved 200mg anthrone in 100ml of ice cold 95% H_2SO_4. Freshly prepared solution was used.

Procedure:

200µl of the plant extract was taken in a test tube and distilled water was added to make final volume of 1ml. 1ml of distilled water was taken in another test tube and marked as blank. 4mL of anthrone reagent was added to both test tubes which were heated thereafter for eight minutes in boiling water bath. Test tubes were cooled rapidly and when green to dark green colour appeared, reading on UV-Visible spectrophotometer at 630nm was taken. The amount of carbohydrate in samples was calculated from standard curve prepared by taking different concentrations of glucose (20-100µg).

4.4.5 *α* Amylase activity (Muentz, 1977)

Reagents:

a) Substrate solution: Added 150mg soluble starch, 600mg potassium dihydrogen phosphate and 20mg of anhydrous calcium chloride in 100ml of distilled water, boiled for 1 minute, cooled and filtered.
b) EDTA solution: Ethylene Diamine Tetra Acetic acid Disodium salt 0.1M in distilled water.
c) Iodine solution: 25.4mg iodine dissolved with 0.4g potassium iodide in 100ml distilled water
Procedure:

The *α*-Amylase specific activity was measured in terms of starch used. 1ml of substrate solution was added to 0.5ml of enzyme extract. The mixture was incubated for half an hour and then 0.1ml of 0.1M EDTA was added. From this

reaction mixture, 0.2ml was taken in a separate test tube. In that test tube 3ml of iodine solution was added and the concentration of left over starch was measured spectrophotometrically at 630nm using starch (50µg/ml) as standard. A parallel blank was prepared by adding water in place of enzyme extract. The enzyme activity was expressed in terms of µg minute/mg/protein.

4.4.6 Protein estimation (Lowry *et al.,* 1951)

Reagents:

a) Reagent A: 2% Sodium Carbonate in 0.1N sodium hydroxide.
b) Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate.
c) Reagent C: 50ml of reagent A was mixed with 1ml of reagent B just before use.
d) Reagent D: Folin Ciocalteau's phenol reagent was diluted with water in 1:1 v/v before use.

Procedure:

2.5ml of reagent C was added to 0.5ml of plant enzyme extract taken in a test tube. The contents were mixed thoroughly and then allowed to stand for 10 minutes at room temperature. After 10 minutes, 0.25ml reagent D was added in the test tube and was mixed properly. After 30 minutes, the contents in the test tube turned blue and the intensity was read at 520nm using UV-Visible spectrophotometer. Standard curve was prepared to calculate protein content in the samples by taking different concentrations of bovine serum albumin (20-100µg).

4.4.7 Protease Assay (Basha and Beavers, 1975)

Reagents:

a) Reagent A: 2% sodium carbonate in 0.1N sodium hydroxide.
b) Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate.
c) Reagent C: 50ml of reagent A was mixed with 1ml of reagent B just before use.
d) Reagent D: Folin Ciocalteau's phenol reagent was diluted with water in 1:1 before use.
e) Casein solution: 1% in 0.1M phosphate buffer, pH 6.0.
f) TCA solution: 15%, w/v in distilled water.

Procedure:

0.5ml of enzyme extract was taken in test tube to which 0.5ml of casein solution was added. Incubated the reaction mixture for 1 hour at 37ºC and then, 1ml of TCA solution was added to this reaction mixture. It led to precipitation of the proteins. Amino acids were released in supernatant by centrifugation at 10,000 rpm for 5 minutes. Estimation of enzyme activity was done using the collected supernatant by using the method of Lowry *et al.,* 1951. Specific activity was calculated against 50g/ml tyrosine as standard and expressed as g/hour/mg protein.

4.4.8 Superoxide dismutase assay (Marklund and Marklund, 1974)

Reagents:

a) Reagent A: Tris HCl buffer 0.1M, pH 8.2.
b) Reagent B: Ethylene Diamine Tetra Acetic acid Disodium salt (EDTA) 6mM in distilled water.
c) Reagent C: Pyrogallol solution 6mM in distilled water.

Procedure:

1.5ml of 0.1M Tris HCl buffer (pH 8.2), 0.5 ml of 6mM EDTA, 1ml of 6mM pyrogallol solution and 0.1ml of enzyme extract was mixed in a cuvette. Absorbance of this reaction mixture was read at 420nm after every 30 seconds upto 3 minutes. A unit of enzyme activity has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank.

4.4.9 Peroxidase Assay (Shannon *et al.,* 1966)

Reagents:

a) Reagent A: Guaiacol in 0.1M phosphate buffer 0.05M, pH 6.5.
b) Reagent B: Hydrogen peroxide (H_2O_2) 0.8M in distilled water.

Procedure:

The reaction mixture was prepared by adding 3ml of 0.05M guaiacol in 0.1M phosphate buffer (pH 6.5) and 0.1ml of 0.8M H_2O_2 to 0.1ml of enzyme extract. The reaction initiated by adding H_2O_2 and rate of change in absorbance was

recorded at 470nm for 3 minutes at an interval of 30 seconds. The reaction mixture without H_2O_2 was measured as a blank. Peroxidase activity has been expressed as change in absorbance/minute/g of tissue.

4.4.10 Statistical analysis

All experiments were performed in a completely randomized block design and performed twice. For each treatment three replicates were maintained. The data collected from dose response study was subjected to one way ANOVA with Tukey's test.

CHAPTER - 5

RESULTS AND DISCUSSION

RESULTS

The results of the entire research work have been divided into four sections depending upon the type of parameters.

Section A

5.1 The effect of Arsenic on selected varieties of *Triticum aestivum* seedlings in terms of

- Percent germination
- Seedling length
- Root length
- Shoot length
- Dry weight

5.1.1 Percent Seed germination in four varieties of *T. aestivum* in control and different concentration of As

The results of the experiments where the four varieties of the *T. aestivum* i.e. PBW 621, PBW 343, PBW 550 and HD 2967 were tested for arsenic show that in test seeds watered with distilled water (control), the germination was 100% (Fig. 1). Further the test seeds watered with solution having different concentration of sodium arsenate showed reduction in percent germination. At lowest concentration of sodium arsenate (125µM), the germination reduced to approximately 76.6% in PBW 343 and PBW 550 while in PBW 621 and HD 2967 it was 86.6% and 93.3% respectively (Fig. 1). With increase in concentration of sodium arsenate from 250

to 750µM, the percent germination was drastically reduced in all test plants of *T. aestivum*.

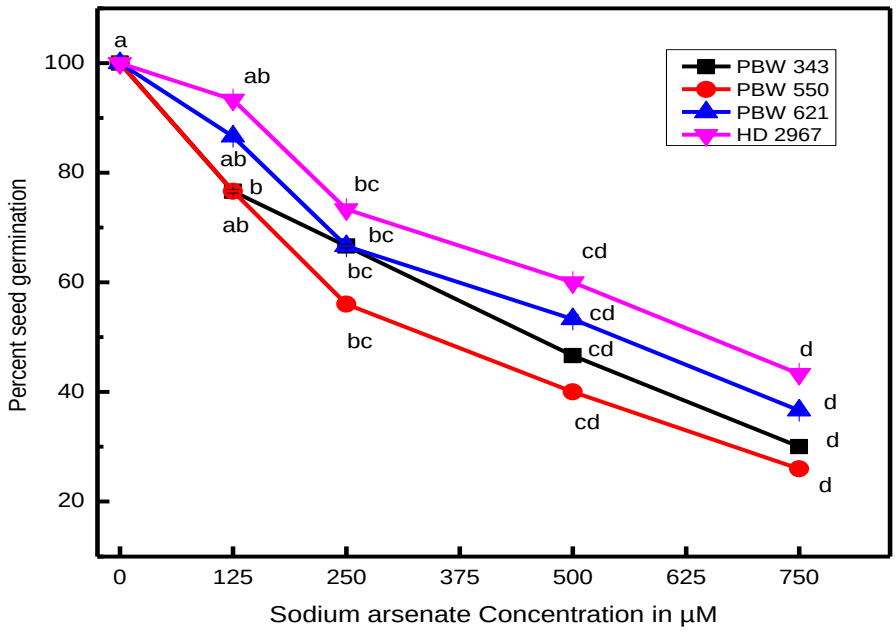
At highest concentration (750µM), the germination was 30% in PBW 343, 26% in PBW 550, 36.6% in PBW 621 and 43.3% in HD 2967 (Fig. 1). This decrease was biologically significant in all varieties indicating toxicity of sodium arsenate for *T. aestivum* plants. Further, it was also observed that HD 2967 was the least affected while PBW 550 was the most affected variety to sodium arsenate toxicity.

Table 4: Seed germination in four varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Number of seed germinated in 4 varieties of *T. aestivum*[#]			
	PBW 343	**PBW 550**	**PBW 621**	**HD 2967**
Control	40.00^{a} ±0	40.00^{a} ±0	40.00^{a}±0	40.00^{a} ±0
125	30.64^{b} ±0.33	30.40^{ab} ±0.88	34.64^{ab} ±0.33	37.32^{ab} ±0.33
250	26.64^{bc} ±0.33	22.40^{bc} ±0.66	26.64^{bc} ±0.88	29.32^{bc} ±0.66
500	18.64^{cd} ±0.66	16.00^{cd} ±0.57	21.32^{cd} ±0.88	24.00^{cd} ±0.57
750	12.00^{d} ±0.57	10.40^{d} ±0.66	14.64^{d} ±0.33	17.32^{d} ±0.33

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.1: Percent seed germination in four varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

At highest concentration of sodium arsenate (750µM), the response to As toxicity was in order of HD 2967(percent germination 43.3) < PBW 621(percent germination 36.6) < PBW 343(percent germination 30) < PBW 550(percent germination 26.6) (Fig.1).

5.1.2 Seedling length in four varieties of *T. aestivum* in control and different concentration of As

Seedling length was measured on sixth day of sowing the different *T. aestivum* varieties watered with distilled water and different sodium arsenate concentration Results as shown in Table 5 indicate that the test plants watered with sodium arsenate solution affected the seedling growth of all four varieties of *T. aestivum*. The mean seedling length in control was 16.4, 14.2, 15.7 and 16.5cm for PBW 343, PBW 550, PBW 621 and HD 2967 varieties of *T. aestivum*, respectively (Table 5). This was the maximum growth for each variety and was assumed 100% for each individual variety (Fig. 2). In all the 4 varieties the seedling length significantly decreased with increase in concentration of sodium arsenate. At lowest concentration (125µM), the reduction in PBW 343 and PBW 550 was 30 and 33% and at highest concentration (750µM), it was approximately 81% (Fig. 2).

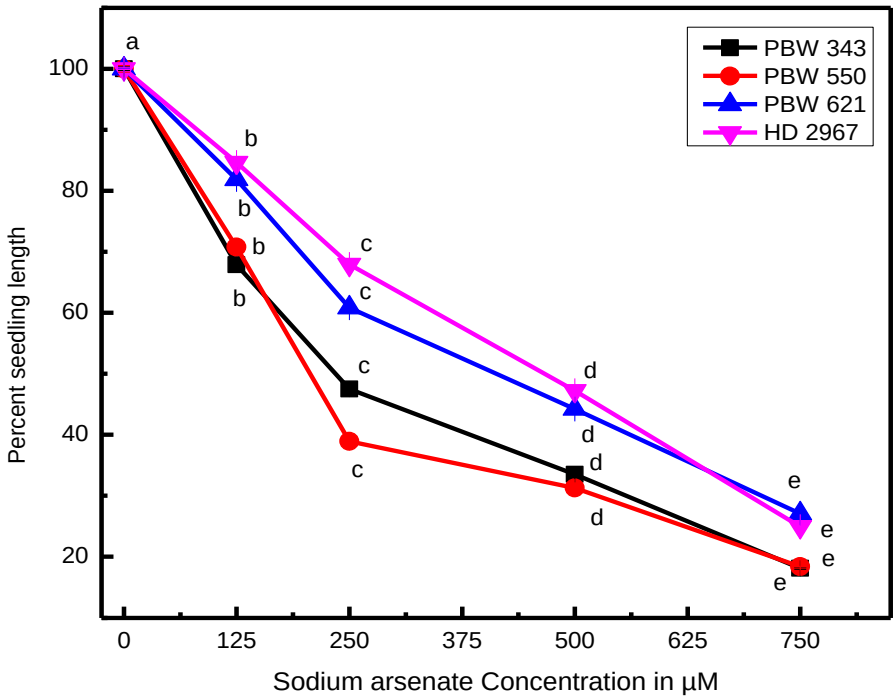
Table 5: Seedling length in four varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Seedling length (cm) in 4 varieties of *T. aestivum*[#]			
	PBW 343	**PBW 550**	**PBW 621**	**HD 2967**
Control	16.40^{a} ±0.28	14.24^{a} ±0.55	15.65^{a} ±0.10	16.49^{a} ±0.12
125	11.13^{b} ±0.20	10.08^{b} ±0.23	12.81^{b} ±0.16	13.96^{b} ±0.12
250	7.79^{c} ±0.24	5.54^{c} ±0.17	9.52^{c} ±0.13	11.20^{c} ±0.19
500	5.50^{d} ±0.25	4.45^{d} ±0.14	6.92^{d} ±0.16	7.79^{d} ±0.44
750	2.97^{e} ±0.14	2.62^{e} ±0.06	4.24^{e} ±0.21	4.11^{e} ±0.24

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Similar trends were observed in other *T. aestivum* varieties under study indicating As toxicity. In case of PBW 621 and HD 2967 at lowest concentration (125µM), the reduction was 15 % and 20% and at highest concentration (750µM), it was up to 73% and 75% (Fig. 2).

Fig. 2: Percent seedling length in four varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

Further, from the Fig. 2, it was observed that there is significant reduction in seedling length in all *T. aestivum* varieties. However, the decrease was more in PBW 343 and PBW 550 compared to the other two varieties. The results indicate that PBW 343 and PBW 550 are more sensitive to sodium arsenate.

Further, the root length and shoot length were measured separately to know which part is more affected by the sodium arsenate.

5.1.3 Root length in four varieties of *T. aestivum* in control and different concentration of As

It was observed that in PBW 343, PBW 550, PBW 621 and HD 2967, the mean root length was 8.94, 7.52, 7.84 and 7.87cm (Table 6) respectively in control. In response to treatment of sodium arsenate, the root length decreased in all *T. aestivum* varieties. At lowest concentration (125µM), in varieties PBW 343, PBW 550, PBW 621 and HD 2967 the reduction was 32.14%, 29.3%, 18.15% and 15.04% (Fig.3) respectively. While at highest concentration (750µM), the reduction in case of PBW 343, PBW 550, PBW 621 and HD 2967 was 85.51%, 86.47%, 77.81% and 76.12% respectively (Fig.3).

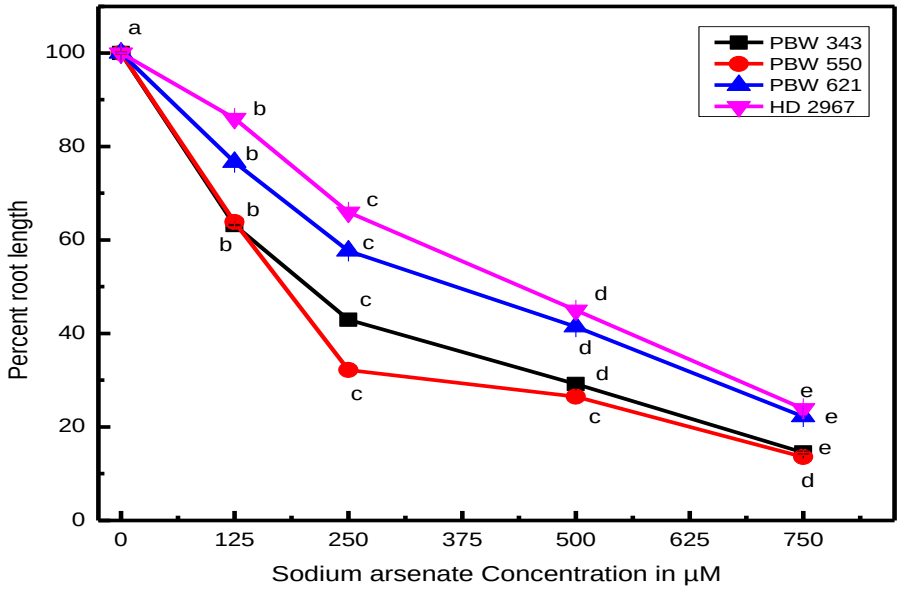
Table 6: Root length in four varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Root length (cm) in 4 varieties of *T. aestivum*[#]			
	PBW 343	**PBW 550**	**PBW 621**	**HD 2967**
Control	$8.94^{a} \pm 0.23$	$7.52^{a} \pm 0.30$	$7.84^{a} \pm 0.12$	$7.87^{a} \pm 0.07$
125	$5.65^{b} \pm 0.14$	$4.80^{b} \pm 0.18$	$6.01^{b} \pm 0.10$	$6.77^{b} \pm 0.11$
250	$3.84^{c} \pm 0.23$	$2.42^{c} \pm 0.10$	$4.52^{c} \pm 0.12$	$5.19^{c} \pm 0.11$
500	$2.61^{d} \pm 0.19$	$1.99^{c} \pm 0.09$	$3.25^{d} \pm 0.08$	$3.54^{d} \pm 0.14$
750	$1.09^{e} \pm 0.31$	$1.21^{d} \pm 0.31$	$1.74^{e} \pm 0.10$	$1.88^{e} \pm 0.18$

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Further, from the Fig. 3, it was observed that root length in all varieties of *T. aestivum* was decreasing significantly to that of control. However, the decrease in root length was more in PBW 343 and PBW 550 than the PBW 621 and HD 2967 which indicates that PBW 343 and PBW 550 are the sensitive varieties while PBW 621 and HD2967 were the resistant varieties.

Fig. 3: Percent root length in four varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

5.1.4 Shoot length in four varieties of *T. aestivum* in control and different concentration of As

The effect of As toxicity was observed in four varieties of *T. aestivum* at lowest concentration (125µM), in varieties PBW 343, PBW 550, PBW 621 and HD 2967 the reduction was 26.55%, 21.43%, 12.94% and 16.01% (Fig.4) respectively. While at highest concentration (750µM), the reduction in case of PBW 343, PBW 550, PBW 621 and HD 2967 was 74.8%, 79.02%, 66.71% and 65.78% respectively (Fig.4).

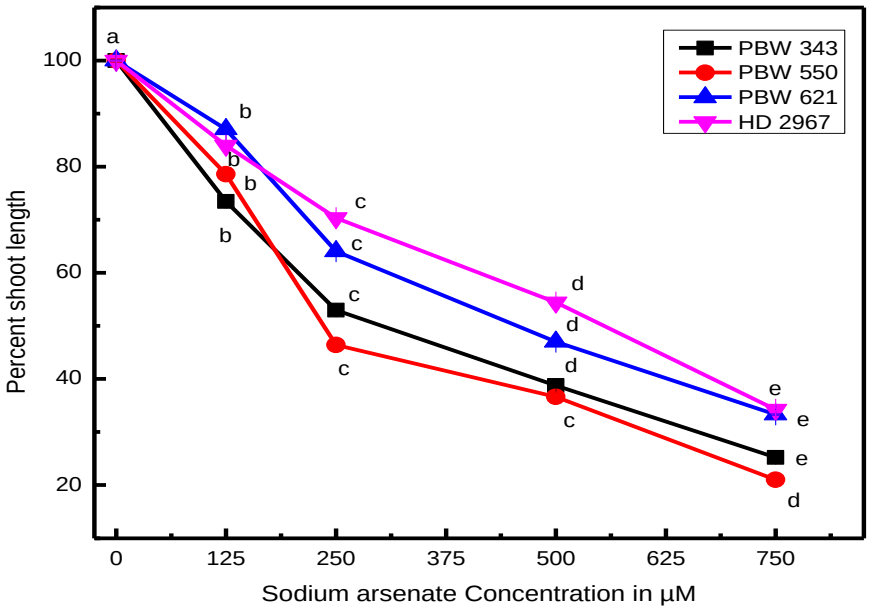
Further, from the Fig. 4, it was observed that shoot length in all varieties of *T. aestivum* was decreasing significantly to that of control. However, the effect was more in PBW 550 and PBW 343 than the other two varieties. Thus these can be regarded as sensitive varieties.

Table 7: Shoot length in four varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Shoot length (cm) in 4 varieties of *T. aestivum*[#]			
	PBW 343	**PBW 550**	**PBW 621**	**HD 2967**
Control	$7.46^{a} \pm 0.22$	$6.72^{a} \pm 0.31$	$7.81^{a} \pm 0.07$	$8.56^{a} \pm 0.11$
125	$5.48^{b} \pm 0.27$	$5.28^{b} \pm 0.25$	$6.80^{b} \pm 0.12$	$7.19^{b} \pm 0.10$
250	$3.95^{c} \pm 0.12$	$3.12^{c} \pm 0.16$	$5.00^{c} \pm 0.11$	$6.02^{c} \pm 0.09$
500	$2.89^{d} \pm 0.14$	$2.46^{c} \pm 0.10$	$3.67^{d} \pm 0.09$	$4.66^{d} \pm 0.09$
750	$1.88^{e} \pm 0.12$	$1.41^{d} \pm 0.06$	$2.60^{e} \pm 0.10$	$2.93^{e} \pm 0.15$

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig. 4: Percent shoot length in four varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

5.1.5 Biomass dry weight in four varieties of *T. aestivum* in control and different concentration of As

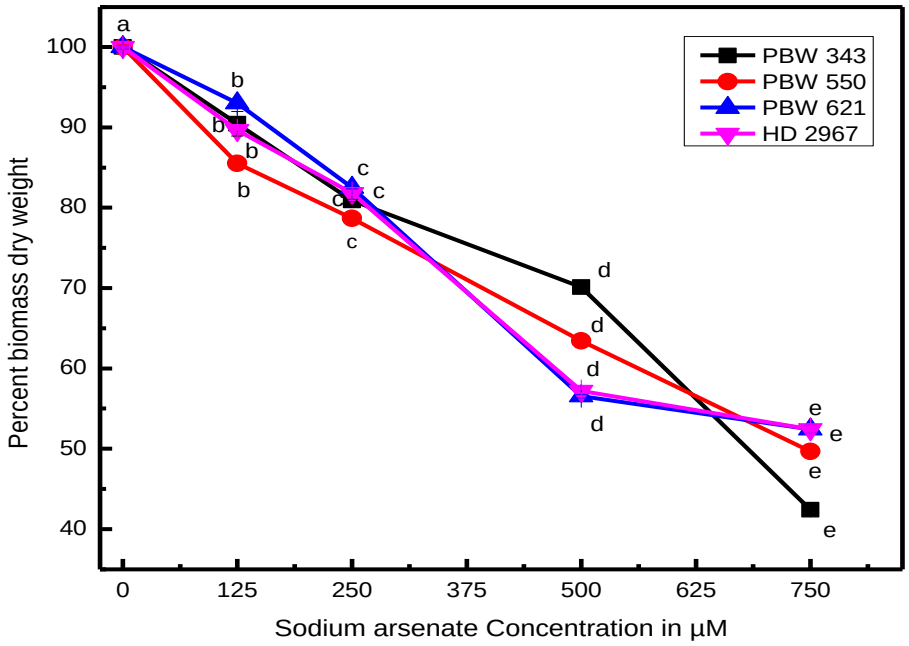
Similar to the decrease in seedling length of test plants in response to As toxicity, the dry weight of test plants also decreased with increase in concentration of sodium arsenate. In test plants grown without any sodium arsenate treatment, all the four varieties show maximum dry weight. But at lowest concentration (125µM), the mean dry weight of PBW 343, PBW 550, PBW 621 and HD 2967 were 46.76, 52.00, 67.73 and 65.26 mg respectively (Table 8) indicating a decrease of 9.56%, 14.48%, 6.97% and 10.36% respectively (Fig. 5). At highest concentration (750µM), the loss in dry weight in PBW 343, PBW 550, PBW 621 and HD 2967 was 57.59%, 50.33%, 47.59% and 47.59% respectively (Fig. 5). The biomass of the seedlings was decreasing with increase in sodium arsenate concentration; the decrease was significant to that of control.

Table 8: Biomass dry weight in four varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Biomass dry weight (mg) in 4 varieties of *T. aestivum*[#]			
	PBW 343	**PBW 550**	**PBW 621**	**HD 2967**
0	$51.70^{a} \pm 0.20$	$60.80^{a} \pm 0.26$	$72.80^{a} \pm 0.15$	$72.80^{a} \pm 0.15$
125	$46.76^{b} \pm 1.53$	$52.00^{b} \pm 0.34$	$67.73^{b} \pm 0.27$	$65.26^{b} \pm 0.27$
250	$41.83^{c} \pm 0.14$	$47.83^{c} \pm 0.54$	$60.06^{c} \pm 0.14$	$59.56^{c} \pm 0.14$
500	$36.26^{d} \pm 0.23$	$38.56^{d} \pm 0.71$	$41.16^{d} \pm 0.20$	$41.63^{d} \pm 0.20$
750	$21.93^{e} \pm 0.35$	$30.20^{e} \pm 0.32$	$38.16^{e} \pm 0.49$	$38.16^{e} \pm 0.49$

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig. 5: Percent biomass dry weight in four varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

Further, from the Fig. 5, it was observed that dry weight in all varieties of *T. aestivum* was decreasing significantly to that of control. Out of four varieties PBW 343 is the most affected and HD 2967 is the least affected variety.

Section B

It was observed that out of four varieties of *T. aestivum* PBW 343 and PBW 550 were showing less growth due to change in season. Thus, for further studies only PBW 621 and HD 2967 varieties of *T. aestivum* were taken.

5.2.1 Chlorophyll a content in two varieties of *T. aestivum* in control and different concentration of As

The photosynthetic pigment Chlorophyll a was affected by sodium arsenate concentrations. It was observed that content of chlorophyll a decreased in both the varieties of *T. aestivum* i.e. PBW 621 and HD 2967. At 125µM concentration, the mean chlorophyll a content was 5.70 and 5.32mg/gfresh weight (Table 9) respectively in HD2967 and PBW 621. But as the concentration increases, the effect was more on variety PBW 621 compared to the other variety. At 500 to 750µM concentration, the chlorophyll content was 56% and 35% in PBW 621 and 55% and 38% in HD 2967 compared to control (Fig. 6). Both the varieties followed decreasing trend.

Table 9: Chlorophyll a content in two varieties of *T. aestivum* in control and different concentration of As

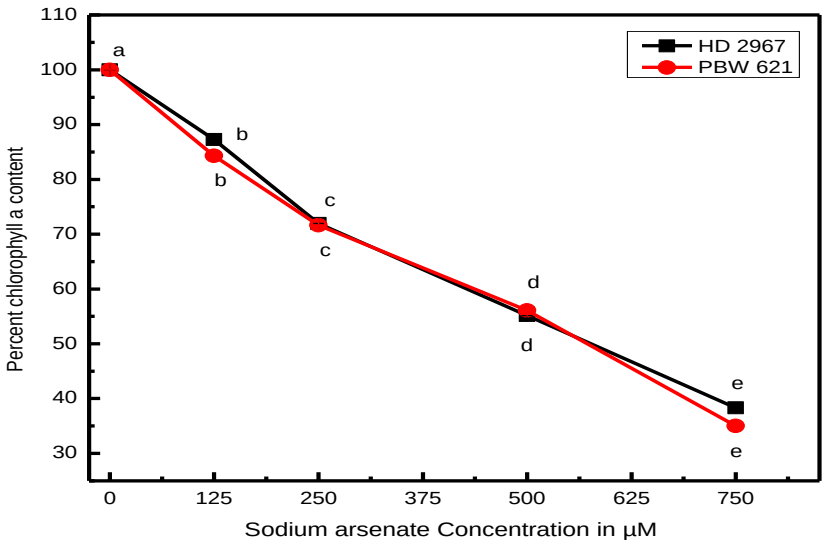
As conc.(µM)	Chlorophyll a content (mg/gfresh weight) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	6.53^{a} ±0.03	6.31^{a} ±0.05
125	5.70^{b} ±0.04	5.32^{b} ±0.08
250	4.70^{c} ±0.04	4.52^{c} ±0.06
500	3.60^{d} ±0.02	3.54^{d} ±0.03
750	2.50^{e} ±0.04	2.21^{e} ±0.02

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

The leaves of test plants watered with 500 and 750µM sodium arsenate showed yellow spots indicating severe affect on chlorophyll content. Both the varieties

followed similar trend and the decrease in chlorophyll content was biologically significant in both the varieties. However among the two varieties, both showed nearly similar reduction and can be concluded that the presence of As affected both equally.

Fig. 6: Percent chlorophyll a content in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

5.2.2 Chlorophyll b content in two varieties of *T. aestivum* in control and different concentration of As

Similar effect of sodium arsenate was observed on chlorophyll b content. At lowest concentration (125µM), the percent chlorophyll b content was 82% in HD 2967 and 72% in PBW 621 compared to control. At 500µM, the chlorophyll b content was 47% in HD 2967 and 45% in PBW 621 indicating more than 50% reduction in chlorophyll b content in both the varieties (Fig.7). However, it was observed that at higher concentration the effect of sodium arsenate on the percent reduction in chlorophyll b was nearly 65% and 70% respectively in HD 2967 and PBW 621. On the basis of results analyzed in Fig.7, conclusion can be drawn that there is sharp reduction in chlorophyll content with increase in As concentration and this

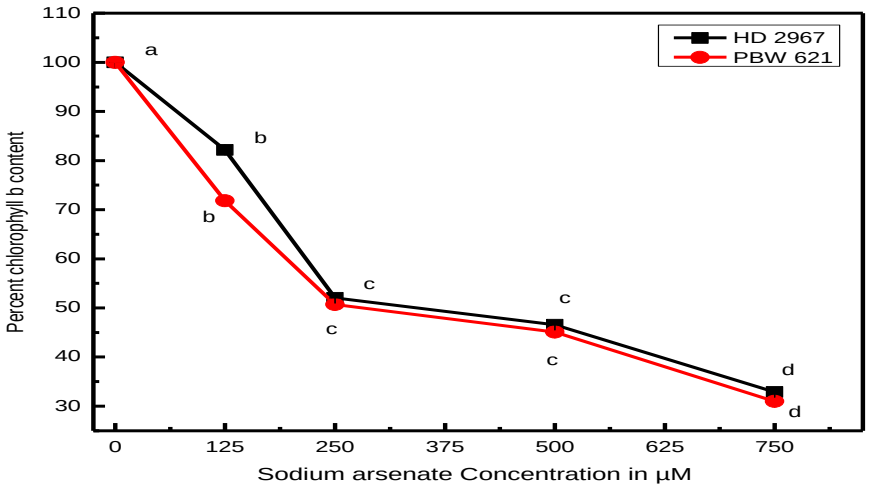
reduction is significant at each concentration with the previous concentration. Among the two varieties, the percent reduction is nearly similar and biologically insignificant with each other.

Table 10: Chlorophyll b content in two varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Chlorophyll b content (mg/g fresh weight) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	0.7390^{a} ±0.0194	0.7140^{a} ±0.0036
125	0.6007^{b} ±0.0048	0.5143^{b} ±0.0078
250	0.3813^{c} ±0.0024	0.3637^{c} ±0.0042
500	0.3483^{c} ±0.0046	0.3573^{c} ±0.0049
750	0.2463^{d} ±0.0044	0.2217^{d} ±0.0023

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.7: Chlorophyll b content in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

The percent reduction in the two test varieties was observed to be insignificant with each other.

5.2.3 Carotenoids content in two varieties of *T. aestivum* in control and different concentration of As

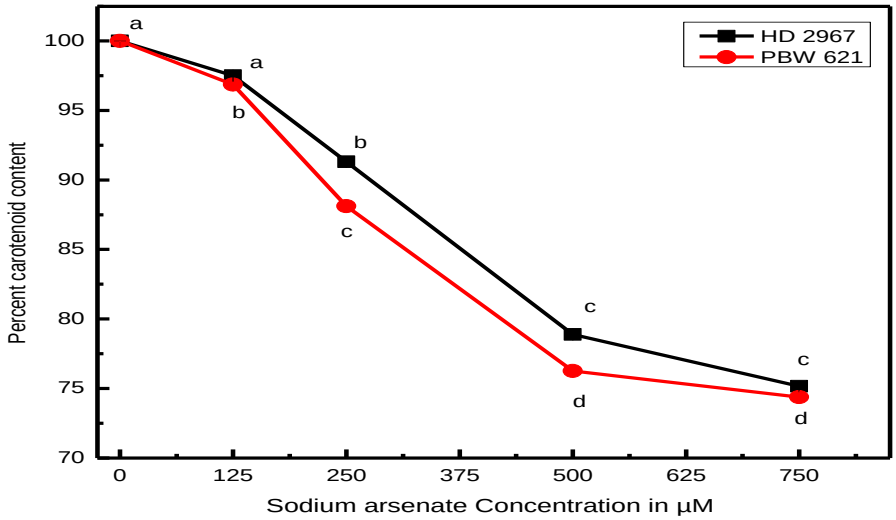
Carotenoids play a significant role in plants photosynthetic activity as well as antioxidant activity. The test results indicate that unlike to chlorophyll a and b the effect on carotenoids was very less. It was observed that at lowest concentration (125µM), no significant change in carotenoids content was observed. But with increase in sodium arsenate concentration there was gradual decreases in the carotenoids content. At 500µM, the reduction was 24% in PBW 621 and 21% in HD 2967, while at 750µM, the reduction was nearly 25% in HD 2967 and 26% in PBW 621 (Fig. 8). Further, between two varieties, the percent reduction was nearly same and followed parallel trend.

Table 11: Carotenoids content in two varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Carotenoids content (µg/gfresh weight) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	1.6150^{a} ±0.0051	1.6003^{a} ±0.0056
125	1.5787^{a} ±0.0153	1.5527^{b} ±0.0027
250	1.4787^{b} ±0.0105	1.4127^{c} ±0.0130
500	1.2760^{c} ±0.0292	1.1943^{d} ±0.0117
750	1.2117^{c} ±0.0094	1.1980^{d} ±0.0145

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.8: Percent carotenoids content in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

5.2.4 Percent cellular respiration in two varieties of *T. aestivum* in control and different concentration of As

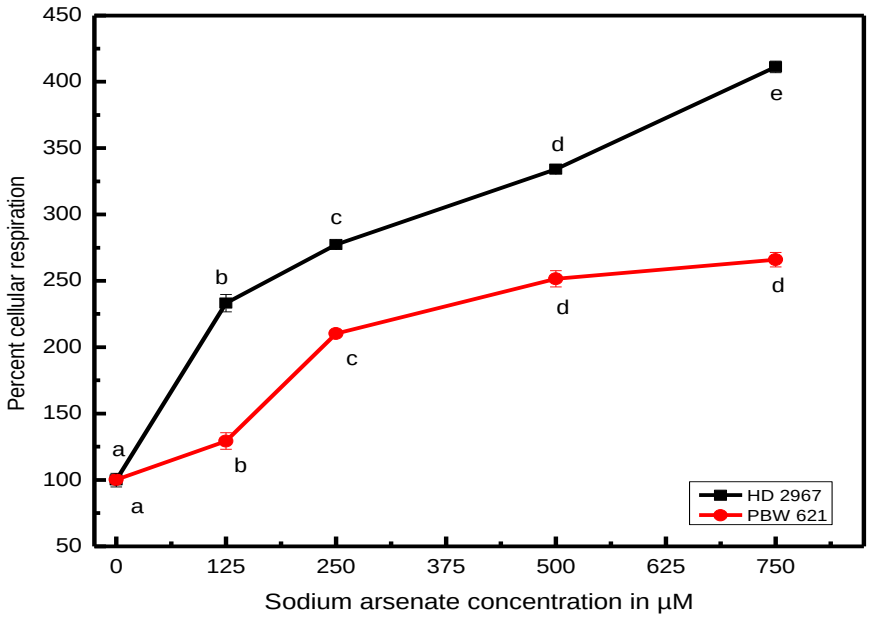
Unlike the above parameters, an increase in cellular respiration was observed in two varieties of *T. aestivum* grown under different concentrations of sodium arsenate. The two varieties showed different response to various concentrations of sodium arsenate. At lowest concentration (125µM), in variety HD 2967 the increase in cellular respiration was 2.33 folds while in case of PBW 621, the increase was 1.29 (Table 12) times to that of control. At 250µM, similar increase in percent cellular respiration was observed in both the varieties. Similarly at highest concentration (750µM), in HD 2967 and PBW 621 the increase was 4.11 folds and 2.65 folds to that of control (Table 12). The above results indicate that both the varieties follow increasing trend but the increase in percent cellular respiration of variety HD 2967 was much higher than PBW 621. Further, the increase in percent cellular respiration was significant compared to control.

Table 12: Percent cellular respiration in two varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Percent cellular respiration in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	99.99[a] ±5.15	100.093[a] ±1.97
125	233.19[b] ±6.59	129.28[b] ±6.29
250	277.21[c] ±2.79	210.21[c] ±2.07
500	334.02[d] ±1.55	251.53[d] ±6.06
750	411.24[e] ±4.29	265.93[d] ±5.46

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig. 9: Percent cellular respiration in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

Section C

5.3.1 Carbohydrate content in two varieties of *T. aestivum* in control and different concentration of As

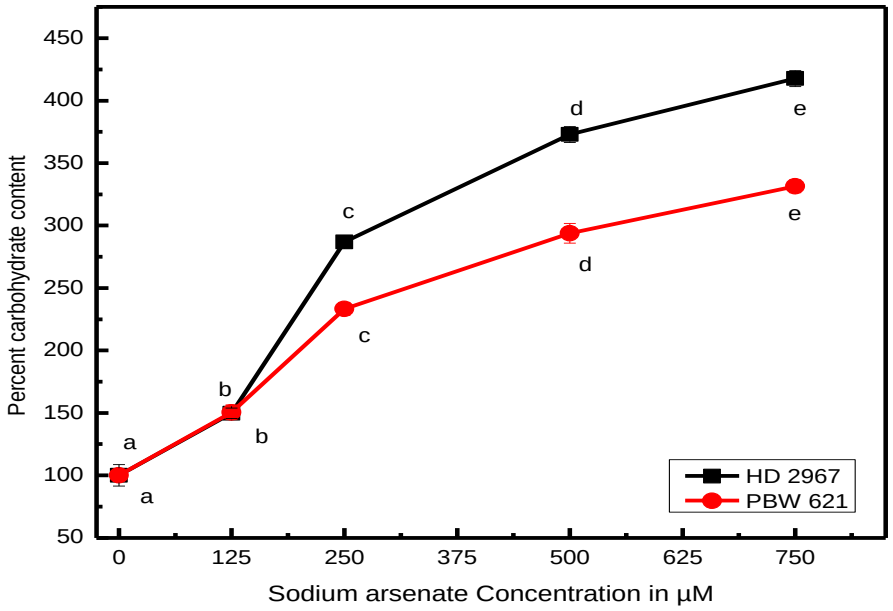
The test results indicate increase in carbohydrate content in test plants watered with sodium arsenate solution. It was observed that with increase in sodium arsenate concentration, the carbohydrate content of both the varieties of test plant *T. aestivum* L. PBW 621 and HD 2967 increased from control to 750µM concentration of sodium arsenate. At lowest concentration (125µM), the carbohydrate content in HD 2967 was increased to 1.45 times and in PBW 621 the increase was 1.95 times (Table 13) compared to that of control. At 500µM concentration, the carbohydrate content was 3.61 times more than the control in variety HD 2967 and 3.81times (Table 13) in variety PBW 621. Similarly at highest concentration (750µM), the carbohydrate content in case of HD 2967 increased 4.04 times and in PBW 621, the increase was 4.30 times in comparison to control (Table 13). The increase in carbohydrate content in test plants watered with distilled water and sodium arsenate solution was significant compared to control and each proceeding concentration of sodium arsenate.

Table 13: Carbohydrate content in two varieties of *T. aestivum* in control and different concentration of As

As conc. (µM)	Carbohydrate content (µg/ml) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	96.87^{a} ±8.65	129.90^{a} ±2.92
125	145.05^{b} ±4.10	195.51^{b} ±5.96
250	277.90^{c} ±3.83	303.10^{c} ±5.11
500	361.27^{d} ±6.36	381.72^{d} ±7.99
750	404.60^{e} ±6.45	430.66^{e} ±5.12

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

.**Fig.10: Percent carbohydrate content in two varieties of *T. aestivum* in control and different concentration of As**



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

The above results indicate that both the varieties follow increasing trend but the increase in carbohydrate content of variety HD 2967 was much higher than PBW 621. In order to know the cause of increase in carbohydrate content, experiment was conducted on the activity of amylase enzyme.

5.3.2 Amylase activity in two varieties of *T. aestivum* in control and different concentration of As

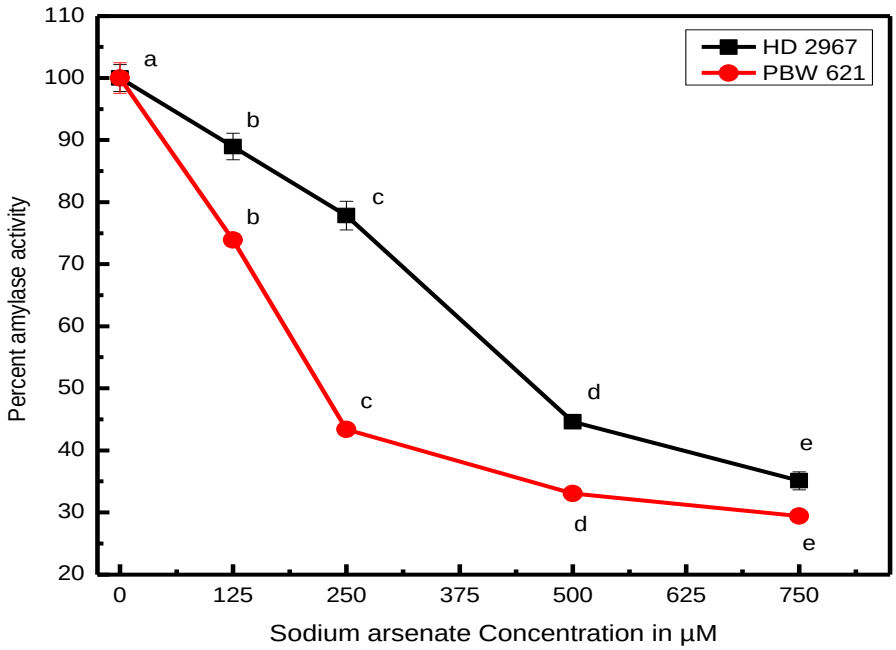
It was observed that different concentrations of sodium arsenate affected the amylase activity of both the varieties of test plant *T. aestivum* L. PBW 621 and HD 2967. In control the mean value of amylase activity was 101 and 99 µg/min/mg/protein in HD 2967 and PBW 621(Table 14). At lowest concentration (125µM), the amylase activity in HD 2967 and PBW 621 was 89% and 74% respectively compared to control (Fig.11). With increase in concentration of sodium arsenate i.e. at 500µM, the activity was further reduced.

Table 14: Amylase activity in two varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	Amylase activity (µg/min/mg/protein) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	101.42[a]±2.18	98.57[a] ±2.44
125	90.23[b]±2.15	72.85[b] ±1.03
250	78.94[c] ±2.32	42.76[c] ±0.12
500	45.23[d] ±1.07	32.56[d] ±0.86
750	35.61[e] ±1.45	28.99[d] ±0.21

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.11: Amylase activity in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

Amylase activity decreases with increase in sodium arsenate concentration, at highest concentration (750µM), the amylase activity reduced by 65% in HD 2967 and 71% in PBW 621 and this decrease was significant to that of control (Fig.11). The decrease in amylase activity indicates as one of the causes of increase in carbohydrate content besides others may also be there.

5.3.3 Protein content in two varieties of *T. aestivum* in control and different concentration of As

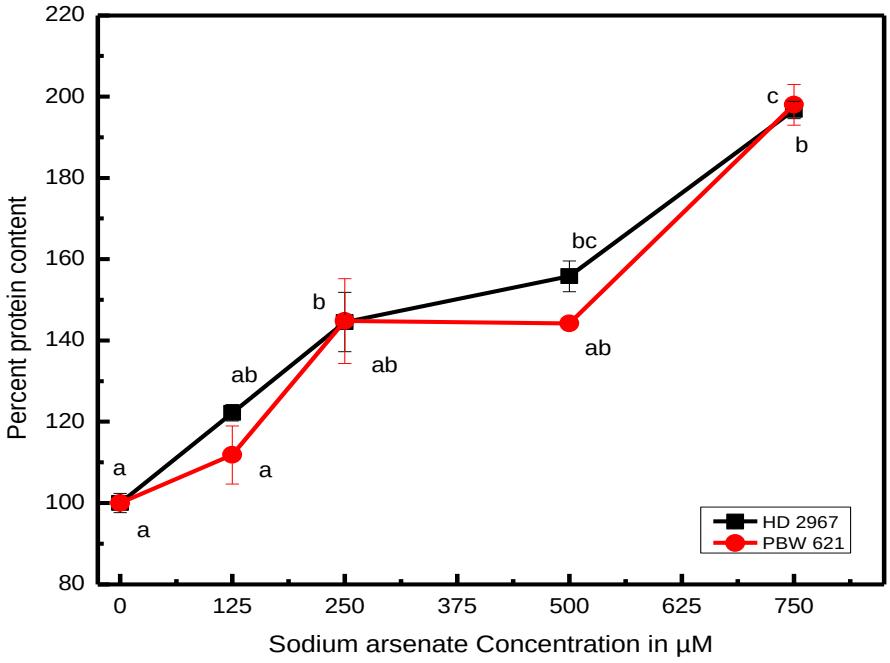
Similar to the carbohydrates, the protein content of the test plants watered with distilled water and 125, 250, 500 and 750µM concentration of sodium arsenate solution was also measured. It was observed that likewise carbohydrates, the content of protein also increased with increase in concentration of sodium arsenate (Table 15). At lowest concentration (125µM), the amount of protein increased by 1.22 times in HD 2967 and 1.11 times (Fig.11) in PBW 621. However, this increase was insignificant compared to control. Further, it was also observed that the percent change in protein content in the two varieties was nearly same and insignificant (Fig.12).

Table 15: Protein content in two varieties of *T. aestivum* in control and different concentration of As

As conc. (µM)	Protein content (µg/l) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	44.46^{a} ±2.37	42.86^{a} ±0.76
125	54.33^{ab} ±2.04	47.93^{a} ±7.16
250	64.26^{b} ±7.27	61.80^{ab} ±0.50
500	69.13^{bc} ±3.81	62.06^{ab}±10.44
750	87.46^{c} ±2.14	84.86^{b} ±5.01

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.12 Percent protein content in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

At 250µM concentration, the percent protein content was 144% in HD 2967 and 145% in PBW 621 (Fig.12). At highest concentration (750µM), the increase in protein content was approximately 197% in HD 2967 and 198% (Fig.11) in PBW 621 varieties and the increase was significant compared to control.

It was observed that protein content was increasing in both varieties of *T. aestivum*. In order to know the cause of increase in protein content, the protease activity was measured.

5.3.4 Protease activtiy in two varieties of *T. aestivum* in control and different concentration of As

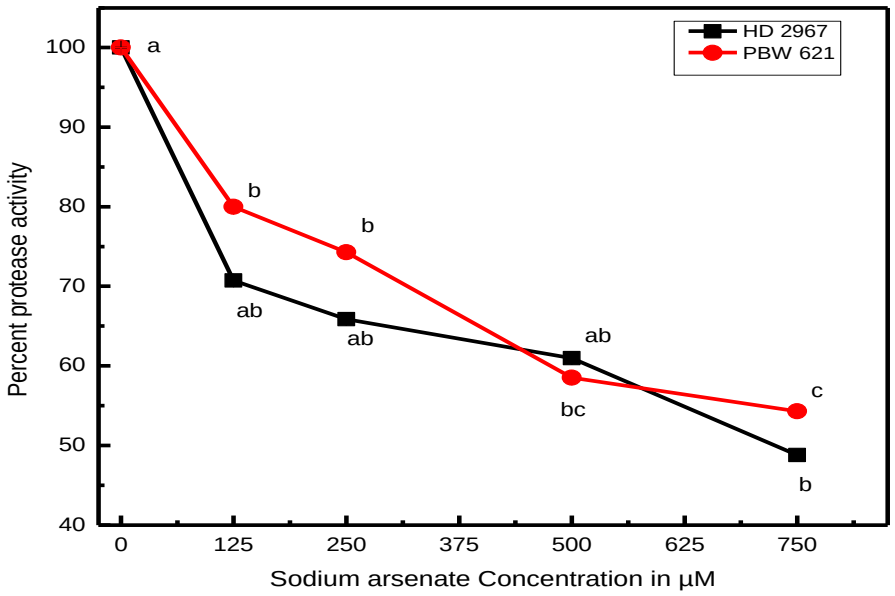
Results indicate that with increase in sodium arsenate concentration from 125 - 750µM the content of protease content decreased.

Table 16: Protease activity in two varieties of *T. aestivum* in control and different concentration of As

As conc. (µM)	Protease activity (µg/h/mg/protein) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	$0.416^{a} \pm 0.054$	$0.358^{a} \pm 0.023$
125	$0.291^{ab} \pm 0.060$	$0.284^{b} \pm 0.001$
250	$0.279^{ab} \pm 0.006$	$0.268^{b} \pm 0.002$
500	$0.259^{ab} \pm 0.002$	$0.245^{bc} \pm 0.004$
750	$0.208^{b} \pm 0.002$	$0.195^{c} \pm 0.004$

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.13: Percent protease activity in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

At lowest concentration (125μM), the decrease was 29% in HD 2967 and 20% in PBW 621. At 250μM, the percent protease content was 34% in HD 2967 and 26% in PBW 621. Similar observations were observed at highest concentration (750μM), in case of HD 2967 the protease content was 51% while in case of PBW 621 it was 46% (Fig.13).

Section D

5.4.1 SOD activity in two varieties of *T. aestivum* in control and different concentration of As

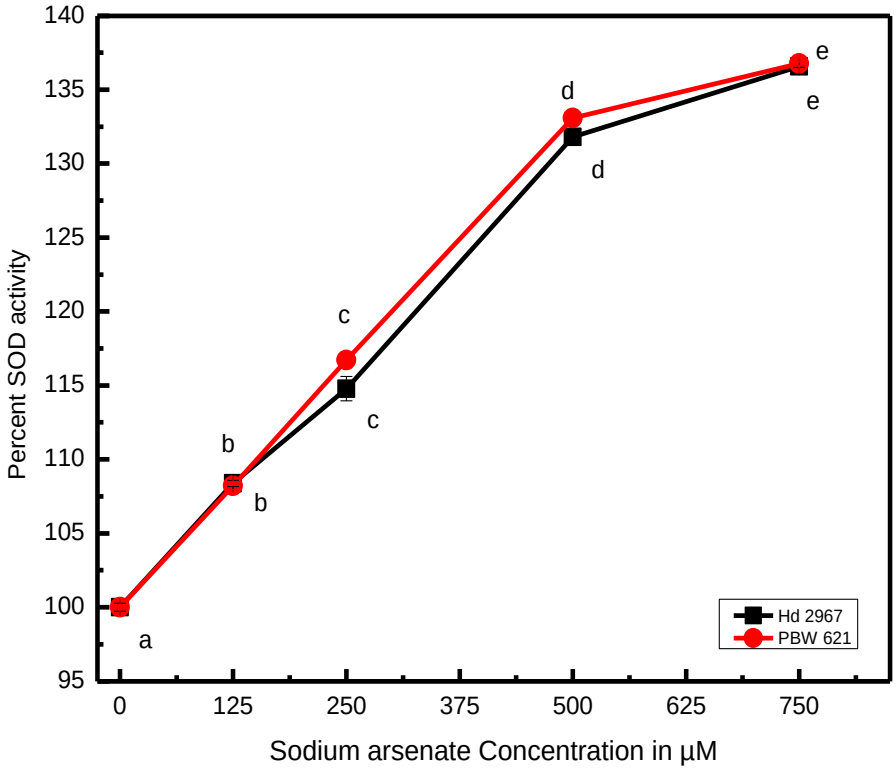
The effect of sodium arsenate on antioxidant activity of two varieties of *T. aestivum* i.e. HD 2967 and PBW 621 was studied and it was observed that the superoxide dismutase activity increased with increase in the concentration. In case of HD 2967 decrease in activity of SOD enzyme at 125, 250, 500, 750µM concentration, was around 108%, 115%, 132% and 137% (Fig.14) the values indicate that the increase was significant when compared to control. Similarly in variety PBW 621 the value of enzyme activity was also showing the increasing trend, the values were 108% at 125µM, 117% at 250µM, 133% at 500µM and 137% at 750µM concentration of the sodium arsenate (Fig.14). The results were significant to that of the control.

Table 17: SOD activity in two varieties of *T. aestivum* in control and different concentration of As

As conc. (µM)	SOD activity (min/g/fresh weight) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	59.37^{e} ±0.27	57.88^{e} ±0.13
125	64.34^{d} ±0.20	62.63^{d} ±0.24
250	68.15^{c} ±0.82	67.56^{c} ±0.33
500	78.26^{b} ±0.37	77.03^{b} ±0.26
750	81.08^{a} ±0.05	79.16^{a} ±0.11

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.14: Percent SOD activity in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

5.4.2 POD activity in two varieties of *T. aestivum* in control and different concentration of As

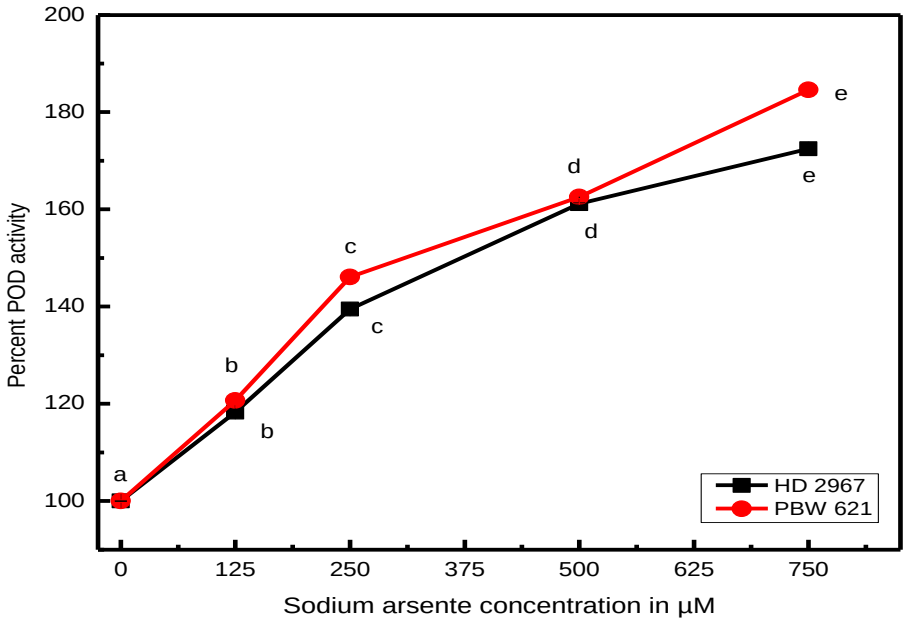
Peroxidase activity in both the varieties of *T. aestivum* followed increasing trend. In variety HD 2967 the increase was 118%, 139%, 161% and 172% (Fig.15) at 125, 250, 500 and 750µM sodium arsenate concentration. The increase in POD activity followed similar trend in PBW 621 at concentration 125, 250, 500 and 750µM the increase was 120%, 146%, 163% and 185% (Fig.15) more than control. The increase was significant in comparison to control.

Table 18: POD activity in two varieties of *T. aestivum* in control and different concentration of As

As conc.(µM)	POD activity (min/g/tissue) in 2 varieties of *T. aestivum*[#]	
	HD 2967	**PBW 621**
Control	11.04^{e} ±0.12	10.20^{e} ±0.27
125	13.06^{d} ±0.22	12.31^{d} ±0.10
250	15.40^{c} ±0.26	14.90^{c} ±0.11
500	17.79^{b} ±0.21	16.58^{b} ±0.28
750	19.04^{a} ±0.13	18.83^{a} ±0.10

[#]Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Fig.15: Percent POD activity in two varieties of *T. aestivum* in control and different concentration of As



Data are expressed as mean values ±S.E. (n=3 each having 40 seeds) and have been analyzed by one way Anova, post hoc multiple comparison Tukey's test. Means followed by the same letter on same line are not significantly different by Tukey's test at the 5% level.

DISCUSSION

Physiological and growth parameters of the four varieties of *Triticum aestivum viz.* PBW 343, PBW 550, PBW 621 and HD 2967 were studied under different concentrations of sodium arsenate and it was observed that the seed germination of the test plants was significantly affected with increase in concentration of sodium arsenate than the control.

As the concentration increased, the percent germination in all the four varieties was drastically reduced. At highest sodium arsenate concentration (750µM), the germination was 30% in PBW 343, 26% in PBW 550, 36.6% in PBW 621 and 43.3% in HD 2967 (Fig.1). The decrease in seed germination was also observed by number of researchers working in this field (Hoffman and Schenk, 2011; Hong *et al.,* 2011; Mallick *et al.,* 2011; Shao *et al.,* 2011; Singh *et al*., 2009).

The effect of sodium arsenate on different varieties of *T. aestivum* indicates that the growth of the plant was inhibited. The varieties PBW 343 and 550 were more sensitive when compared to the PBW 621 and HD 2967. The root length and shoot length, both were reduced as a result of increase in sodium arsenate concentration. Fig. 1 to 5 indicates the reduction in germination percentage, seedling length and dry weight of the four varieties of the *T. aestivum.* The above observations are in line with the pervious observations done by number of researchers Nickson *et al.,* 1998; Abedin *et al.,* 2002; Mallick *et al.,* 2011; Shao *et al.,* 2011; Singh *et al*., 2009; Choudhury *et al.,* 2008; Liu and Zhang*,* 2007; Chaturvedi, 2006a. It has been reported that arsenic causes water stress in the plants, as As concentration is increased the availability of the water to the seedling is drastically reduced and thus disturbs the nutrient balance of the plant which causes reduced growth and reduction in dry weight (Shaibur *et al.,* 2006). According to Ter-Walle and Slater (1967) arsenate interferes with the mechanism of the phosphorylation as it binds with the ADP and thus no ATP is formed which ultimately leads to the reduction in growth of the plants. Also the effect is more on root length than the shoot length of the test plants as roots are the first site of contact with the arsenic thus inhibition is more on the roots than the shoots (Abedin *et al.,* 2002).

The effect of sodium arsenate was observed on photosynthetic pigments of the test plants PBW 621 and HD 2967 and it was observed that percent chlorophyll a,

b and carotenoids were reducing with increase in the concentration of sodium arsenate. The above observations are in line with the pervious observations made by other researchers Chun-xi *et al*., 2007; Mascher *et al.,* 2002; Moreno-Jiménez *et al.,* 2009. According to Van and Clijsters (1990) when As enters to the cell, it gets accumulated and interferes with chlorophyll by combing with the –SH base of the protein, which further leads to disruption of the chloroplast and thus the content of chlorophyll a and b are reduced.

Percent Cellular respiration in both the varieties followed the increasing trend with increase in sodium arsenate concentration (Fig. 9). The above observations are in line with the pervious observations made by Singh *et al.,* 2007; Wang *et al.,* 2001; Shao *et al*., 2011. The energy required for the growth of plant is provided by the respiration process. Under heavy metal stress the physiological and biochemical processes are disturbed because the percent cellular respiration is disturbed. According to Wang *et al.* (2001) the rate of respiration increases with increase in As concentration due to enhancement in respiratory enzymes and tricarboxylic cycle which leads to high production of energy by the respective cells. Shao *et al*. (2011) reported that every plant has its own mechanism to counter the heavy metal stress and thus certain physiological changes takes place for the betterment of the plant.

It is evident from the results that higher concentration of sodium arsenate leads to increase in the carbohydrate content. It was observed that at highest concentration (750µM), the carbohydrate content was 4.18 times in HD 2967 and 3.31 times in PBW 621. The above observations are similar to previous reports that arsenic increases carbohydrate content of the plant (Dubey and Singh, 1999; Chun-xi *et al*., 2007).

Carbohydrate is the substrate of respiration process and as the above results indicate that respiratory ability of the plant under As concentrations is increasing thus the carbohydrate content is also increasing. According to Chun-xi *et al.* (2007) under metallic stress, the plant needs more respiratory substrate for production of more energy thus more soluble sugars are formed in order to counter the toxicity of the metals which helps the plant cellular components to combat the dehydration in the cells. Thus under As toxicity, the cells accumulate more soluble sugars and as a result the carbohydrate content is increased.

High level of Arsenic leads to alter the content of the proteins in the plants. Results indicate that compared to control, the protein content in both varieties watered with sodium arsenate solution was much higher. In case of HD 2967, the protein content at 250µM, concentration was 145% (Fig.11) and at 750µM, the content was 197% compared to control. While in case of PBW 621, the content of protein was 145% at 250µM while at 750µM, the content was 198% compared to control (Fig.11). The above results are similar to the observations of Chun-xi *et al*., 2007; Mallick *et al*., 2011 etc. on different crops. Acc to Yu *et al*. (1995) under As toxicity, the growth of the plant is reduced, so less protein is used for the metabolic reactions which ultimately leads to accumulation of proteins in the cells and thus the protein content in the seedling is increased.

The carbohydrates and proteins are metabolized by hydrolyzing enzymes amylases and proteases, the content of both the enzymes reduced with increase in sodium arsenate concentration. At 750µM, the value of *α* amylase was 35.11% and 29.11% (Fig.12) in HD 2967 and PBW 621 respectively and the decrease was significant with control. In protease, similar observations were observed at highest concentration (750µM), in HD 2967, the protease content was decreased 51% while in case of PBW 621 it was 46% compared to control (Fig.13). Similar observations were studied by Liu *et al*., 2005; Jha and Dubey, 1999 in which they reported that the increase in Arsenic content leads to reduction in the content of the hydrolyzing enzymes of the treated plant.

Effect of Arsenic was studied on the scavenging enzymes of the cells. It was observed that As leads to the release of ROS which interferes with the cell metabolism and in a protective manner the SOD and POD act upon the cells. SOD is the major O_2- scavenger and thus protects the cell from damage (Chun-xi *et al*., 2007). Plants grown under stress conditions leads to the release of reactive oxygen species which harms the cells metabolism thus in protection against the ROS the cell releases scavenging enzymes superoxide dismutase and peroxidase. It was observed that high concentration of sodium arsenate leads to enhancement in the activity of the SOD and POD in PBW 621 and HD 2967.

CONCLUSION

The effect of sodium arsenate on different varieties of *Triticum aestivum* was studied in the present work. Four varieties of *T. aestivum viz*. PBW 343, PBW 550, PBW 621 and HD 2967 were chosen which are majorly grown in south western parts of the Punjab. Effect of arsenic on the growth, physiological, biochemical and antioxidant parameters was studied and following conclusions were obtained:

- High concentrations of sodium arsenate inhibited the seed germination of all the four varieties, thus reduction in percent germination was observed.
- Reduction in seedling length was observed with increase in concentration of sodium arsenate; and it was also observed that roots were more affected than shoots which indicates that roots are more sensitive to the toxicity.
- As affects the growth of the seedling thus increase in sodium arsenate concentration leads to reduction in dry weight of biomass.
- Photosynthetic pigments chlorophyll a, b and carotenoids contents were reduced with increase in sodium arsenate concentration. While the percent cellular respiratory activity was increased with increase in As concentration.
- Enhancement in content of macromolecules was observed under sodium arsenate concentration. The hydrolyzing enzymes i.e *α* amylase and protease activity was decreased with increase in concentration.
- High concentration of sodium arsenate results in increase in level of antioxidant enzymes. SOD and POD activity increased with increase in As concentration.

At last it can be concluded that presence of arsenic leads to abrupt changes in the growth, physiological and biochemical parameters of the *T. aestivum.*

Further, studies can be carried out in field conditions where full plants can be grown and the accumulation potential of the plant can be studied. Arsenic is a known carcinogen and mutagen, thus studies at chromosomal levels will help us in better understanding of the effects undergoing inside the plant cells.

SUMMARY

Arsenic is present naturally in the environment. But due to its uncontrolled usage in pesticides, industrial applications such as paint manufacturing, embalming fluids, batteries etc. its amount has been increasing beyond permissible limits. As is regarded as Group A carcinogen which leads to carcinogenic and mutagenic changes in the cells. Southwestern parts of Punjab are affected with high amount of As in the water and soil thus As toxicity is being reported from these areas. The crops grown in this area of Punjab might be effected by the As toxicity as ground water is used for irrigating fields.

The present study was formulated to understand the effect of Arsenic on the *T. aestivum* crop grown in southwestern parts of the Punjab. Four different varieties of *T. aestivum viz.* PBW 343, PBW 550, PBW 621 and HD 2967 were chosen for the study as these varieties are majorly grown in southwestern parts of the Punjab.

It was observed that when test plants of *T. aestivum* were grown under different sodium arsenate concentrations the percent germination of all the four varieties was altered. The effect was highest on the PBW 550 as the germination was much reduced in this variety. HD 2967 was the most resistant variety, as the percent germination in this variety was comparatively less hampered than the other *T. aestivum* varieties. The reduction in seedling length, shoot length, root length and dry weight of biomass of test plants were also observed under high As concentration.

The effect of sodium arsenate was observed on photosynthetic pigments of the *T. aestivum* varieties *viz.* PBW 621 and HD 2967. Reduction was observed in chlorophyll a, b and carotenoids content with increase in the concentration of sodium arsenate. Cellular respiratory ability in both the varieties followed the increasing trend with increase in As concentration.

It was observed that sodium arsenate affects the content of macromolecules and their hydrolyzing enzymes. Results indicates that the carbohydrate content of the plant was enhanced with increase in the sodium arsenate concentration and it may be due to the accumulation of the soluble sugars in the cells which leads to increase in carbohydrate content. Likewise, the protein content also followed the

same trend. The content of protein was much higher to that of control and the difference was significant in both the varieties of the *T. aestivum*.

The content of water soluble macromolecules increased with increase in concentration of As, however, the content of their hydrolyzing enzymes followed opposite trend. The carbohydrate catalyzing enzyme, *α* amylase decreased with increase in As concentration in both varieties PBW 621 and HD 2967. The effect of sodium arsenate was also studied on the protease enzyme, which helps in degradation of proteins. It was observed that with increase in As concentration the protease activity was decreased.

Plants grown under stress conditions leads to the release of reactive oxygen species which harms the cells metabolism. As a result cell releases scavenging enzymes like superoxide dismutase and peroxidase. It was observed that the activity of the SOD and POD increased with increase in concentration of As indicating induction of stress under high As concentration.

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