MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF LENTIL CULTIVARS TO DIFFERENT LEVELS OF ARSENIC

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CERTIFICATE

*This is to certify that thesis entitled, "**MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF LENTIL CULTIVARS TO DIFFERENT LEVELS OF ARSENIC**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in AGRONOMY**, embodies the result of a piece of bona fide research work carried out by **Md. Mahmodul Hasan Sohag**, Registration No. **11-04492** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation, has duly been acknowledged.

Dated:
Place: Dhaka, Bangladesh

_____
Professor Dr. Md. Shahidul Islam
Supervisor

DEDICATED TO

Professor Dr. Md. Shahidul Islam

Professor Dr. Mirza Hasanuzzaman

And my beloved parents

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MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF LENTIL CULTIVARS TO DIFFERENT LEVELS OF ARSENIC

ABSTRACT

An experiment was conducted at the Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Japan to investigate the morpho-physiological and biochemical responses of four lentil (*Lens culinaris* Medik.) cultivars viz. BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 under arsenic (As) stress. Seven-day-old seedlings grown in hydroponic culture were treated with different levels of As (0 µM, 80 µM, 160 µM, 320 µM as $Na_2HAsO_4.7H_2O$) for four days. Arsenic treatments reduced plant biomass and water content. Arsenic induced oxidative stress in lentil through the overproduction of reactive oxygen species (ROS) which is indicated by higher malondialdehyde (MDA), other aldehyde, electrolyte leakage (EL) and hydrogen peroxide (H_2O_2) contents. Plant tried to cope up oxidative stress by antioxidant defense mechanisms depending on the cultivars and As doses. Dry weight, fresh weight, shoot length, water content, chlorophyll content, carotenoid content, ascorbate were higher in BARI Lentil-1, BARI Lentil-2 compared to BARI Lentil-5, BARI Lentil-6 at 320 µM As concentration with concomitant reduction in lipid peroxidation (MDA content), other aldehyde, EL, proline, H_2O_2 content and translocation factor (TF). Activities of antioxidant enzymes-catalase (CAT), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX) were decreased while monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), superoxide dismutase (SOD) activity were increased in BARI Lentil-5, BARI Lentil-6 compared to BARI Lentil-1, BARI Lentil-2. Considering the morpho-physiological parameters and antioxidant metabolism, BARI Lentil-1 and BARI Lentil-2 could be recommended as relatively As tolerant over BARI Lentil-5, BARI Lentil-6.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	ELABORATIONS
ABA	Abscisic acid
APX	Ascorbate peroxidase
As	Arsenic
AsA	Ascorbic acid
BRRI	Bangladesh Rice Research Institute
CAT	Catalase
Chl	Chlorophyll
Car	Carotenoid
DAS	Days after sowing
DHAR	Dehydroascorbate reductase
DHA	Dehydroascorbate
DW	Dry weight
EL	Electrolyte leakage
et al.	and others
FAO	Food and Agricultural Organization
FW	Fresh weight
FC	Field capacity
Gly I	Gyoxalase I
Gly II	Gyoxalase II
GR	Glutathione reductase
GSSG	Oxidized glutathione
GPX	Glutathione peroxidase
GSH	Reduced glutathione
GST	Glutathione-S-transferase
LOX	Lipoxygenase
LSD	Least Signicance Difference
MDA	Malondealdehyde

LIST OF ABBREVIATIONS (cont'd)

ABBREVIATIONS	ELABORATIONS
MDHAR	Monodehydroascorbate reductase
MDHA	Monodehydroascorbate
MG	Methylglyoxal
NADPH	Nicotinamide adenine dinucleotide phosphate
Pro.	Proline
POD	Peroxidase
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase

CHAPTER 1
INTRODUCTION

Arsenic (As) is one of the toxic and carcinogenic metalloid to human as well as plant. Due to crop cultivation at As polluted region or irrigation by As polluted water, As have been accumulated in the edible portion of plant and becoming a threat to the world (Lindsay *et al.*, 2017). Arsenic found all over the world and it is the 52th abundant element in nature. The average concentration of As is 20 µM to 30 µM at lithosphere (Adriano, 2001, Bianucci *et al*., 2017).

Arsenic has both organic and inorganic form. Among the inorganic form arsenate (AsV) and arsenite (AsIII) are predominant in soil. The two form of As have different way to disrupt the plant metabolisms and finally led to plant death. AsV is the chemical analogue of phosphorous (P) and easily enters into plant cell through the phosphorous transporters. In cell, AsV reacts with the cellular metabolisms where phosphorous are involved as example phosphorylation and ATP synthesis etc. After entering the plant cell, AsV is converted to AsIII by arsenate reductases. Arsenite can absorb through the nodulin26-like intrinsic proteins which are the sub family of aquaporin. Furthermore, AsIII can transported by silicon transporters as the similar structure of silicon. AsIII has the ability to bind the sulfhydryl groups and inactive the various enzymes (Finnegan *et al.*, 2012, Lindsay *et al.*, 2017).

Arsenic affects physiological, biochemical and morphological features of different plant. Arsenic inhibits the shoot and root growth and reduces shoot and root dry weight. Shoot and root length also decreases under As stress (Rahman *et al.*, 2015, Du *et al.*, 2017, Bianucci *et al.*, 2017). Structural changes of the plant cells occur by Arsenic through disrupting the chloroplast ultrastructure, mitochondrial structure and nucleus (Du *et al.*, 2017). Arsenic induces osmotic stress by decreasing relative water content (RWC) and increasing proline (pro) accumulation. Reduction of photosynthetic pigments occurs by As through decreasing the chlorophyll (chl) content in leaves (Hasanuzzaman *et al.*, 2013, Rahman *et al*., 2015).

In the recent studies it is well established that As induced oxidative stress through the overproduction of reactive oxygen species (ROS). Reactive oxygen species such as singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl

radical (·OH) are produced by different mechanisms in plant cell (Yadav, 2010). During the conversion of AsV to AsIII generates ROS and inhibits the activity of key enzymes is the another way to produce ROS. Reduction of AsV to AsIII is followed by methylation and this is a redox driven reaction so that it produces ROS. Due to the methylation of As different kind of compounds are produced such as monomethylarsonic acid, dimethylarsinic acid, trimethylarsonium oxide, tetramethyarsonium ions, arsenosugar, arsenocholine and arsenobetaine. This compounds are highly reactive in nature and react with oxygen which generate ROS in plant cell. Beside that in mitochondria, dimethylarsinic acid separate iron (Fe) from ferritin. Iron is a redox active metal so it produces ROS by Haber-Wesis reaction. Reactive oxygen species disrupt the cell's normal functions and react with proteins, lipids, DNA as well as disrupt antioxidant defense system. (Sharma, 2012, Abbas *et al*., 2018).

At normal condition plant maintains a balance between ROS and ROS scavenging antioxidant defense mechanisms. The ROS are detoxified by both enzymatic and non-enzymatic antioxidants. Ascorbate (AsA), glutathione (GSH), phenolic compounds, non-proteins amino acids, carotenoid are non-enzymatic antioxidants and superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione peroxidase (GPX), glutathione *S*-transferase (GST) are antioxidants enzymes. Under stress, the equilibrium condition between ROS and antioxidants are disrupted and overproduced ROS that create oxidative stress (Hasanuzzaman *et al.,* 2012a).

Lentil (*Lens culinaris* Medik) is a very common and important crop all over the world. It belongs to the sub family Faboideae under the family Fabaceae. It is an important source of protein, minerals and vitamins for human consumption. It fixes nitrogen in soil from the atmosphere. It covers 5481120 hectares of land in the world and its annual production is 6315858 tones. Bangladesh ranks the seventh position in the world total lentil production (FAO, 2016). It is cultivated in 2.493 hectares of land in Bangladesh with an annual production of 269 thousand metric tons and average yield of 1.080 ton ha^{-1}. It occupies the second position among the pulse crops in terms of area and production but in case of consumer preference, it occupies the first rank in Bangladesh (BBS, 2016).

Bangladesh is considered as one of the most arsenic affected country in the world. In our country, most of the food composites contain a remarkable inorganic arsenic (Ahmed et al. 2016). On the other hand, arsenic affected areas are increasing day by day and lentil is an important plant of that affected area. So, it is high time to take arsenic mitigation measures. In this regard breeding approach is an important measure and for breeding, it needs a tolerance variety. To find out tolerant variety, it is the best way screening the existing variety. Under this circumstance, the proposed study was undertaken to examine the morpho-physiological and biochemical responses of lentil cultivar with the following objectives:

i. To investigate the morpho-physiological and biochemical changes of lentil cultivars under arsenic stress
ii. To understand the arsenic tolerance mechanisms of lentil cultivars
iii. To find out arsenic tolerant lentil cultivars

CHAPTER 2

REVIEW OF LITERATURE

2.1. Abiotic stress

Plants are a sessile organisms so that plant faced a lot of stresses during its life cycle. Stress can be defined as an adverse or abnormal condition that decreased the plant's growth, development and, productivity. There are two types of stress; one is biotic stress and another is abiotic stress (Pandey *et al.,* 2017). If stress becomes very high or continues long period it may lead to changes in morphological structure, physiological and, biochemical activities. In severe cases, it leads to plant death. There are different types of abiotic stressors in plants like salinity, drought, toxic metals/metalloids, high temperature, low temperature, flooding, nutrient imbalance, high light intensity, ultraviolet (UV) radiation, herbicides, ozone, other pollutants and so on (Hasanuzzaman *et al.,* 2012). It was reported that due to abiotic stress 50% crop loss occurred every year. So abiotic stress becoming a threat to food security all over the world (Haggag *et al.,* 2015). In agriculture, it is not possible to grow plants without abiotic stress. However, based on stress duration and level of stress toxicity plant try to cope up stress through developing specific mechanisms.

2.2. Toxic metals/metalloids stress

Among the various abiotic stress toxic metals/metalloids becoming a global environmental threat day by day. In the recent years, the concentration of toxic metals/metalloids is increasing in soil that makes a serious problem worldwide in crop production as well as human health (Sarma, 2011). Mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), antimony (Sb) are predominate toxic metals/metalloids in soil (Shah *et al.,* 2010).

2.3. Effect of Arsenic on crop attributes

Arsenic is one of the most harmful toxic metalloid in nature. Low concentration As can damage the morphological, physiological and, biochemical functions of plant.

2.3.1. Effect on growth

Arsenic affected the plant growth by reducing the plant height, fresh weight (FW), Dry weight (DW). A hydroponic experiment was carried out by Ahmad *et al.* (2012) with two cultivars of rice (*Oryza sativa*) treated with 50, 150 and 300 µM arsenite. They observed that the shoot length and root length of both cultivars were harshly affected by As toxicity.

Malik *et al.* (2011) reported that As inhibited the growth of the roots and shoots (as dry weight) by 65% and 60%, respectively, over controls in *Cicer arietium* L. The shoot/root ratio declined from 4.3 in the control to 3.5 in As-treated plants.

Talukdar *et al.* (2013b) reported that 50 µM As caused a significant reduction in length and DW of both shoots and roots of common bean legume (*Phaseolus vulgaris* L.) seedlings. Compared with control, shoot and root lengths were decreased by about 50 and 67%, respectively. Dry weight of shoot was reduced by 31%, while that of root was reduced by 60%.

Rahman *et al.* (2015) found that DW decreased with the increased of As concentration. Compared with control, 28 and 35% DW decreased in rice seedling (*Oryza sativa*) at 500 and 1000 µM As stress respectively.

An experiment was conducted by Du *et al.* (2007) to investigate the effect of different level of As stress on physiological, biochemical, and morphological characteristics in seedlings of two cultivars of maize (*Zea mays* L.). They found that shoot length, root length, and DW were reduced due to As stress.

Bianucci *et al.* (2017) observed a significant reduction of root DW at 6 µM As stress but significant reduction of shoot DW at 100 µM As stress in peanut (*Arachis hypogaea*) plant. They also observed a significant reduction of shoot length and root length at 20 µM As stress.

Anjum *et al*. (2017) found that 200 µM As reduced plant growth, plant height, number of leaves per plant, leaf area, stem diameter, and shoot fresh and dry weight in maize (*Zea mays* L.)

Niazi *et al.* (2017) reported that As reduced the growth attributes like plant height, leaf area, number of leaves, shoot and root biomass in *Brassica napus* and *Brassica juncea* in comparison to their respective controls.

2.3.2. Effect on plant physiology

Arsenic inhibited the rate of photosynthesis by disrupting the light harvesting apparatus with a reduction in chlorophyll (Chl) concentration and photosynthetic activity-II or suppression important occurrence of photosynthesis process (Abbas *et al*., 2018).

Malik *et al.* (2011) reported that the accumulation of amino acids such as lysine, methionine + cystine, phenylalanine + tyrosine, proline, threonine, tryptophan, and valine was inhibited significantly in the seeds of As-applied plants compared to the control.

A field experiment was conducted by Dwivedi *et al*. (2012) to analyze the amino acid (AA) profile of sixteen rice genotypes differing in grain As accumulation. They observed that, both essential and non-essential amino acids were decreased as the grain As concentration was increased in high As accumulating rice genotypes. They also observed Non-essential amino acids were increased in low As accumulating rice genotypes.

Tripathi *et al.* (2017) reported that As reduced the essential and non- essential amino acid in chickpea (*Cicer arietinum* L.).

An experiment was conducted by Mascher *et al.* (2002) to investigate the effects of different soil concentrations of arsenate on red clover plants (*Trifolium pratense* L. cv. Renova). They observed that decreased in chlorophyll (chl) and carotenoid concentrations correlated with increasing arsenic content in plants.

Malik *et al.* (2o11) found accumulated the greatest As (7 mg kg−1 dry weight), followed by stem (4.8 mg), leaves (4.0 mg), and seeds (0.7 mg)... A marked increase in membrane damage coupled with reduction in chlorophyll and relative leaf water content occurred in As-treated plants. The contaminated plants showed 34% and 25% decrease over control in sucrose content in their leaves and seeds, respectively

Hasanuzzaman *et al.* (2013) reported that in Wheat (*Triticum aestivum* L.) Chl *a+b* content was reduced by 15% and 40% at 250 and 500 µM As concentration respectively. They also reported that RWC content was reduced by 15% and 17% at 250 and 500 µM As concentration respectively.

Srivastava *et al.* (2013) claimed that As declined the Chl content and rate of photosynthesis in *Hydrilla verticillata.*

Singh *et al*., (2015) found that As uptake caused reduction in chlorophyll fluorescence, nitrogen content concentrations of H_2S and nitric oxide (NO). The activities of cysteine desulfhydrase and nitrate reductase were also decreased.

Rahman *et al.* (2015) observed that, 33% and 44% decrease of Chl *a+b* content was found in in rice (*Oryza sativa* L.) under 500 and 1000 µM As concentration respectively. They further observed that, 20% and 27% decrease of RWC was found in under 500 and 1000 µM As concentration respectively.

Mubarak *et al.* (2016) found that As reduced Chl concentration as well as relative water content (RWC) in *Boehmeria nivea* L. They found that, ss the As concentration increased, all chlorophyll content measurements (chl *a*, chl *b*, and chl *a+b*) significantly decreased by 11–54%, 22–54%and 14–54%, respectively, relative to the control.

Srivastava *et al.* (2017) reported that due to As toxicity Chl *a*, Chl *b*, total chlorophyll and carotenoids decreased in black gram (*Vigna mungo* L.).

An experiment was conducted by Anjum *et al*. (2017) to examine the influence of Cd and As stresses on morpho-physiological growth and yield of two contrasting maize cultivars. They reported that, As reducd gas exchange attributes such as photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 and chlorophyll contents.

An experiment was carried dy Niazi *et al.* (2017) to find out the potential role of phosphate on growth, gas exchange attributes, and photosynthetic pigments of *Brassica napus* and *Brassica juncea* under arsenic (As) stress. They found that gas exchange attributes-photosynthetic rate, transpiration rate, stomatal conductance and photosynthetic pigments as well as water use efficiency were reduced due to As stress.

2.3.3. Effect on plant anatomy

Tripathi *et al.* (2017) observed As changed the anatomy of cells and cortex region was most affected in chickpea (*Cicer arietinum* L.). The layers of collenchymatous cells were reduced. Collenchymatous cells loosely arranged and became deformed. Sclerenchyma cells shriveled and flaccid.

Armendariz *et al.* (2017) reported that the effect of As on *Glycine max* was reduction in root cortex area, broken cells in the outer cortical layer and cell death of root tips. Dark deposits in cortex cells and within phloem cell walls and xylem vessel elements.

An experiment was conducted by Du *et al.* (2017) to determine the effect of arsenic stress on physiological, biochemical, and morphological characteristics in seedlings of two cultivars of maize with different arsenic tolerance. They found that chloroplast ultrastructure and mitochondrial structure changed due to As toxicity.

2.3.4. Effect on nutrient

A field experiment was carried out by Tripathi *et al.* (2017) at chickpea (*Cicer arietinum* L.) treated with 64 µM AsV. They observed that iron (Fe) concentration was reduced due to As toxicity.

An experiment was conducted by Malik *et al.* (2011) to investigate the As uptake, distribution, and effects on growth, yield, and quality of seeds. They found that minerals content such as calcium (Ca), phosphorus (P), and iron (Fe) declined greatly in the seeds of As-treated plants.

Gunes *et al*. (2010) conducted an study to examine the combined effect of soil-applied phosphorus (P) and arsenic (As) on P, As, potassium (K), calcium (Ca), magnesium (Mg), silicon (Si), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), titanium (Ti), rubidium (Rb), strontium (Sr), barium (Ba), lantanium (La), and cerium (Ce) concentrations of sunflower plants under glasshouse conditions. They reported that As toxicity caused significant increased in the concentrations of Mn, La and Ce, but it decreased K, Ca, Mg, Si, Fe, Zn, Cu, Rb, and Sr concentrations.

2.3.5. Effect on yield

Tripathi *et al.* (2017) found that seed setting of chickpea was affected by As. The number of flowers and pods was nearly absent. Compared with control the number of seed was reduced about 91% due to As toxicity.

Anjum *et al*. (2017) reported that yield, number of ears per plant, number of kernels per ear, and 100-kernel weight were reduced due to As toxicity in maize cultivars.

According to Malik *et al.* (2011) noticed that seed yield and number of pods plant^{-1} decreased by 66 and 53%, respectively, over controls in *Cicer arietinum* L. due to AS stress. They also noticed that the accumulation of seed reserves such as starch, proteins, sugars, and minerals was inhibited significantly due to As-treated plants.

2.4. Arsenic induced oxidative stress and antioxidant defense system

Now it is well established that As induced oxidative stress in plant through the overproduction of reactive oxygen species (ROS- $^1O_2$, $O_2^{\cdot-}$, H_2O_2). This ROS are detoxified enzymatic and non-enzymatic antioxidant. Ascorbate (AsA), glutathione (GSH), phenolic compounds, non-proteins amino acids, carotenoid are non-enzymatic antioxidants and superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione peroxidase (GPX), glutathione *S*-transferase (GST) are antioxidants enzymes. Plant maintains a balance between ROS and antioxidant. Under stress condition this balance is disrupted and plant faced oxidative stress (Hasanuzzaman *et al*., 2012a).

Non-enzymatic antioxidant directly reacts with ROS to detoxifie them. Superoxide dismutase (SOD) is the first line defense against ROS which reduced $O_2^{\cdot-}$ to H_2O_2 and O_2. Catalase (CAT) convert H_2O_2 to H_2O and O_2. This ROS are detoxified through AsA-GSH cycle. The enzymes of AsA-GSH cycle are APX, MDHAR, DHAR and GR. Ascorbate peroxidase (APX) reduced H_2O_2 to H_2O and monodehydroascorbate (MDHA) by using AsA. This MDHA recycling to AsA thorough the activity of MDHAR (Hasanuzzaman *et al*., 2012a). Due to the activity of DHAR dehydroascorbate (DHA) is reduced to AsA as well as oxidized glutathione (GSSG) produced by using GSH (Chen *et al.*, 2003). GR catalyses the NADPH-dependent reduction of disulphide

bond of GSSG and is thus important for maintaining the GSH pool (Chalapathi Rao and Reddy, 2008). Glutathione peroxidase (GPX) reduce H_2O_2 and organic and lipid hydroperoxides (LOOHs) to H_2O by using GSH (Noctor *et al*., 2002). GST catalyse the binding of various xenobiotics (including numerous pesticides) and their electrophilic metabolites with GSH to produce less toxic and more water-soluble conjugates (Edwards *et al*., 2000).

Oxidative stress marker such as lipid peroxidation (MDA), H_2O_2 content, Electrolyte leakage (EL) increase under As stress. In order to study by Rahman *et al.* (2015), MDA content was increased in *Oryza sativa* due to As stress. They also observed that H_2O_2 content amplified 65 and 89% compared with control under 500 and 1000 µM As concentration. They also reported that AsA content decreased whereas DHA content increased due to As stress. The amount of GSH and GSSG increased but GSH/GSSG ratio decreased. Due to As treatment the enzymes activity of APX, MDHAR, GR, SOD up regulated wgereas DHAR, GST, GPX down regulated

Du *et al.* (2015) stated that As increased the MDA content both As tolerant and sensitive cultivars in *Zea mays*. Shoot contained more MDA content than root. At As concentration of 20 and 40 mgL^{-1}, the shoot MDA content was 12.90 and 8.49% higher in sensitive cultivar compared to tolerant cultivar.

According to Hasanuzzaman *et al.* (2013), 58 and 180% increase of MDA content was found in *Triticum aestivum* under 250 and 500 µM As concentration respectively as well as 41 and 95% increase of H_2O_2 content was found under 250 and 500 µM As concentration respectively. They also reported that AsA content decreased whereas GSH content increased due to As stress. Due to As treatment the enzymes activity of APX, GST, GR up regulated; DHAR, GPX down regulated whereas MDHAR, CAT remain statistically similar compared to control.

Ruíz-Torres *et al*. (2017) reported that arsenic uptake increase the arsenic chelating and reduced the antioxidant defense enzyme activities at root in garlic plant.

2.5. Effect of Arsenic on Lentil plants

A few experiment was conducted to identify the effect of As on lentil plants. Talukdar (2013a) conducted a pot experiment on eight genotypes of lentil (*Lens culinaris* Medik.)

to find out the bioaccumulation and transport of As. Significant variations among eight genotypes were observed in bioaccumulation and transport of As in different plant parts. Generally, lentil plant contained higher amount of As in roots then shoot. This indicated lowering of bioaccumulation factors but rise in the value of bioconcentration factors. He claimed that among the eight L 414 and L 830 are relatively safe for edible purposes.

An experiment was carried out by Talukdar and Talukdar (2014) to find out the response of sulfate transporters, thiol metabolism, and antioxidant defense system in roots of two lentil (*Lens culinaris* Medik.) genotypes (As tolerant genotype L 414 and As sensitive genotype DPL 59) grown in arsenic (10, 25, and 40 μM AsV). They found that, in case of L 414, there was no significant change of length, shoot and root dry mass under 25 and 40 μM As treatment. But in case of DPL 59 root length and shoot length were reduced 3 fold and 2 fold as well as root and shoot dry mass were reduced 2.5 fold and 2 fold compared with control plant under 25 μM As treatment. At 40 μM As treatment, more reduction was found. GSH content increased significantly in roots of L 414 at 25 μM, while it was changed marginally in relation to control at 10 and 40 μM. GSH content declined in DPL 59 roots, and the lowest level was measured at 40 μM. GSSG content was significantly low in L 414 but increased (2-4 fold) progressively in DPL 59 in a concentration-dependent manner. SOD activity increased in both cultivars in a concentration-dependent way but at higher magnitudes in L 414. APX, DHAR, and GR activities changed non-significantly in L 414 at 10 and 25 μM, followed by their marked rise in L 414 at 40 μM. APX activity was 1.5-fold lower than that in control in DPL 59 at 10 μM and declined further as the concentration increased DHAR activity changed marginally in DPL 59 throughout the treatments. GR level reduced in DPL 59 about 1.3-fold at 10 μM, 2-fold at 25 μM, and 3.5-fold at 40 μM.

Ahmed *et al.* (2012) reported that shoot dry weight, root dry weight, flower number, pod dry weight, root tolerance index and relative shoot height per pot decreased with increasing concentration of arsenate in irrigation water. They also reported that nitrogen fixation, N and P content in lentil decreased significantly with increasing arsenic concentration in irrigation water.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

This experiment was conducted at the Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Japan during the period from March, 2017 to February, 2018.

3.2. Plant materials

Four lentil cultivars (*Lens culinaris* Medik.) viz. BARI Lentil-1 (V1), BARI Lentil-2 (V2), BARI Lentil-5 (V5) and BARI Lentil-6 (V6) were used for this experiment.

3.3. Sources of plant materials

All plant materials were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Dhaka, Bangladesh.

3.4. Treatments

There were two factors in this experiment, one was variety and other was Arsenic (As) level.

Factor A: Variety

i. BARI Lentil-1
ii. BARI Lentil-2
iii. BARI Lentil-5
iv. BARI Lentil-6

Factor B: Arsenic

i. 0 µM
ii. 80 µM
iii. 160 µM
iv. 320 µM

3.5. Design and Layout

Healthy and uniform size seeds of four lentil cultivars were washed several times with deionized water and soaked with deionized water for 24 h. Then the seeds were placed on six layer (9 cm) moistened filter paper in Petri dishes and kept in dark germinator for 72 h. Well germinated seeds were transferred to growth chamber under control environment (light, 350 µmol photon m^{-2} s^{-1}; temperature, 25±2°C; relative humidity, 65–70%). Each Petri dish contained 40±2 seedlings. The seedlings were provided 5000-fold diluted Hyponex (Tokyo, Japan) solution for nutrient. Finally, seven-day-old seedlings were treated with different levels of As (0 µM, 80 µM, 160 µM, 320 µM as $Na_2HAsO_4.7H_2O$) with Hyponex solution. Nutrient solution was changed every two days to reduce the fluctuation of As concentration. After four days of treatment, shoot and root were harvested for taking data. The experiment was conducted following completely randomized design with three replications.

3.6. Collection of data

3.6.1. Growth parameter

- Shoot length
- Shoot fresh weight
- Shoot dry weight

3.6.2. Physiological parameters

- Relative water content (RWC)
- Shoot water content
- Photosynthetic pigments
- Arsenic (As) content

3.6.3. Biochemical parameters

- Lipid peroxidation
- Electrolyte leakage
- H_2O_2 content
- Proline

- Ascorbic acid content
- Glutathione content
- Activities of antioxidant enzymes (CAT, APX, DHAR, MDHAR, GR, GPX, SOD)

3.7. Measurement of As content and translocation factor (TF)

To measure the As content, shoots and roots were dried at 80°C for 72 h. Dried samples were digested separately with 5 mL acid mixture [HNO_3:$HClO_4$ (5:1 v/v)] at 80°C for 48 h. Arsenic was measured with an atomic absorption spectrophotometer and calculated from standard curve. Translocation factor (TF) refer ratio of As in plant shoot to root and TF was calculated according to Malik et al. (2010) using the following formula:

TF = Metals (shoot)/ Metals (roots)

3.8. Measurement of shoot length, fresh weight and dry weight of seedling

To measure the fresh and dry weight 15 seedlings from each treatment were selected. The shoot and root of these selected seedlings were separated carefully, weighed in a digital balance; data were recorded and considered as fresh weight (FW). Dry weight (DW) was determined after drying the seedlings at 80°C for 48 h. Fresh weight and dry weight were expressed as mg seedling^{-1}. Shoot length was measured by meter scale and expressed as cm.

3.9. Measurement of lipid peroxidation and other aldehyde

The level of lipid peroxidation was measured by estimating malondealdehyde (MDA) content according to Heath and Packer (1968) with slight modification by Hasanuzzaman *et al.* (2012b). Leaf samples (0.5 g) were homogenized in 3 mL 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (1 mL) was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged again at 11,500 × g for 10 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. MDA content was

calculated by using extinction coefficient 155 $mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} FW. The absorbance of the colored supernatant was measured at 455 nm for measurement other aldehyde. Other aldehyde content was calculated by using extinction coefficient 45.7 $mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} FW. This extinction coefficient obtained from five aldehydes include propanol, butanol, hexanol and propanol dimethylacetal (Meir *et al.*, 1992).

3.10. Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yang *et al.* (2007). Fresh l eaf sample (0.5 g) was homogenized with 3ml 5% (w/v) trichloroacetic acid (TCA) by using ice cold mortar and pestle and centrifuged the homogenate at 11,500g for 15 min. Plant supernatant (500 µl) was mixed properly with 500 µl 10 mM K-P buffer (pH 7) and 1 ml of 1M KI reagent. A blank sample was prepared with 5% TCA instead of leaf extract. Then these mixture was incubated at 25 ^{0}C for 1 hr. The optical absorption of the supernatant was measured spectrophotometrically at 390 nm. The amount of H_2O_2 was calculated from the standard curve.

3.11. Determination of electrolyte leakage (EL)

Electrolyte leakage was measured according to Dionisio-Sese and Tobita (1998) with slight modification by Hossain *et al*. (2017). Shoot sample (0.2 g) was cut into smaller pieces and then placed in a test tube containing 20 ml distilled deionized water. Covering with caps, test tubes were heated at 40 °C for 10 min. After cooling, initial electric conductivity (EC_1) was measured using a Eutech CON 700 conductivity meter, Singapore. Again, test tubes were heated at 121 °C for 20 min using an autoclave. After cooling at room temperature, the final electric conductivity (EC_2) was recorded. Electrolyte leakage was calculated using the following formula:

Electrolyte leakage, $EL = (EC_1/ EC_2) \times 100$

3.12. Determination of water content

To determine water content of shoot, fresh weight (FW) and dry weight (DW) were measured. For DW, seedlings were oven dried at 80 °C until the weight became

constant. Finally, water content was calculated according to Hossain *et al*. (2017) using the following formula:

WC (%) = {(FW − DW)⁄DW} × 100.

3.13. Determination of Leaf Relative Water Content (RWC)

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Leaves from randomly chosen plants were taken. Leaves were weighed as FW and then immediately floated on distilled water in a petri plate for 24 h in the dark. Turgid weights (TW) of leaves were obtained after removing excess surface water with paper towels. Dry weights (DW) of leaves were measured after drying at 80°C for 48 h. Then, RWC was calculated using the following formula:

RWC (%) = [(FW-DW)/ (TW-DW)] ×100

3.14. Determination of Proline (Pro) Content

Free Pro in leaf tissues was measured following the protocol of Bates et al. (1973). Fresh leaf tissue (0.25 g) was homogenized well in 5 ml of 3% sulfo-salicylic acid on an ice cooled morted on ice. The homogenate was centrifuged at 11,500×g for 15 min. Two ml of the supernatent was than mixed with 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and 1 ml of glacial acetic acid. The mixture was placed at 100°C in water bath for 1 h, then transferred in to test tube and kept in ice to be cooled, after a while when it was cooled, 2 ml of toluene was added and mixed thoroughly by vortex mixture. After sometimes by transferring the upper aqueous layer the optical density of the chromophore containing toluene was read spectrophotometrically at 520 nm using toluene as a blank. The amount of Pro was calculated from the standard curve using laboratory grad Pro.

3.15. Determination of Chlorophyll and Carotenoid Content

Chlorophyll (Chl) content was determined by taking fresh leaf samples (0.25 g) from randomly selected seedlings. The samples were homogenized with 10 ml of acetone (80% v/v) using pre-cooled pestle and mortar and the homogenate was centrifuged at 10,000 × g for 10 min. The absorbance of the supernatants was measured with a UV-

visible spectrophotometer at 663nm, 645 nm and 470nm for Chl a, chl b and carotenoid (Car) respectively. Chl contents were calculated using the following equations proposed by Arnon (1949):

Chl *a* (mg g^{-1} FW) = (12.7 × A_{663} – 2.69 × A_{645}) × V/1000 × W

Chl *b* (mg g^{-1} FW) = (22.9 × A_{645} – 4.68 × A_{663}) × V/1000 × W

Chl *a*+*b* = Chl *a* + Chl *b*

Where:
A_{663} and A_{645} = Absorbance at these wavelength
V = Final extract volume (ml)
W = Weight of sample (g)

Car contents were calculated according to Lichtenthaler and Wellburn (1983) using the following equations:

Car (mg g^{-1} FW) = (1000 × A_{470} - 3.27 × Chl *a* - 104 × Chl *b*)/229

Where:
A_{470} = Absorbance at these wavelength

3.16. Extraction and analysis of ascorbate and glutathione

Fresh wheat leaves (0.5 g) were homogenized in 3 mL ice-cold 5% meta-phosphoric acid containing 1 mM ethylenediaminetetraacetic acid (EDTA) using a mortar and pestle. The homogenate was centrifuged at 11,500 × g for 12 min at 4 °C, and the supernatant was collected to analyze for AsA and GSH. Ascorbate content was determined following the method of Huang *et al.* (2005) with some modifications. The supernatant was neutralized with 0.5 M K-P buffer (pH 7.0), and the oxidized fraction was reduced by 0.1 M dithiothretitol. AsA was assayed spectrophotometrically at 265 nm in 100 mM K-P buffer (pH 7.0) with 0.5 units of ascorbate oxidase (AO). A specific standard curve of AsA was used for quantification. The GSH pool was assayed according to Yu *et al.* (2003) with modifications as described by Paradiso *et al.* (2008). Aliquots (0.2 mL) of supernatant were neutralized with 0.3 mL of 0.5 M K-P buffer

(pH 7.0). Based on enzymatic recycling, GSH is oxidized by 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) and reduced by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of GR, and GSH content was evaluated by the rate of absorption changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. Oxidized glutathione (GSSG) was determined after removing GSH by 2-vinylpyridine derivatization. Standard curves with known concentrations of GSH and GSSG were used. The content of GSH was calculated by subtracting GSSG from total GSH.

3.17. Determination of protein

Protein concentration of each sample was measured following the method of Bradford, (1976) using BSA (Bovin Serum Albumin) as standard.

3.18. Enzyme extraction and assays

Using a pre-cooled mortar and pestle, 0.5 g of wheat leaf tissue was homogenized in 1 ml of 50 mM ice-cold K-P buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM β-mercaptoethanol and 10% (w/v) glycerol. The homogenates were centrifuged at 11,500× g for 15 min and the supernatants were used for determination of enzyme activity. All procedures were performed at 0−4ºC.

3.18.1. Ascorbate peroxidase (APX, EC: 1.11.1.11)

APX (EC: 1.11.1.11) activity was assayed following the method of Nakano and Asada (1981). The reaction buffer solution contained 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM EDTA, and enzyme extract in a final volume of 700 µl. The reaction was started by the addition of H_2O_2 and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

3.18.2. Monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4)

MDHAR (EC: 1.6.5.4) activity was determined by the method of Hossain *et al.* (1984). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 0.2 mM NADPH, 2.5

mM AsA, 0.5 unit of AO and enzyme solution in a final volume of 700 µl. The reaction was started by the addition of AO. The activity was calculated from the change in absorbance at 340 nm for 1 min using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

3.18.3. Dehydroascorbate reductase (DHAR, EC: 1.8.5.1)

DHAR (EC: 1.8.5.1) activity was determined by the procedure of Nakano and Asada (1981). The reaction buffer contained 50 mM K-P buffer (pH 7.0), 2.5 mM GSH, and 0.1 mM DHA. The reaction was started by adding the sample solution to the reaction buffer solution. The activity was calculated from the change in absorbance at 265 nm for 1 min using extinction coefficient of 14 $mM^{-1}cm^{-1}$.

3.18.4. Glutathione Reductase (GR, EC: 1.6.4.2)

GR (EC: 1.6.4.2) activity was measured by the method of Hasanuzzaman *et al.* (2011). The reaction mixture contained 0.1 M K-P buffer (pH 7.0), 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and enzyme solution in a final volume of 1 ml. The reaction was initiated with GSSG and the decrease in absorbance at 340 nm was recorded for 1 min. The activity was calculated using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$.

3.18.5. Catalase (CAT, EC: 1.11.1.6)

CAT (EC: 1.11.1.6) activity was assayed following the method of Hasanuzzaman et al. (2012b) by monitoring the decrease in absorbance at 240 nm for 1 min caused by the decomposition of H_2O_2. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2, and enzyme solution in a final volume of 700 µL. The reaction was initiated with the enzyme extract and activity was calculated using extinction coefficient 39.4 $M^{-1}cm^{-1}$.

3.18.6. Glutathione peroxidase (GPX, EC: 1.11.1.9)

GPX (EC: 1.11.1.9) activity was assayed using the method of Elia *et al.* (2003). The reaction mixture consisted of 100 mM K-P buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN_3), 0.12 mM NADPH, 2 mM GSH, 1 unit GR, 0.6 mM H_2O_2 (as a substrate), and 20 µL of sample solution. The oxidation of NADPH was recorded at 340 nm for 1 min and the activity was calculated using extinction coefficient 6.62 $mM^{-1}cm^{-1}$

3.18.7. Superoxide dismutase (SOD, EC: 1.15.1.1)

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured based on the xanthine-xanthine oxidase system following the method of El-Shabrawi *et al.* (2010). The reaction mixture contained K-P buffer (50 mM), 2.24mM NBT, catalase (0.1 units), xanthine oxidase (0.1 units), xanthine (2.36 mM), and enzyme extract. A change in absorbance was read at 560 nm. Superoxide dismutase activity was expressed as units (amount of enzyme required to inhibit NBT reduction by 50%) min^{-1} mg^{-1} protein.

3.19. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and the mean differences were compared by Fisher's LSD using XLSTAT v.2015 software (Addinsoft, 2015). Differences at $P \leq 0.05$ were considered significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Growth parameter

4.1.1. Phenotypic appearances

Plant response varied in phenotypic appearances with different variety and arsenic concentration. It this experiment, clear differences in phenotypic appearances were found among the four variety at 320 µM As. BARI Lentil-5 and BARI Lentil-6 severely affected by As compared to BARI Lentil-1 and BARI Lentil-2 (Plate 1).

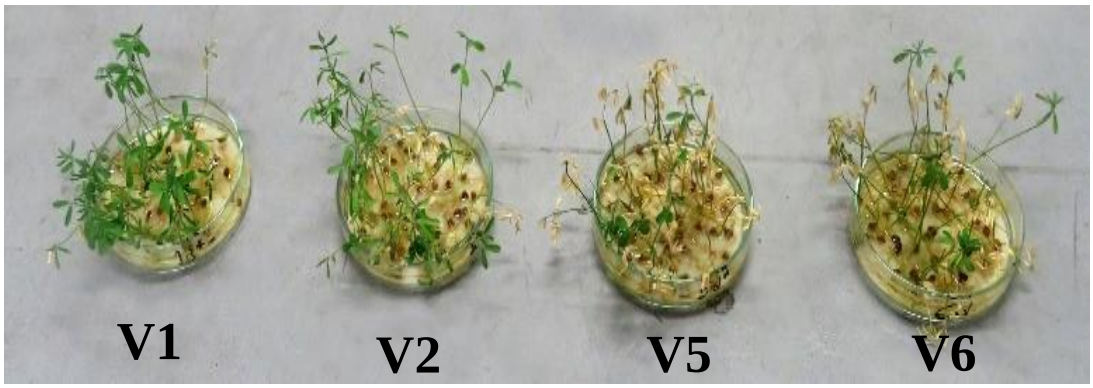

Plate 1. Morphological features of four lentil cultivars under 320 µM arsenic stress

Here,
V1, V2, V5, V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

4.1.2. Shoot length

This experiment showed As stress decreased the shoot length in a roughly concentration-dependent manner of four cultivars of lentil seedlings compared with control. Compared with control (0 µM As) shoot length was decreased by 19.25%, 25.12%, 19.35% in BARI Lentil-1; 27.63%, 22.41%, 23.81% in BARI Lentil-2; 34.11%, 22.19%, 25.45% in BARI Lentil-5; 32.62%, 38.68% 38.50% in BARI Lentil-6 under 80 µM, 160 µM, 320 µM As respectively. At 80 µM As stress there was no significant difference between BARI Lentil-2 and BARI Lentil-6; At 160 µM As stress there was no significant difference among BARI Lentil-1, BARI Lentil-2, BARI Lentil-5; At 320 µM As stress there was no significant difference between BARI Lentil-2 and

BARI Lentil-5(Table 1) and Table 1 shown higher reduction of shoot length observed at BARI Lentil-5 and BARI Lentil-6 compared to BARI Lentil-1 and BARI Lentil-2. Arsenic is a toxic element to plant. It affects the plant growth and development. Shoot length are inhibited due to As toxicity of various plant such as rice (Ahmad *et al.*, 2012), maize (Du *et al.*, 2017), peanut (Bianucci *et al.*, 2017) etc. A study by Talukdar and Talukdar (2014) was reported that As sensitive cultivar showed a higher reduction of shoot length in comparison with tolerant cultivar.

Table 1. Shoot length of lentil seedlings under 0 µM, 80 µM, 160 µM, 320 µM arsenic stress

Shoot length (cm plant^{-1})				
Treatment	**BARI Lentil-1**	**BARI Lentil-2**	**BARI Lentil-5**	**BARI Lentil-6**
0 µM	10.23 ± 0.03 b	9.95 ± 0.15 bc	9.82 ± 0.08 c	11.22 ± 0.38 a
80 µM	8.26 ± 0.04 d	7.20 ± 0.38 gh	6.47 ± 0.11 i	7.56 ± 0.18 e-g
160 µM	7.66 ± 0.14 ef	7.72 ± 0.60 e	7.64 ± 0.24 ef	6.88 ± 0.16 h
320 µM	8.25 ± 0.11 d	7.58 ± 0.04 e-g	7.32 ± 0.12 fg	6.9 ± 0.14 h
$LSD_{(0.05)}$ 0.38 CV (%) 2.93				

Mean (±SD) was calculated from three replications for each treatment. Values in column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test.

4.1.3. Shoot FW and DW

Toxic metal or metalloid reduced the plant biomass. Reduction of plant growth occurred through inhibition cell elongation and decrease mitotic activity due to As toxicity of plant (Dho *et al.*, 2010). In this present experiment, the FW and DW of shoot significantly decreased of four cultivars of lentil seedlings under all As concentration (80 µM, 160 µM, 320 µM) compared with control (Table 2). Reduction of shoot FW and DW at As toxicity also observed in rice (Rahman *et al.*, 2015) and common bean (Talukdar *et al.*, 2013b).

Table 2. Shoot FW and DW of lentil seedlings under 0 µM, 80 µM, 160 µM, 320 µM arsenic stress

Shoot FW (mg plant^{-1})				
Treatment	**BARI Lentil-1**	**BARI Lentil-2**	**BARI Lentil-5**	**BARI Lentil-6**
0 µM	55.90 ± 0.50 d	62.05 ± 0.25 c	69.70 ± 0.20 b	74.03 ± 0.13 a
80 µM	47.40 ± 0.75 f	42.93 ± 0.53 gh	40.63 ± 2.33 h	51.55 ± 1.85 e
160 µM	45.40 ± 2.90 fg	47.05 ± 2.45 f	44.50 ± 2.20 fg	50.95 ± 3.10 e
320 µM	46.85 ± 2.35 f	40.93 ± 0.63 h	40.15 ± 3.35 h	31.63 ± 0.48 i
$LSD_{(0.05)}$ 3.20 CV (%) 3.88				
Shoot DW (mg plant^{-1})				
Treatment	**BARI Lentil-1**	**BARI Lentil-2**	**BARI Lentil-5**	**BARI Lentil-6**
0 µM	8.73 ± 0.18 c	9.65 ± 0.10 b	10.78 ± 0.08 a	11.13 ± 0.08 a
80 µM	7.90 ± 0.31 e-g	7.68 ± 0.53 f-h	6.88 ± 0.63 i	8.55 ± 0.10 cd
160 µM	7.58 ± 0.13 gh	8.20 ± 0.70 de	7.23 ± 0.33 hi	8.11 ± 0.03 d-f
320 µM	8.03 ± 0.03 e-g	7.18 ± 0.23 hi	7.53 ± 0.43 gh	6.94 ± 0.31 i
$LSD_{(0.05)}$ 3.20 CV (%) 3.88				

Mean (±SD) was calculated from three replications for each treatment. Values in column with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test.

Compared with control (0 µM As) relative decrease of shoot FW observed 15.20%, 18.74%, 16.16% in BARI Lentil-1; 30.81%, 24.16%, 34.04% in BARI Lentil-2; 41.72%, 36.14%, 42.40% in BARI Lentil-5; 30.36%, 31.17%, 57.27% in BARI Lentil-6 under 80 µM, 160 µM, 320 µM As respectively. In all arsenic concentration higher relative decrease of shoot FW found at BARI Lentil-5 and BARI Lentil-6 compared to BARI Lentil-1, BARI Lentil-2 and highest reduction found at 320 µM As in BARI Lentil-6 (57.27%). In case of shoot DW roughly same trend found like shoot FW. Compared with control (0 µM As) relative decrease of shoot DW observed 7.60%, 11.40%, 6.14% in BARI Lentil-1; 21.28%, 15.89%, 26.41% in BARI Lentil-2; 35.74%, 32.47%, 29.67% in BARI Lentil-5; 23.66%, 27.67%, 38.02% in BARI Lentil-6 under 80 µM, 160 µM, 320 µM As respectively. Every arsenic concentration lower relative decrease of shoot DW recorded at BARI Lentil-1 and BARI Lentil-2 compared to BARI

Lentil-5, BARI Lentil-6. A study between two maize cultivar reported that As sensitive cultivar showed a higher relative decrease of DW compared to tolerant cultivar (Du *et al.*, 2017).

4.2. Physiological parameter

4.2.1. Relative water content (RWC)

Relative water content (RWC) of plants is an effective parameter to determine degree of relative tolerance under osmotic stress caused by As. In this experiment, As stress reduced leaf RWC of the lentil seedlings. At 80 µM As stress, there was no significant difference among the four lentil cultivars except BARI Lentil-1 and BARI Lentil-6 as well as at160 µM As stress there was no significant difference among the four lentil cultivars. But at 320 µM As stress, a significant difference was found among the four lentil cultivars, showing 10.73%, 16.15%, 27.23%, 23.38% reduction in leaf RWC at BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, BARI Lentil-6, respectively as compared to control plant (Figure 1). Decreased RWC indicated that plant faced water stress which disrupt the normal function of plant. Higher reduction of leaf RWC observed at BARI Lentil-5 and BARI Lentil-6 compared to BARI Lentil-1, BARI Lentil-2. Reduction in RWC due to As toxicity has also been reported by Stoeva *et al.* (2005) in bean, Malik *et al.* (2011) in chickpea, Hasanuzzaman *et al.* (2013) in wheat and Rahman *et al.* (2015) in rice.

4.2.2. Water content

In this experiment, As stress declined shoot water content at the roughly similar trend of RWC. So, it is clear indicated that plant faced osmotic stress due to As toxicity. At 80 and 160 µM As treatment there was no significant difference among BARI Lentil-1, BARI Lentil-5 and, BARI Lentil-6. Significant difference among the four lentil cultivars observed at 320 µM As treatment (Figure 2). In this case higher reduction of shoot water content observed at BARI Lentil-5 (4%) and BARI Lentil-6 (8%) compared to BARI Lentil-1 (1%), BARI Lentil-2 (2%). Arsenic might be disrupted the water balance and decreased water uptake as well as e suppressed the short distance water transfer in the symplast as well as in the apoplast (Rucińska-Sobkowiak, 2016).

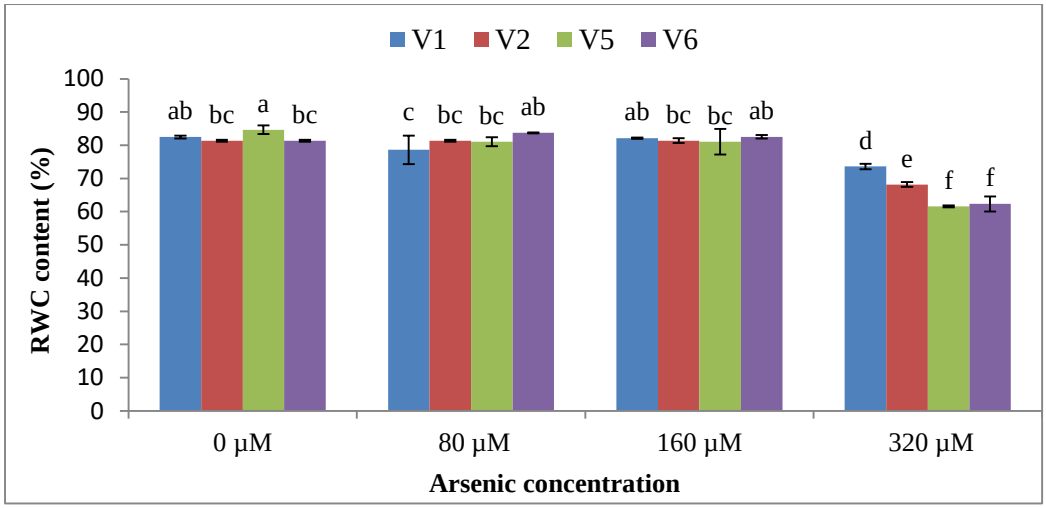

Figure 1. Relative water content (RWC) of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

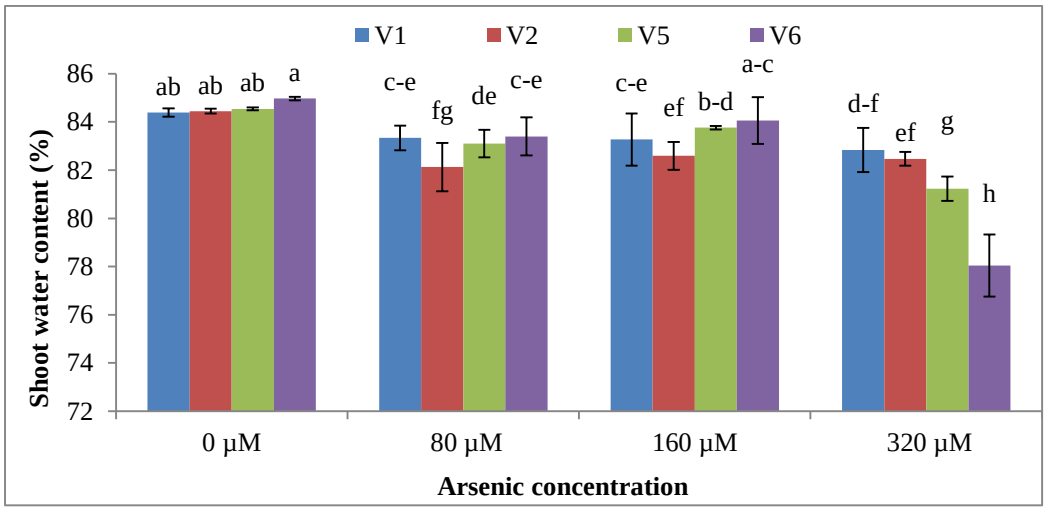

Figure 2. Shoot water content (%) of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively

4.2.3. Photosynthetic pigments

Photosynthesis is an essential process to produce food for plants. One of the common effect of As is reduction of photosynthetic pigment of plant. The amount of chlorophyll (Chl *a*, Chl *b*, Chl *a*+*b*) and carotenoids (Car) was found to reduce in plant species under the influence of As toxicity (Srivastava *et al.*, 2013). In this experiment, decay of Chl and Car found in lentil leaves under As treatment. Chl *a* reduced roughly As concentration dependent manner in all cultivars. In BARI Lentil-2, no significant reduction of Chl *a*, Chl *b*, Chl *a*+*b* content at 80 and 160 µM As treatment was found compared to control. A clear significant difference of Chl *a* among four lentil cultivars was observed at 320 µM As treatment. Higher reduction of Chl *a* found in BARI Lentil-6 (20.34%), BARI Lentil-5 (10.11%) compared to BARI Lentil-1 (4.64%), BARI Lentil-2 (4.55%) at 320 µM As (Figure 3).

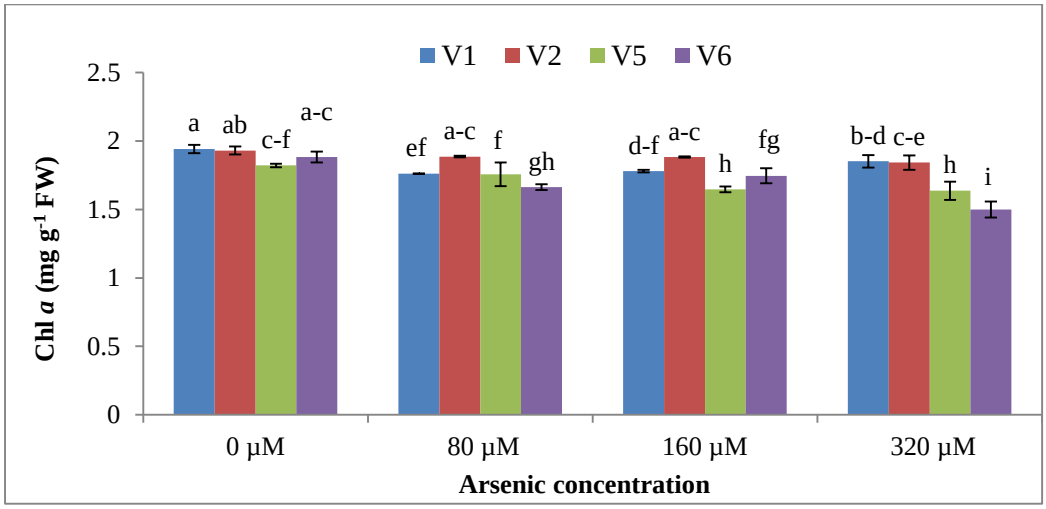

Figure 3. Chl *a* content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively

In case of Chl *b*, similar result found like Chl *a*. At 80 µM As treatment, there was no significant difference among the cultivars except BARI Lentil-2 as well as at160 µM As treatment, there was no significant difference among the cultivars except BARI Lentil-5. Significant difference of Chl *b* among the cultivars was observed at 320 µM. As like Chl *a*. Higher reduction of Chl *b* was found in BARI Lentil-6 (28.55%) and BARI Lentil-5 (16.29%) compared to BARI Lentil-2 (8.87%) and BARI Lentil-1 (7.65%) at 320 µM As treatment (Figure 4).

In case of Chl a+*b*, similar trend was observed. Significant difference was found among the cultivars at 320 µM As stress (Figure 5). Higher reduction of Chl a+*b* was found at 320 µM As treatment in BARI Lentil-6 (21.93%) and BARI Lentil-5 (11.32%) compared to BARI Lentil-2 (5.36%) and BARI Lentil-1 (5.22%).

In case of Car, an identical result was found like Chl *a*. The significant variation was found among the cultivars at 320 µM As stress. The reduction of Car by 6.47%, 9.42%, 17.69%, and 23.95% was observed in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6, respectively at 320 µM As treatment (Figure 6).

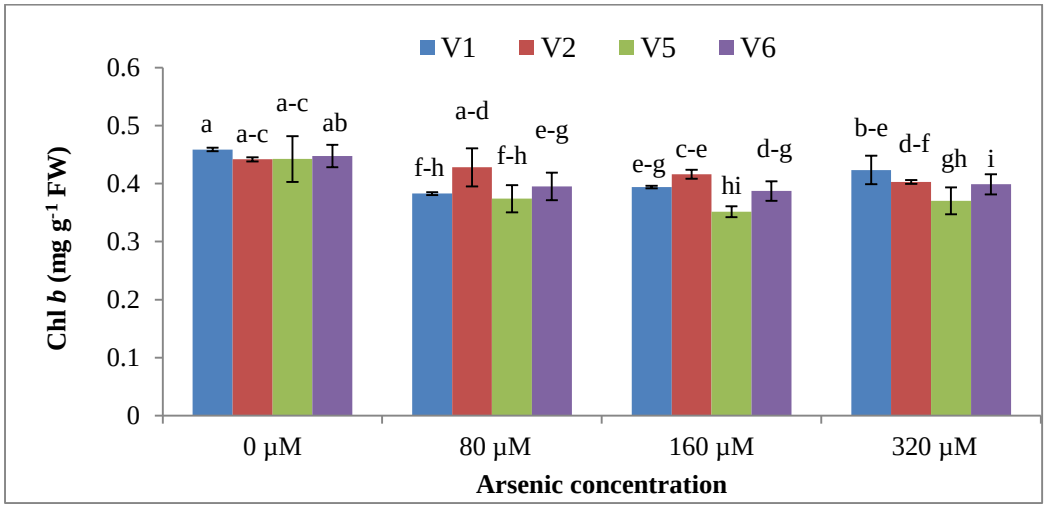

Figure 4. Chl *b* content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

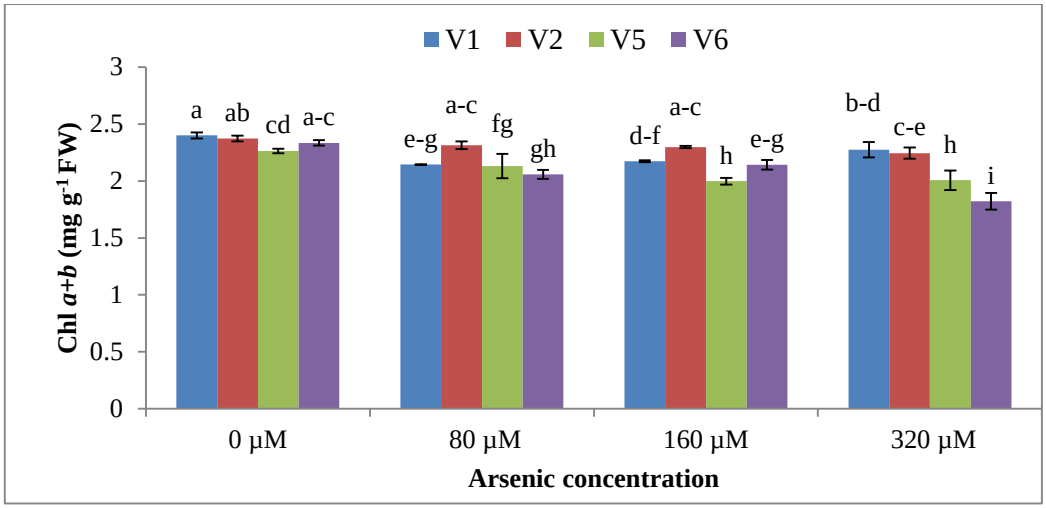

Figure 5. Chl *a+b* content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

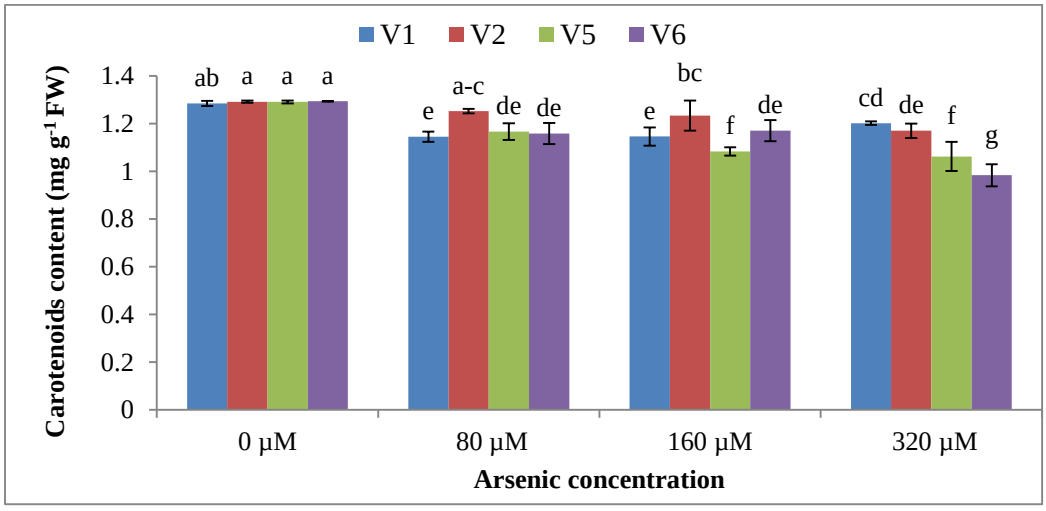

Figure 6. Carotenoids content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

Earlier studies showed that As reduced chl and car content in *Trifolium pretense* (Mascher *et al.*, 2002), *Lemna monor* L. (Mubarak *et al.*, 2016), *Vigna mungo* (Srivastava *et al.*, 2017). On the other hand, three was no significant variation found among Chl *a*, Chl *b*, Chl *a+b* and Car in lettuce plants under As exposure by Gusman *et al.* (2013). Chlorophyll and carotenoids is a simple and dependable indicator of toxic metals/metalloids toxicity. Toxic metals/metalloids substitute their Magnesium (Mg) in the Chl molecule that disrupts the normal function of photosynthesis. Photosynthetic pigments are important internal factor that can reduce the photosynthesis rate. They are the targets of harmful As impact as reported by Miteva and Merakchiyska (2002). Besides that, Car accumulation is considered one of the tolerance mechanisms under oxidative stress (Mallick and Mohn, 2000). Under the present study, BARI Lentil-1 and, BARI Lentil-2 showed a relative tolerance compared to BARI Lentil-5 and, BARI Lentil-6 in terms of Chl *a*, Chl *b*, Chl *a+b*, and Car at 320 µM As treatment.

4.2.4. Arsenic (As) content and Translocation factor (TF)

The level of As toxicity in plant depends on the amount of As accumulation and distribution into the plant cells. In the present experiment, As accumulation increased at a linear trend both root and shoot with the As concentration. However, root accumulated more As than shoot. The root of BARI Lentil-1 contained 187.28, 297.87, 498.71 µg g^{-1} DW As; BARI Lentil-2 contained 160.92, 345.80, 456.33 µg g^{-1} DW As; BARI Lentil-5 contained 147.53, 294.21, 390.65 µg g^{-1} DW As; BARI Lentil-6 contained 174.42, 276.16, 385.61 µg g^{-1} DW As at 80, 160, 320 µM As treatment, respectively (Figure 7). At 80 and 160 µM As concentration, there was no significant accumulation of As in shoot among all the lentil cultivars. At 320 µM As stress, significantly BARI Lentil-1 and BARI Lentil-2 contained higher As amount than BARI Lentil-5 and BARI Lentil-6. But in case of shoot, As accumulated in reverse trend of root. The shoot of BARI Lentil-1 contained 24.41, 226.58 µg g^{-1} DW As; BARI Lentil-2 contained 37.13, 251.67 µg g^{-1} DW As; BARI Lentil-5 contained 66.42, 269.86 µg g^{-1} DW As; BARI Lentil-6 contained 47.83, 317.75 µg g^{-1} DW As at 160, 320 µM As treatment, respectively (Figure 8).

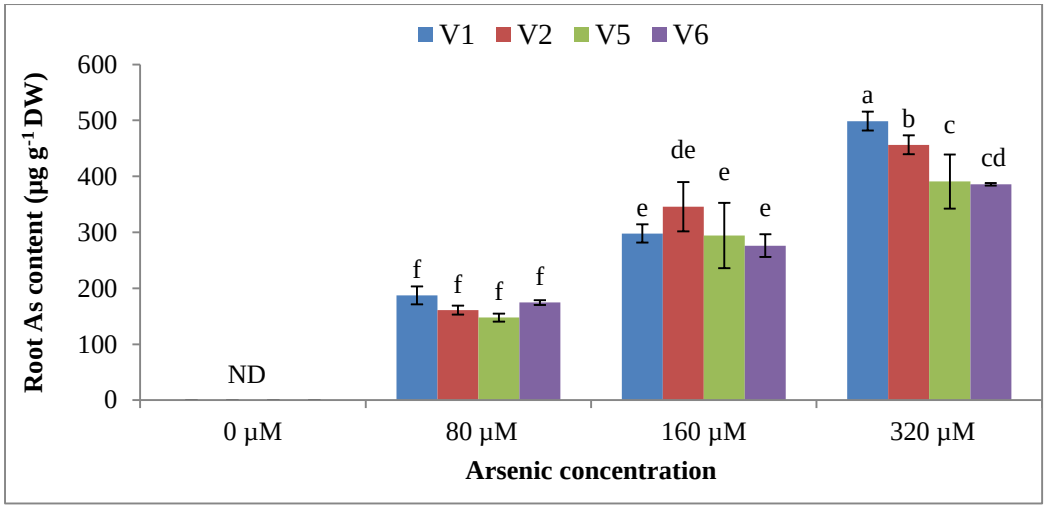

Figure 7. Root As content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively and ND means non detectable.

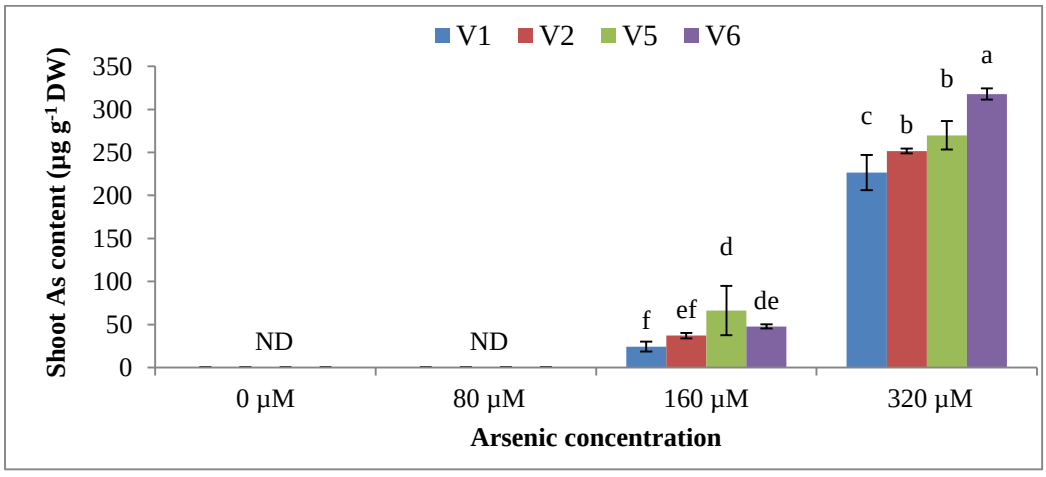

Figure 8. Shoot As content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively and ND means non detectable.

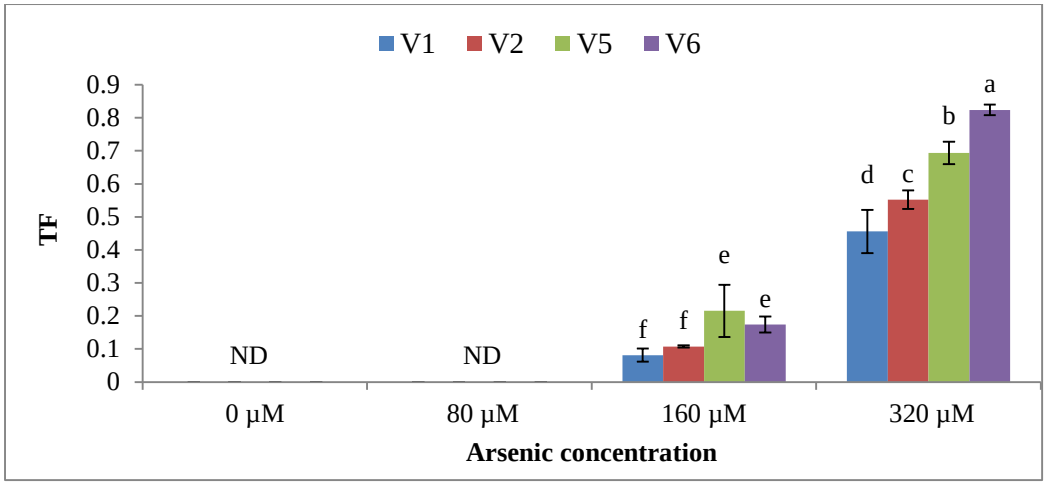

Figure 9. Translocation factor (TF) of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively and ND means non detectable.

BARI Lentil-1 and BARI Lentil-2 contained lower As amount than BARI Lentil-5 and BARI Lentil-6. Translocation factor (TF) refer the ratio of As in plant shoot to root. It indicates that how much As transfer from root to shoot. In the present experiment, both As concentration BARI Lentil-1 and BARI Lentil-2 showed lower TF compared to BARI Lentil-5 and BARI Lentil-6 (Figure 9). However, 0.08, 0.10, 0.21, 0.17 were the TF of BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, BARI Lentil-2 at 160 µM As treatment, respectively and 0.45, 0.55, 0.69, 0.82 were the TF of BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, BARI Lentil-6 at 320 µM As treatment, respectively. Plant with TF more than one (TF>1) referred as hyperaccumulator. From the present data, it was clear that lentil was not As hyperaccumulator plant. Non- hyperaccumulator plant tried to cope up metals/metalloids toxicity by remaining the metals/metalloids in the root or detoxify different mechanisms. In this present study, BARI Lentil-1and BARI Lentil-2 uptake more As in root but transfer less As to shoot and in case of BARI Lentil-5 and BARI Lentil-6 this was vice versa. This might be a reason of relative As tolerance of BARI Lentil-1and BARI Lentil-2 compared to BARI Lentil-5and BARI Lentil-6. This result was supported by Talukdar and Talukdar (2014) who reported that As sensitive lentil cultivar showed a higher TF in comparison with tolerant cultivar

4.3. Biochemical parameters

4.3.1. Oxidative stress markers

4.3.1.1. Lipid peroxidation

It is well established that As induced oxidative stress through the overproduction of reactive oxygen species (ROS). This overproduced ROS causes breakdown of cell membrane through the lipid peroxidation. In stress condition, lipid peroxidation used as a marker of ROS generated damage. Malondialdehyde (MDA), a cytotoxic compound is one of the final product of lipid peroxidation (Srivastava *et al.,* 2017). In the present experiment, MDA content increase roughly dose-dependent manner. However, all cultivars showed a significant increase of MDA content than control plant. There was no significant difference among all the cultivars at 80 µM As treatment. At 160 µM As treatment, only BARI Lentil-6 showed significant increase of MDA content. The increase of MDA content by 129.68%, 225.23%, 301.47%, and 483.52% were found respectively in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 due to 320 µM As compared to their control (o µM As) plants (Figure 10). It might be higher cell membrane damage occurred through the lipid peroxidation in BARI Lentil-5 and BARI Lentil-6, therefore, this cultivars showed higher increase of MDA content. This result was similar to Talukdar and Talukdar (2014), they observed higher increase of MDA content found in lentil root at As sensitive variety. A Comparative analysis of two maize cultivars under As stress, higher increase of MDA content was found at As sensitive maize cultivar in both root and shoot (Du *et al.,* 2017).

4.3.1.2. Other aldehyde content

Other aldehyde content is the indicator of cell membrane damage. Other aldehyde content increases at various abiotic stress condition. In the present study, other aldehyde content increase in concentration-dependent fashion.

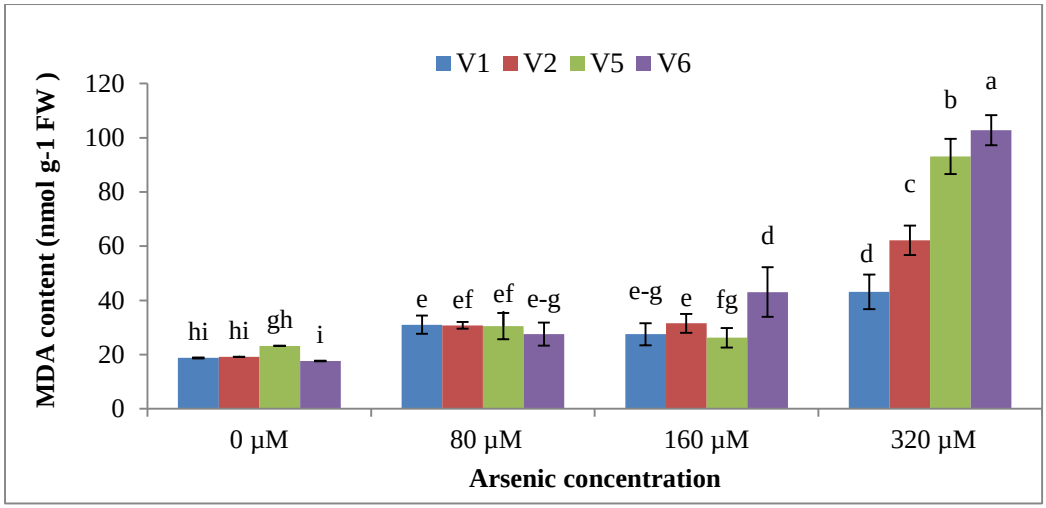

Figure 10. MDA content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

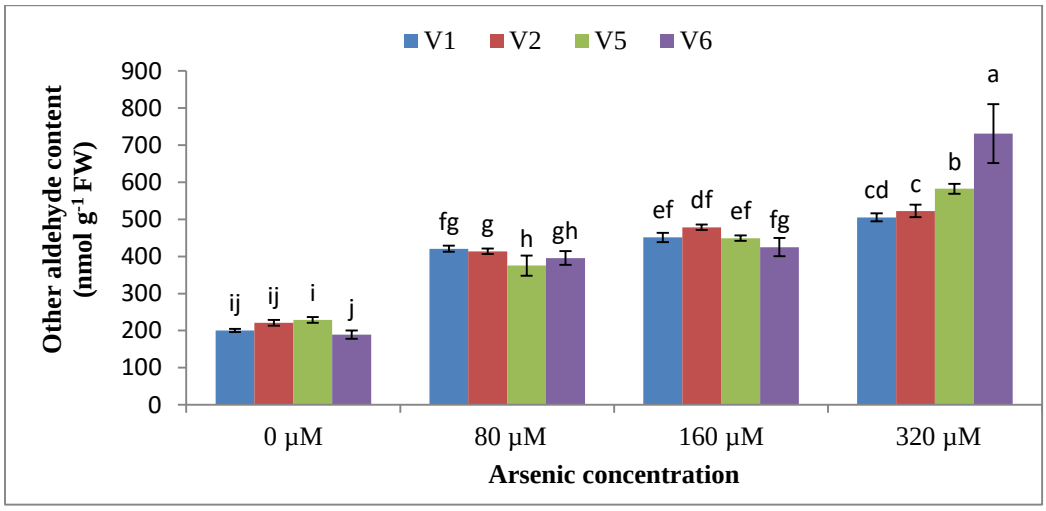

Figure 11. Other aldehyde content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

There was no significant increase among the cultivars at 80 µM As treatment except BARI Lentil-5. No significant increase occurred among all the cultivars at 160 µM As treatment. Like MDA, Higher increase of content other aldehyde observed in BARI Lentil-6 and BARI Lentil-5 compared to BARI Lentil-1 and BARI Lentil-2 at 320 µM As treatment (Figure 11). Keramat *et al.* (2010) reported that in soybean plant increase other aldehyde content due to heavy metal like Cd toxicity.

4.3.1.3. Electrolyte leakage (EL)

Electrolyte leakage (EL) indicates the degree of cell membrane damage in plants. Increasing EL due to As toxicity was observed by Malik *et al.* (2011) in *Cicer arietinum* and Duman *et al.* (2010) in *Lemna minor*. Electrolyte leakage was increased in lentil plan due to cadmium (Cd) toxicity (Talukdar, 2012). This experiment showed As stress increased the EL (%) at concentration-dependent manner of four cultivars of lentil seedlings compared with control. There was no significant increase among the cultivars at 80 µM As treatment except BARI Lentil-6. Compared with control (0 µM As) EL was increased by 108.68%, 124.58%, 146.87%, and 195.32% in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 at 320 µM As respectively (Figure 12). BARI Lentil-1 and BARI Lentil-2 might be maintain a membrane stability, so that they their EL comparatively lower than BARI Lentil-5 and BARI Lentil-6 at 320 µM As. This results were supported by Begum *et al.* (2016) where two cultivars of rice seedlings treated with As and observed the EL was higher in sensitive cultivar.

4.3.1.4. H_2O_2 content

During abiotic stress, oxygen (O_2) reduced to unstable high reactive superoxide ($O_2^{\bullet-}$) within short period which is converted to stable hydrogen peroxide (H_2O_2). Hydrogen peroxide (H_2O_2) can inactive the enzymes activities by oxidizing their thiol groups. (Hasanuzzaman *et al,* 2012a). The accumulation of H_2O_2 increased in plant cell and increased oxidative damage in the cell membrane due to As toxicity (Nath *et al.*, 2014, Rafiq *et al.*, 2017). In the present study, the content of H_2O_2 increased significantly in all the cultivars of lentil seedlings.

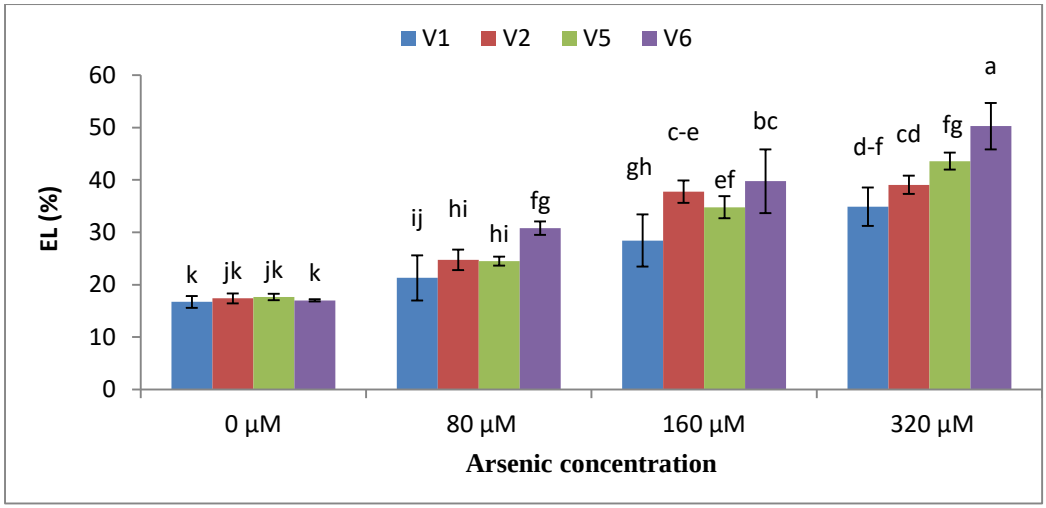

Figure 12. Electrolyte leakage (EL) of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

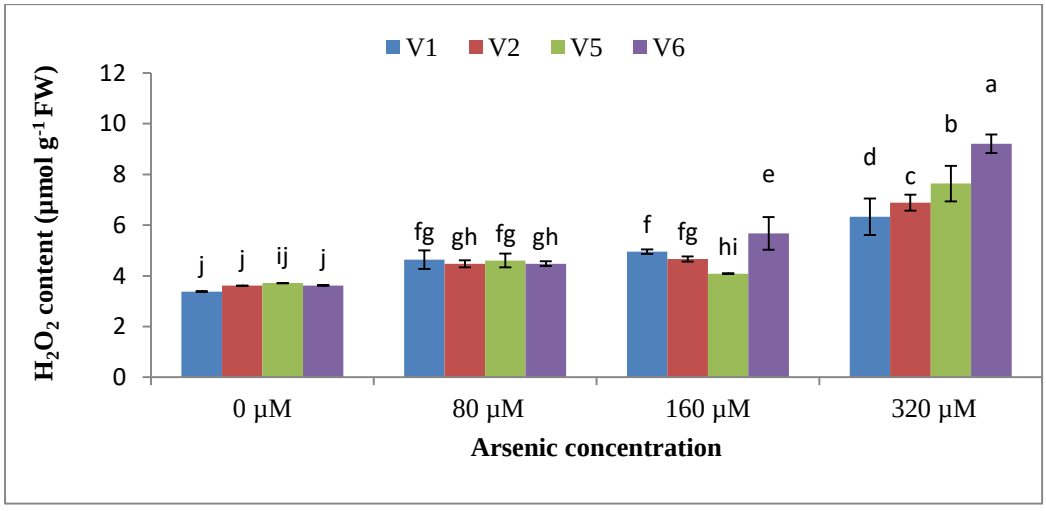

Figure 13. H_2O_2 content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

There was no significant increase among the cultivars at 80 µM As treatment. At 160 µM As treatment, lesser H_2O_2 content was found in BARI Lentil-5 whereas higher H_2O_2 content found at BARI Lentil-6. A distinguished difference was found among the cultivars at 320 µM As treatment. Compared with control (0 µM As) H_2O_2 content was increased by 87.46%, 90.63%, 105.73%, and 154.49% in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 at 320 µM As respectively (Figure 13). Talukdar and Talukdar (2014) noted that lentil plants exposed to As exhibit an increase in H_2O_2. This result supported the present investigation.

4.3.1.5. Proline content

Proline (Pro) is well established as an osmotic stress marker and when plants exposed to various abiotic stresses including toxic metals/metalloids accumulation of Pro content increases manifold (Alam *et al*., 2013, Al Mahmud *et al*., 2017). In the present experiment, Pro content increased in a concentration-dependent manner of the four cultivars of lentil seedlings at every concentration of As. At 80 µM As treatment, there was significant difference between BARI Lentil-1 and BARI Lentil-6. At 160 and 320 µM As treatment, BARI Lentil-5 and BARI Lentil-6 showed significant increase of Pro content compared to BARI Lentil-1 and BARI Lentil-2 (Figure 14). Proline accumulation in plant is an indicator of water loss. At 320 µM As treatment, Lentil-5 and BARI Lentil-6 showed higher Pro content due to water loss that was proved by lower RWC content ((Figure 1) and water content ((Figure 2). Rahman *et al.* (2015) reported that water loss due to As stress reduced RWC and increased Pro accumulation in plant.

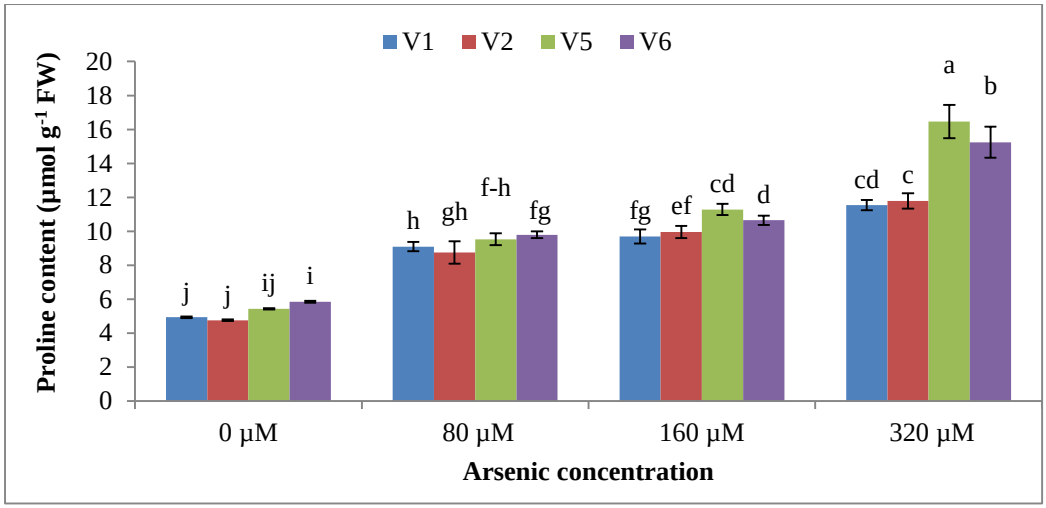

Figure 14. Proline content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

4.3.2. Antioxidant defense system

4.3.2.1. Ascorbate and glutathione contents

Ascorbate (AsA) is one the most abundant non-enzymatic antioxidant which acts as a stress tolerance marker in plants. It can reduced membrane damage through the direct react with ROS such as H_2O_2, $O_2^{\bullet-}$ and $^1O_2$. It can protect photosynthetic machinery as well as regenerate carotenoids and α-tocopherol through the AsA-GSH cycle (Singh *et al.*, 2006). In the present experiment, AsA content reduced as a result of As toxicity. At 80 µM As treatment, there was no significant difference among the cultivars except BARI Lentil-5. The reduction of AsA was statistically similar in BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 under 160 µM As treatment. But at 320 µM As treatment, AsA content reduced drastically in BARI Lentil-5 and BARI Lentil-6, whereas comparing with control AsA content increased in BARI Lentil-1 and BARI Lentil-2 (Figure 15). Singh *et al.* (2006) observed significant increase in AsA content in fronds of arsenic hyperaccumulator *Pteris vittata* then arsenic- sensitive *Pteris ensiformis*.

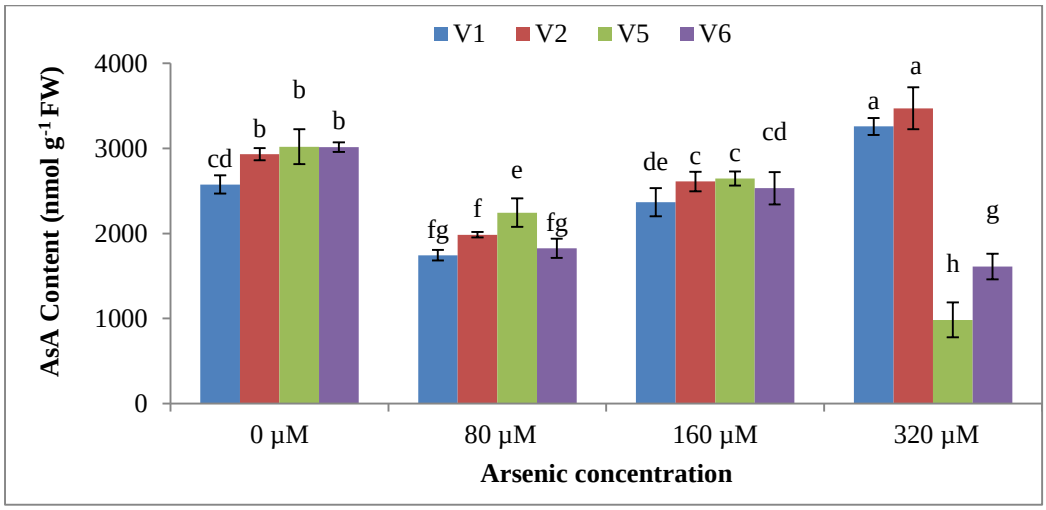

Figure 15. Ascorbate (ASA) content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

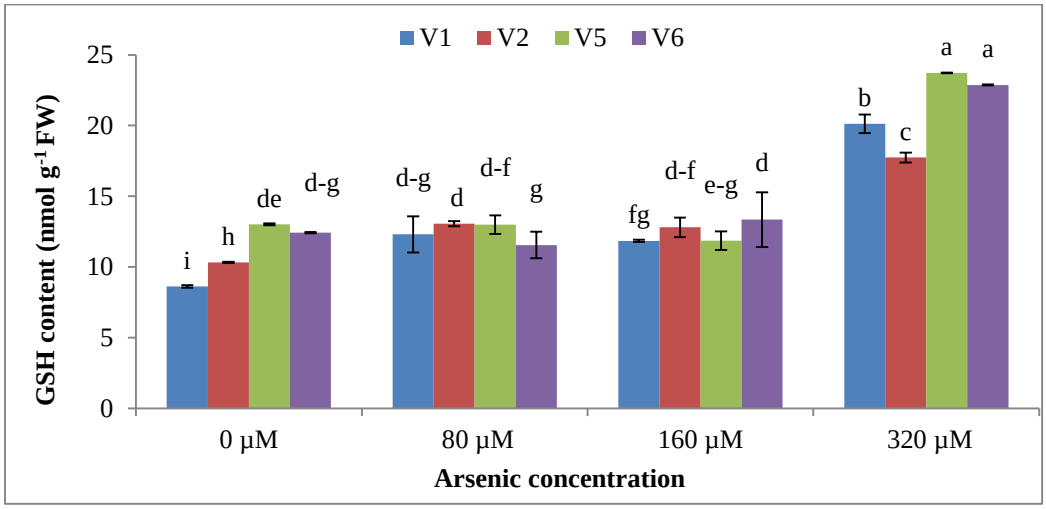

Figure 16. GSH content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

Glutathione is another non-enzymatic antioxidant which maintain the redox balance in pant cell as well as play an important role in detoxification toxic metals and metalloids through phytochelatin synthesis. Glutathione can provide defense against oxidative damage through the AsA-GSH cycle by directly bind with ROS and detoxified it (Sharma, 2012). Whoever, the changing ratio of reduced glutathione and oxidized glutathione (GSH/GSSG) during ROS detoxification process is an important phenomenon in redox signaling pathways (Li and Jin, 2007). Hasanuzzaman *et al.* (2013) suggested that increase of GSH content indicated as relative tolerance under As stress. Reduced Glutathione is oxidized to GSSG and increased the level of GSSG during the scavenging reaction of ROS (Rahman *et al*., 2015). In the present study, At 80 and 160 µM As treatment, there was no significant change among BARI Lentil-1, BARI Lentil-2, and BARI Lentil-5. At 320 µM As treatment, although GSH content was lower in cultivars BARI Lentil-1, compared with control it was higher (Figure 16). Compared with control (0 µM As) GSH content was increased by 133.39%, 71.80%, 82.31%, and 83.97% in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 at 320 µM As respectively. In case of GSSH, there was no significant change among BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 under 80 µM As stress. At 160 µM As stress, there was no significant change among all the cultivars. At 320 µM As stress, BARI Lentil-1and BARI Lentil-2 showed lower GSSG content compared to BARI Lentil-5 and BARI Lentil-6 (Figure 17). In case of GSH/GSSR ratio there was no significant change among BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 under 80 µM As stress. At 160 µM As stress, there was no significant change among all the cultivars. At 320 µM As stress, the GSH/GSSG ratio was statically similar in BARI Lentil-1, BARI Lentil-2, and BARI Lentil-5. Lower GSH/GSSG ratio was found in BARI Lentil-6 (Figure 18).

In this experiment, higher GSH content and lower GSSG content were found at BARI Lentil-1, BARI Lentil-2. This result was similar with Talukdar and Talukdar (2014). They reported that As tolerant cultivar showed higher GSH content and lower GSSH content compared to sensitive cultivar. In the AsA-GSH cycle, GSH provide electron to regenerate AsA and as a substrate for GPX. Higher GSH might be regenerate AsA, So that higher AsA content was found in BARI Lentil-1and BARI Lentil-2 (Noctor and Foyer, 1998; Tausz *et al.*, 2004).

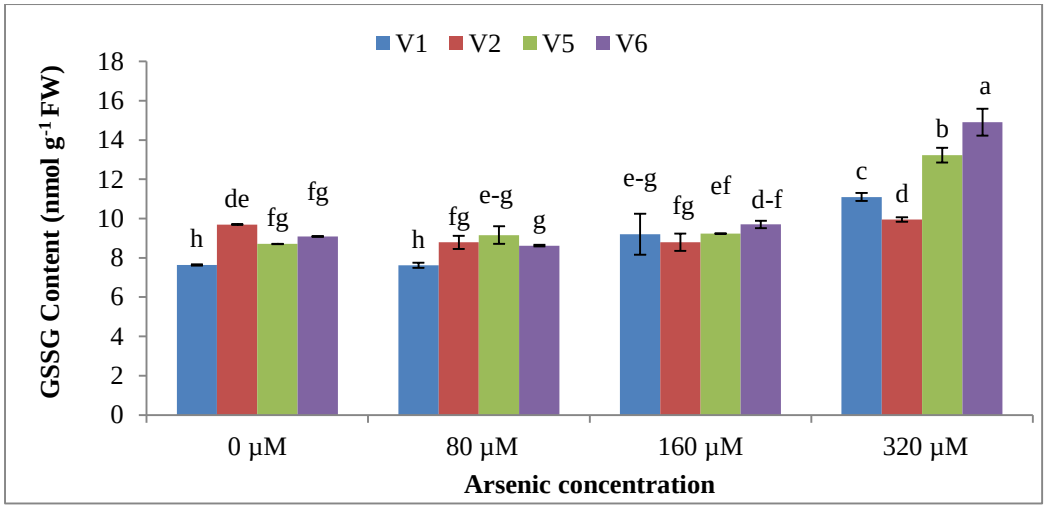

Figure 17. GSSG content of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

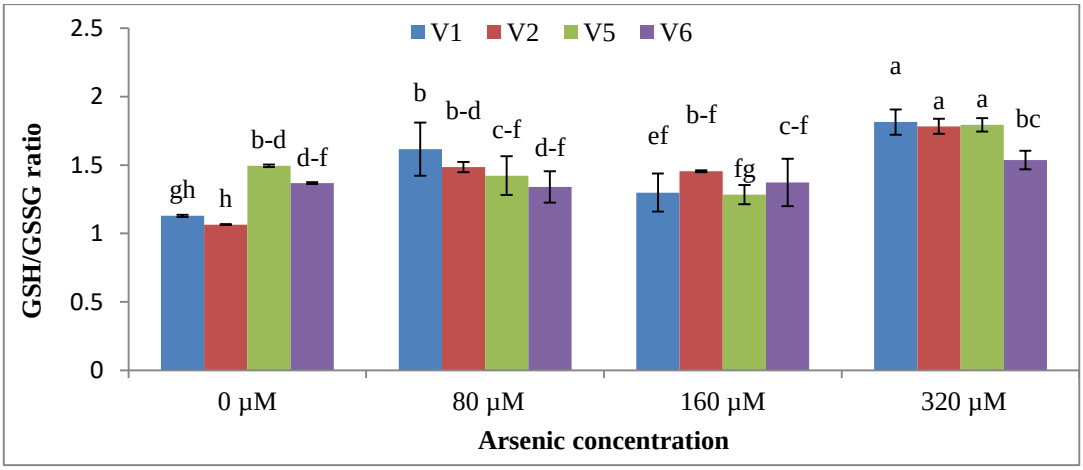

Figure 18. GSH/GSSG ratio of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

4.3.2.2. Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) is an antioxidant enzyme which is considered the first line defense in the antioxidant system. Superoxide dismutase detoxifies $O_2^{\bullet-}$ to less reactive H_2O_2 (Hasanuzzaman *et al.*, 2012a). The present study showed the activity of SOD increase in a concentration-dependent fashion in all cultivars. The activity of SOD at 80 µM As stress, BARI Lentil-1 and BARI Lentil-2 were statistically similar; BARI Lentil-5 and BARI Lentil-6 were statistically similar as well as BARI Lentil-2 and BARI Lentil-5 were statistically similar. At 160 and 320 µM As stress, the activity of SOD was lower in BARI Lentil-1 and BARI Lentil-2 compared with BARI Lentil-5 and BARI Lentil-6 (Figure 19). Previous studies showed that As stress up-regulated the SOD activity (Tripathi *et al.*, 2015, Rahman *et al.*, 2015). No significant variation of SOD activity in lentil root between As sensitive and tolerant culvivar was reported by Talukdar and Talukdar (2014). The higher activity of SOD might be a reason of higher H_2O_2 content in BARI Lentil-5 and BARI Lentil-6 under 320 µM As stress (Figure 13).

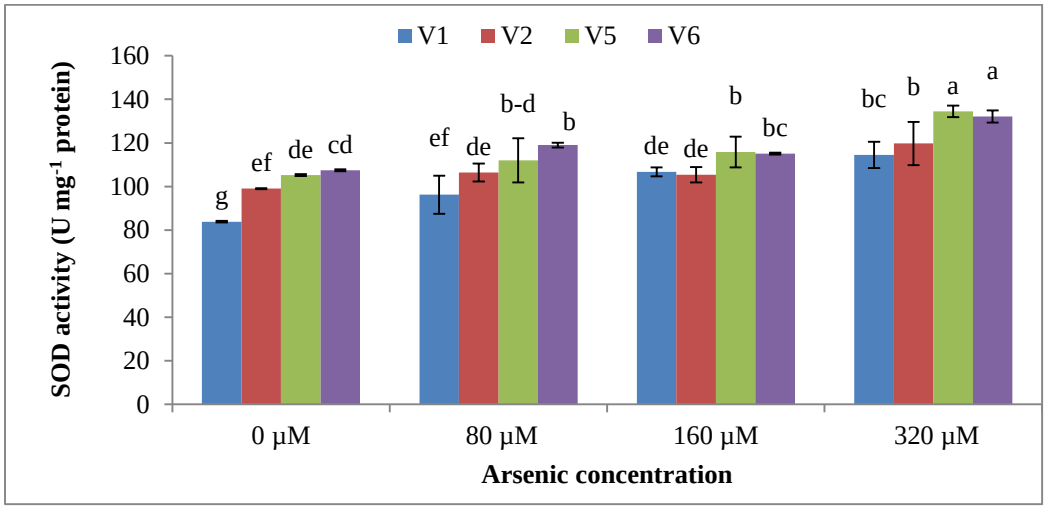

Figure 19. Superoxide dismutase (SOD) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

4.3.2.3. Catalase (CAT) activity

Catalase (CAT) is a tetrameric heme-containing another H_2O_2 decomposing enzyme found in peroxisomes, glyoxysomes, and related organelles where H_2O_2 generating enzymes are located. It has a capacity to rapidly degrade H_2O_2 into H_2O and O_2, thus preventing cells from oxidative damage (Sa´nchez-Casas and Klesseg, 1994). One molecule of CAT can convert around six million molecules of H_2O_2 to H_2O and O_2 per minute (Gill and Tuteja, 2010). In the present study, CAT activity increased due to arsenic stress. At 80 µM As stress, no significant difference was found among the cultivars BARI Lentil-1, BARI Lentil-5, and BARI Lentil-6. At 160 µM As stress, no significant difference was found among the cultivars BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6. Compared with control (0 µM As) CAT activity was increased by 44.50%, 32.10%, 0.0 %, and 22.16% in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 at 320 µM As respectively (Figure 20). The higher activity of CAT might be a reason of lower H_2O_2 content in BARI Lentil-1 and BARI Lentil-2 under 320 µM As stress (Figure 13). Shrivastava *et al.* (2005) reported that higher activity of CAT has been shown in arsenic-tolerant chinese brake fern (*Pteris vittata*) than arsenic-sensitive slender brake fern (*Ptries ensiformis*) and boston fern (*Nephrolepis exaltata*).

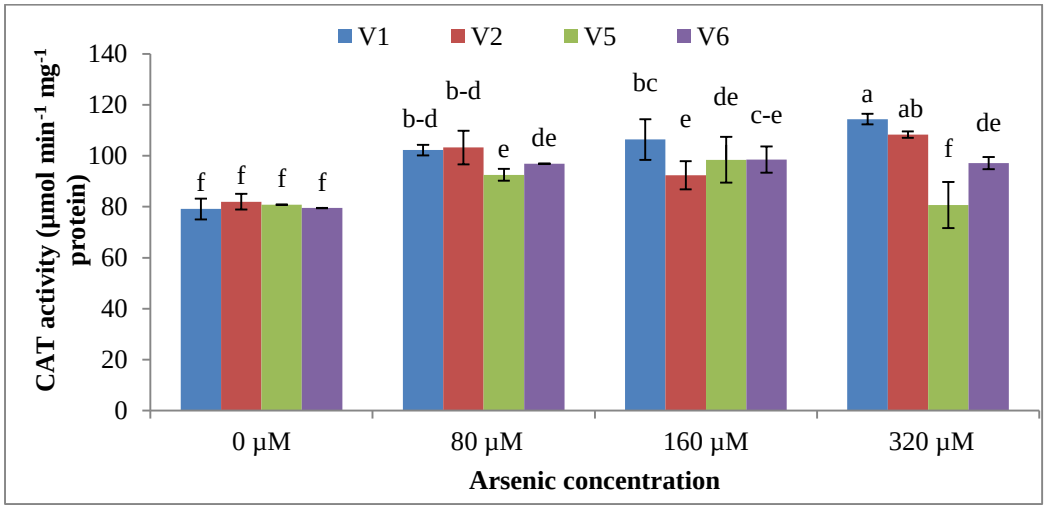

Figure 20. Catalase (CAT) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

4.3.2.4. Glutathione peroxidase (GPX) activity

GPX is a foremost cellular enzyme and member of a large peroxidase family. It reduced H_2O_2 and organic and lipid hydroperoxides (LOOHs) through using GSH, therefore protect plant cells from oxidative stress. It also capable of repairing membrane lipid peroxidation and is an important protectant against oxidative membrane damage (Kühn and Borchert, 2002). In the present study, comparing with control GPX activity increase or stable in BARI Lentil-1 and BARI Lentil-2, on the other hand GPX activity decrease or stable in BARI Lentil-5 and BARI Lentil-6 due As toxicity. At 80 µM As stress, no significant difference was found among the cultivars BARI Lentil-1, BARI Lentil-2, and BARI Lentil-6. At 160 µM As stress, the activity GPX was increased 51. 41%, 11.59%, and 4.84% in BARI Lentil-1, BARI Lentil-2, and BARI Lentil-6 respectively, whereas the activity GPX was decreased 9.82% in BARI Lentil-5. At 320 µM As stress, the activity GPX was increased 20.71% and 12.59%, in BARI Lentil-1and BARI Lentil-2 respectively, whereas the activity GPX was decreased 32.74% and 65.53% in BARI Lentil-5 and BARI Lentil-6 respectively (Figure 21).

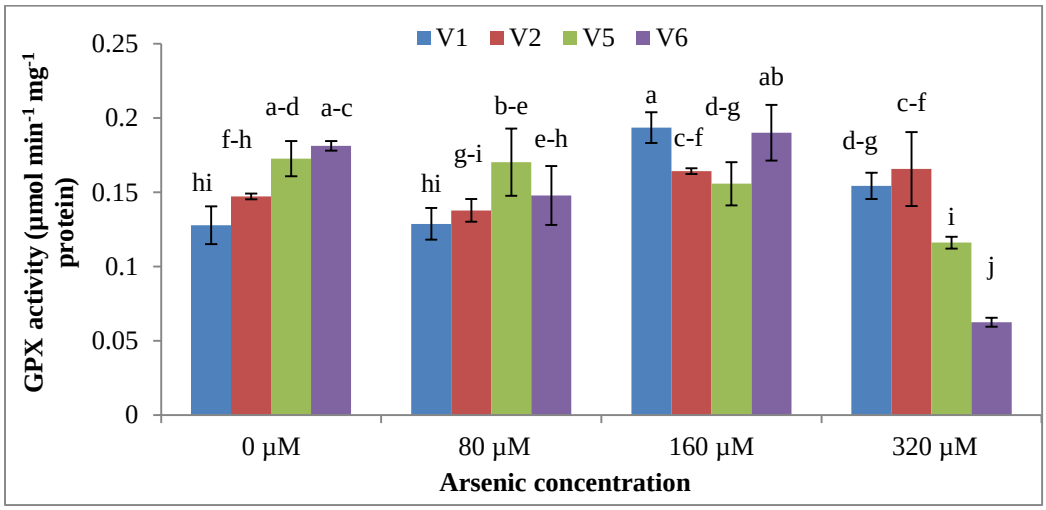

Figure 21. Glutathione peroxidase (GPX) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

Lower MDA (Figure 10), other aldehyde (Figure 11), EL (Figure 12), H_2O_2 (Figure 13) in BARI Lentil-1, BARI Lentil-2 under 320 µM As stress might be due to the higher activity of GPX. Hasanuzzaman *et al.* (2013) and Rahman *et al.* (2015) suggested that increase GPX activity help to reduce oxidative damage in plant.

4.3.2.5. Dehydroascorbate reductase (DHAR) activity

Dehydroascorbate reductase (DHAR) is an important enzyme of AsA-GSH cycle and the key component of AsA recycling under oxidative stress. It regenerate AsA from its oxidized state (DHA) to maintain cellular redox balance. It is vital for tolerance to various abiotic stresses (Hasanuzzaman *et al.*, 2012b). In the present study, DHAR activity was varied with different variety and arsenic concentration. At At 80 µM As stress, no significant difference of DHAR activity was found among all the cultivars. At 160 µM As stress, BARI Lentil-1 and BARI Lentil-2 showed lower DHAR activity compared to BARI Lentil-5 and BARI Lentil-6. At 320 µM As stress, the activity DHAR was increased 37.76% and 11.89%, in BARI Lentil-1 and BARI Lentil-2 respectively, whereas the activity DHAR was decreased 24.34% and 11.45% in BARI Lentil-5 and BARI Lentil-6 respectively (Figure 22). The higher DHAR activity might be the reason of higher AsA content in BARI Lentil-1 and BARI Lentil-2 (Figure 15) under 320 µM As stress. According to Talukdar and Talukder (2014), As tolerant cultivar showed higher DHAR activity than As sensitive cultivar.

4.3.2.7. Monodehydroascorbate reductase (MDHAR) activity

Monodehydroascorbate reductase (MDHAR) is another important enzyme of AsA-GSH cycle. It help to regenerate AsA from Monodehydroascorbate (MDHA). This MDHA is produced by the oxidation of AsA. If MDHA is not reduced again to AsA by MDHAR, it will spontaneously disproportionate into AsA and DHA. The regeneration of AsA could be regulated in this cycle mainly by NADPH-dependent MDHAR activity (Mittova *et al*., 2000) and thus it is crucial for AsA regeneration and essential for maintaining a reduced pool of AsA (Martínez and Araya, 2010) . In the present study, MDHAR activity was increased due to As stress. At At 80 µM As treatment, no significant change of MDHAR activity was found among all the cultivars.

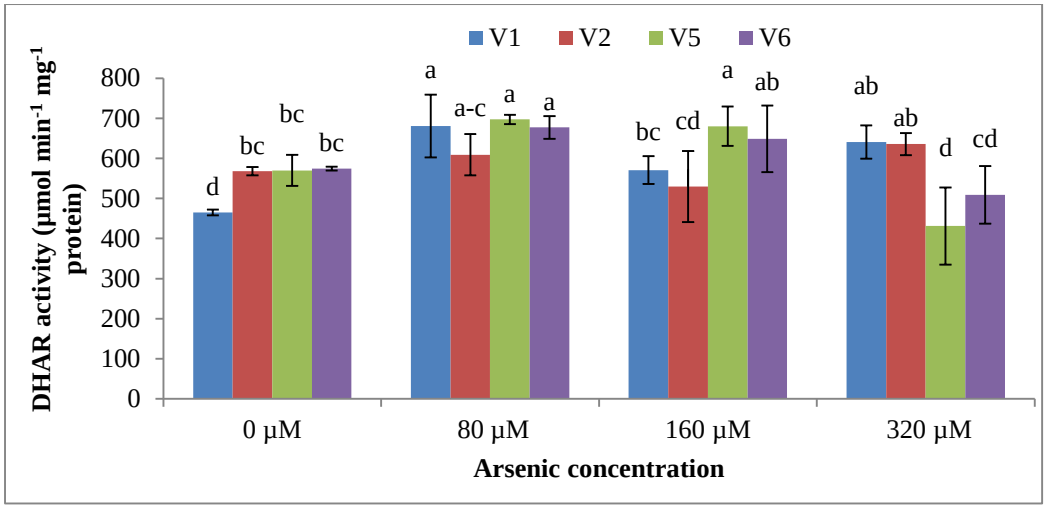

Figure 22. Dehydroascorbate reductase (DHAR) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

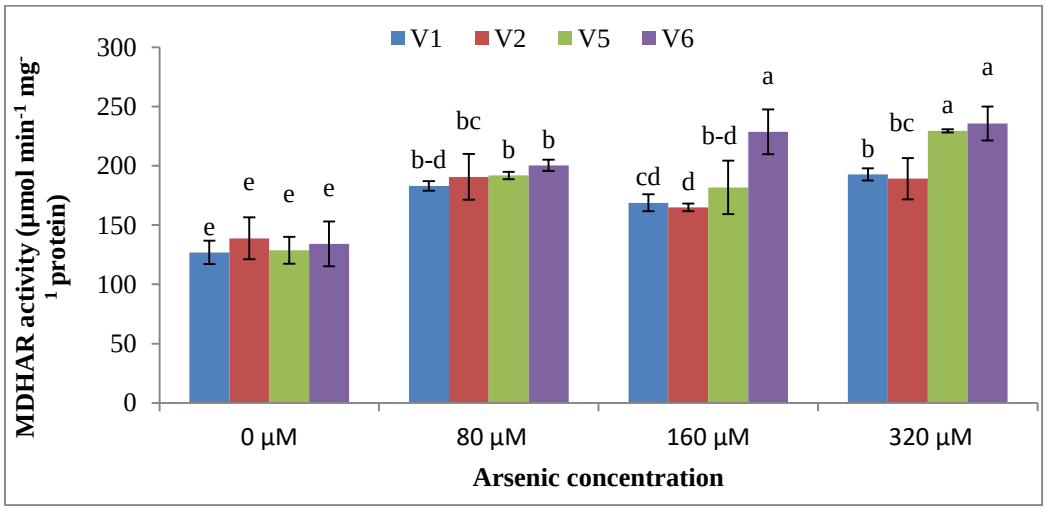

Figure 23. Monodehydroascorbate reductase (MDHAR) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

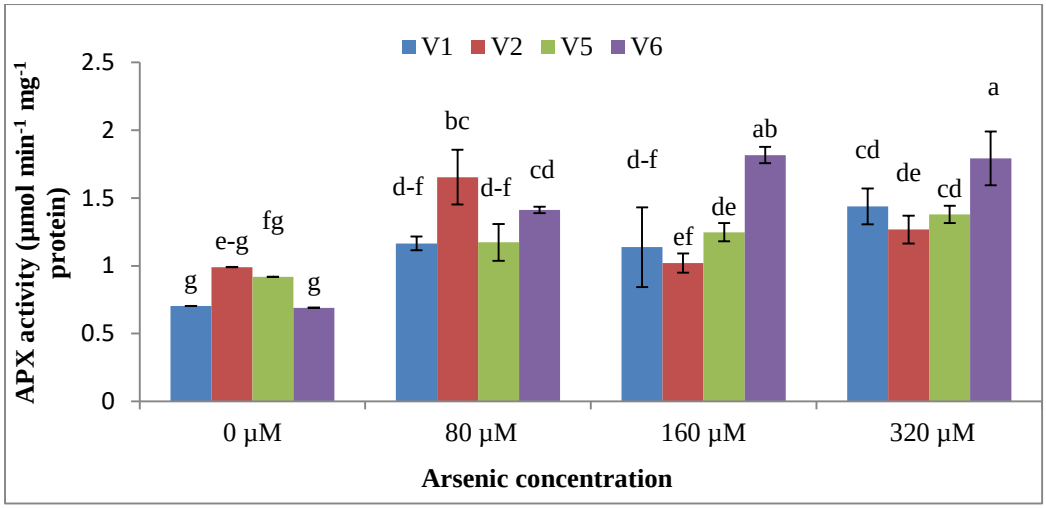

Figure 24. Ascobate peroxidae (APX) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

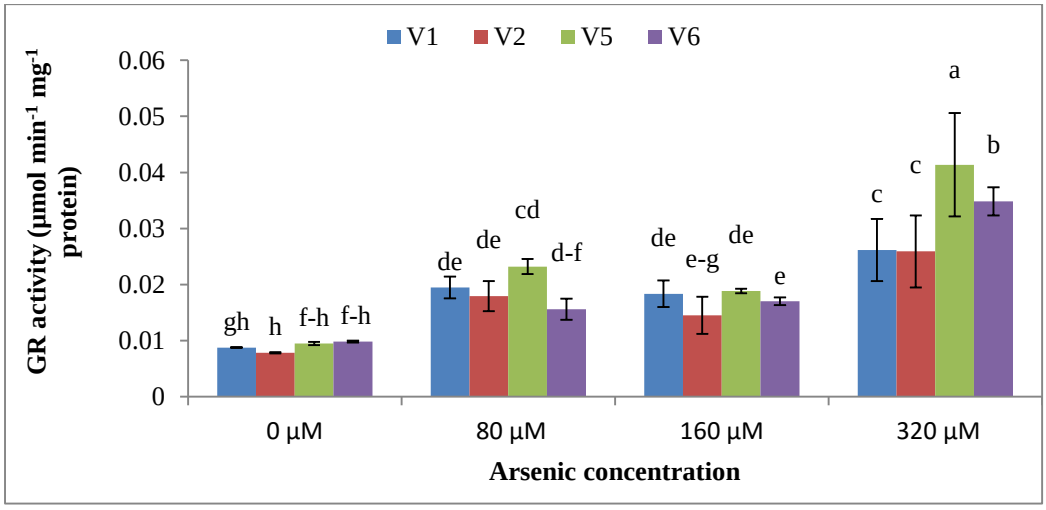

Figure 25. Glutathione reductase (GR) activity of four lentil cultivars under different arsenic concentrations

Mean (±SD) was calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying the Fisher's LSD test).

Here,

V1, V2, V5, and V6 indicate as BARI Lentil-1, BARI Lentil-2, BARI Lentil-5 and BARI Lentil-6 respectively.

At 160 µM As treatment, there was no significant variation among the cultivars except BARI Lentil-6. Compared with control (0 µM As) MDHAR activity up regulated by 51.91%, 36.23%, 78.19%, and 75.63% in BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 at 320 µM As respectively (Figure 23). Monodehydroascorbate reductase can participate in superoxide ($O_2^{\bullet-}$) production in membranes (del Río *et al.*, 2002).

4.3.2.7. Ascobate peroxidae (APX) activity

Ascobate peroxidae (APX) is the first line enzyme of AsA-GSH cycle. It convert H_2O_2 to H_2O using AsA (Hasannuzzaman *et al.*, 2013). In the present experiment, APX activity increase in all the cultivars due to As Stress. At 80 µM As treatment, there was no significant variation among the cultivars except BARI Lentil-2. There was no significant change of APX activity in BARI Lentil-1, BARI Lentil-2, and BARI Lentil-5 under 160 and 320 µM As stress (Figure 24). Upregulation of APX activity was found in *Triticum aestivum* L. (Hasannuzzaman *et al.*, 2013), *Oryza sativa* L. (Rahman *et al.*, 2015), and *Vigna mungo* L. (Srivastava *et al.*, 2017) exposed to As.

4.3.2.8. Glutathione reductase (GR) activity

Glutathione reductase (GR) is a potential enzyme of the AsA-GSH cycle and plays an essential role in the defense system against ROS. It catalyses the NADPH-dependent reduction of disulphide bond of GSSG and is thus important for maintaining the GSH pool (Hasannuzzaman *et al.*, 2012a). In the present experiment, the activity of GR increase due to As stress. At 80 and 160 µM As stress, there was no significant variation among the cultivars. At 320 µM As stress, BARI Lentil-1 and BARI Lentil-2 showed lower GR activity compared to BARI Lentil-5 and BARI Lentil-6 (Figure 25). Upregulation of GR activity was observed by Rai *et al.* (2011) in rice under As stress.

CHAPTER 5

SUMMARY AND CONCLUSION

An experiment was conducted to find out the morpho-physiological and biochemical effects of lentil cultivars due to arsenic stress and find out the tolerance mechanisms of lentil cultivar under arsenic stress as well as find out arsenic tolerant lentil cultivars. Arsenic reduced plant shoot length, FW, DW, RWC, shoot water content, Chl a, Chl b, Chl a+b, Car content and increase lipid peroxidation (MDA content), other aldehyde, EL, proline, H_2O_2 content. Activities of antioxidant enzymes-catalase (CAT), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), superoxide dismutase (SOD) up regulated and down regulated due to As stress.

At 80 μM and 160 μM As concentrations, all the cultivars faced more or less similar stress. The clear difference among the cultivars was observed at 320 μM As concentration. At 320 μM As concentration, lower lipid peroxidation (MDA content), other aldehyde, EL, proline, H2O2 content observed in BARI Lentil-1 and BARI Lentil-2, so that they faced less oxidative stress. That's why photosynthetic pigment, relative water content found more in this cultivars. BARI Lentil-1 and BARI Lentil-2 uptake more As, but transfer less to root. The activity of CAT, GPX, DHAR was higher as well as AsA and GSH content higher in BARI Lentil-1 and BARI Lentil-2. This provide a higher defense against oxidative damage.

Considering the morpho-physiological parameters and antioxidant metabolism, BARI Lentil-1 and BARI Lentil-2 could be recommended as relatively tolerant over BARI Lentil-5, BARI Lentil-6 under As toxicity

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APPENDICES

Appendix I. Mean square values of MDA, other aldehyde, H_2O_2, and EL of BARI Lentil-1,BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 seedlings as influenced by arsenic

Source of variation	df	Mean square value of			
		MDA	Other aldehyde	H_2O_2	EL (%)
Replication	2	210.23	2610	8891	40.83
Variety	3	735.98	3455	21186	166.37
Treatment	3	7283.49	289998	340652	1421.11
Variety × Treatment	9	583.34	10585	13111	28
Error	30	7.47	441	677	6.42

Appendix II. Mean square values of Chl *a* , Chl *b*, Chl *a*+*b*, and Car of BARI Lentil-1,BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 seedlings as influenced by arsenic

Source of variation	df	Mean square value of			
		Chl *a*	Chl *b*	Chl *a*+*b*	Car
Replication	2	9.9×10^{-6}	0.00067	0.00104	0.00223
Variety	3	7.4×10^{-4}	0.0039	0.14153	0.02033
Treatment	3	1.6×10^{-3}	0.01151	0.14379	0.07314
Variety × Treatment	9	2.9×10^{-5}	0.00199	0.02884	0.00954
Error	30	1.1×10^{-6}	0.00036	0.00381	0.00108

Appendix III. Mean square values of proline, RWC, shoot water content, shoot length, and shoot FWof BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 seedlings as influenced by arsenic

Source of variation	df	Mean square value of				
		Proline	RWC	Shoot water content	Shoot length	Shoot FW
Replication	2	2.907	0.37	2.7712	0.0169	0.89
Variety	3	11.226	10.064	1.5436	1.2674	35.91
Treatment	3	143.188	712.047	24.5516	24.438	1468.78
Variety × Treatment	9	3.164	35.496	5.0772	1.0133	123.76
Error	30	0.227	2.955	0.3207	0.0572	3.69

Appendix IV. Mean square values of shoot DW, AsA, GSSG, GSH and GSH/GSSG ratio BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 seedlings as influenced by arsenic

Source of variation	df	Mean square value of				
		Shoot DW	AsA	GSSG	GSH	GSH/ GSSG ratio
Replication	2	0.3701	25028	0.4545	0.871	0.00034
Variety	3	0.9993	727260	6.9383	14.382	0.01865
Treatment	3	17.9116	1837114	36.5111	253.544	0.49543
Variety × Treatment	9	1.7748	1362781	3.8921	7.738	0.07138
Error	30	0.0931	20163	0.1243	0.487	0.00967

Appendix V. Mean square values of CAT, DHAR, MDHAR, GR, and APX of BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 seedlings as influenced by arsenic

Source of variation		Mean square value of				
	df	CAT	DHAR	MDHAR	GR	APX
Replication	2	36.23	15344.7	90.3	0.oooo13	0.00124
Variety	3	332.66	647.1	2499.9	0.00024	0.31651
Treatment	3	1075.5	37715.1	13861.6	0.00084	1.074
Variety × Treatment	9	167.08	19361.1	688	0.000042	0.20784
Error	30	22.53	3655.2	179.5	0.0000012	0.03254

Appendix VI. Mean square values of SOD, GPX, root As, shoot As, and TF of BARI Lentil-1, BARI Lentil-2, BARI Lentil-5, and BARI Lentil-6 seedlings as influenced by arsenic

Source of variation		Mean square value of				
	df	SOD	GPX	Root As	Shoot As	TF
Replication	2	889935	0.0000024	197	305	0.00234
Variety	3	8599486	0.000018	4491	3290	0.05486
Treatment	3	0.00000014	0.0055	237849	291624	1.25495
Variety × Treatment	9	418091	0.0033	2816	2347	0.03133
Error	30	216916	0.00018	640	317	0.00221