Gibberellic acid ameliorates cadmium induced morphological and anatomical variations in Barley (*Hordeum vulgare* L.)

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Abstract

Present study was designed to investigate the ameliorative effect of gibberellic acid under cadmium stress in barley (Hordeum vulgare L.) genotypes (Jou-17 and Haider). Barley genotypes (Jou-17 and Haider) showed great variations in morphological as well as in anatomical parameters under cadmium stress. A pot experiment was conducted in soil filled pots. Germinated seedlings were subjected to various levels of $CdCl_2$ *(control, 10 mM, 20 mM and 30 mM) with and without gibberellic acid (control, 0.05 mg/l). The results showed that application of gibberellic acid improved plant growth, shoot and root length, shoot and root fresh and dry weight when applied in combination with* $CdCl_2$*. Microscopic study of root and stem of both genotypes (Jou-17 and Haider) showed that gibberellic acid improved root and stem anatomy by improving epidermis and endodermis thickness, cortical cells, and vascular bundle cells area under cadmium stress. According to these findings, it is concluded that gibberellic acid has protective role on plant morphology, and root and stem anatomy under cadmium stress.*

Keywords: Gibberellic acid, Barley (Hordeum vulgare L.), Drought, Plant growth, Root anatomy, Stem anatomy

Introduction

Heavy metals are naturally occurring elements that can be toxic to plants in elevated concentrations. They are often present in soil and water due to both natural processes and human activities such as mining, industrial pollution, and the use of certain agricultural chemicals. Some common heavy metals that can be detrimental to plants include lead, cadmium, mercury, arsenic, and zinc (Rascio and Navari-Izzo, 2011). Heavy metals addition in agricultural soil is a common threat of the

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world (Ashraf *et al*., 2017). High concentration of metalloids and traces of metals caused soil pollution. The quantity of theses metals increased in the atmosphere due to rapidly expanding industrial development, coal combustion residues, wastewater for irrigation (Zhang *et al*., 2010), high use of pest and insect controlling medicines, mine tailing, disposal of high metal waste, paints, high use of fertilizers petrochemical spillage and atmospheric deposition (Akhtar *et al*. 2017; Kintlova *et al*. 2017). Traces of metals is toxic and poisonous event at very low level (Zulfiqar *et al*., 2019). Trace metals include elements such as cadmium (Cd), alumium (Al), lead (Pb), copper (Cu), iron (Fe), nikel (Ni), zinc (Zn), chromium (Cr), mercury (Hg), thallium (Tl), beryllium (Be) and arsenic (As) (Karimpour *et al*., 2018). Metal contaminants reduce the soil fertility, terminate the micro-organisms population and their natural processes.

Heavy metals stress is non biological stress cause many harmful effects on plants. Cadmium (Cd) can cause many physiological and morphological changes in plants. It badly affects the plant growth (shoot and root length), water uptake, respiration, photosynthesis, enzymes activities and metabolism of carbohydrates. High quantity of cadmium by entering the food chain impairs ecosystem as well as human health by direct ingestion of food, drinking polluted water, direct contact with polluted soil, reduction of land suitable for farming and reduce in food quality (Hassan *et al*., 2019; Hussain *et al*., 2021a). In non-tolerant plants, cadmium directly interferes with mitosis (Shi *et al*., 2014), nucleoli toxicity (Qin *et al*., 2013) and with various physio-metabolic processes.

Barley is a versatile and nutritious grain that has been used as a food source for thousands of years. It's known for its nutty flavor and chewy texture. It retains the bran, germ, and endosperm layers, making it the most nutritious option. It's commonly used in soups, stews, and salads (Baik and Ullrich, 2008). Plants have ability to decrease the Cd effect by activating their defense mechanism. Some higher plants like *Vicia faba*, *Allium cepa*, *Zea mays* and *Hordeum vulgare* provide a valuable genetic system for screening and monitoring pollutants in the environment (Andrioli *et al*., 2012). Plants lessen the heavy metal toxicity by binding to cell wall, by producing extracellular spaces and by regenerating damaged cell organelles. Plants activate mechanism of hormone synthesis which play an important role against abiotic stresses know as growth regulators (PGRs) (Wang *et al.*2018; Sharma *et al*. 2019). Phytohormones such as salicylic acid (SA), ethylene and gibberellic acid (GA_3) exhibit important role against Cd stress (Faraz *et* *al.* 2019). These PGRs contain many enzymes that improve plant evolution and development (Ma *et al*., 2009). Gibberellic acid (GA_3) is the most significant plant hormone. They control the stem elongation seed development, prevent leaf senescence, promote flowering, and disrupt the seed dormancy. Gibberellin is the most essential group of the plant hormone and very vigorous in nature. It has been reported Gibberellic acid declined the antagonistic effect of Cd stress (Masood and Khan2013; He *et al.* 2015). Gibberellins act as potent signaling molecules to enhance growth and developmental processes to cope with stress conditions and strengthen the immune system in plants (Colebrook *et al*., 2014: Saijo and Loo, 2020).

Cereals are mostly annual grass families with long stems like wheat, rice, rye, sorghum, barley, and maize. The word cereal derived from Latin word 'cerealis' its Botanical meaning 'grain' a sort of fruit called caryopsis. Barley (*Hordeum vulgare* L.*)* is the utmost cereal crop belongs to Poaceae (Bolechova *et al.,* 2015). Barley is used as dietary food for both human and animal throughout the world (Bchini *et al*., 2010; Kalai *et al*., 2013). It is the major source of dietary fiber, due to this fact barley has excessive interests to study from ancient times for human benefits (Kalai *et al*., 2013).

Materials and Methods

The experiment was conducted in Research Laboratory of Botany, Government College Women University Faisalabad. Two varieties of barley genotypes (Jou-17 and Haider) were obtained from Ayub Agriculture Research Institute of Faisalabad. Seeds were sown in soil and sand filled plastic pots and both genotypes were irrigated with half strength of Hoagland solution and corresponding with the application of different concentration of $CdCl_2$ (10 mM, 20 mM and 30 mM) and GA_3 (0.05 mg /l). After 30 days' morphological characters such as shoot and root length, shoot fresh and dry weight, and root fresh and dry weight were measured.

Stem and roots were collected for each treatment to study the various modifications in stem and root anatomical characteristics and to check the ameliorative effect of gibberellic acid under cadmium stress. Samples measuring 1.5 cm of the thickest stem and root were excised kept in Formalin acetic alcohol (FAA) (v/v ethyl alcohol 50% distilled water 30%formilin 10 % acetic acid 5%) for 48 h then transferred into acetic alcohol (v/v ethanol 75% acetic acid 25%) solution. Anatomical features of stem and root epidermis area, cortex thickness, cortical cell (length, width and area), and vascular tissues (xylem, and phloem) were recorded and subjected to statistical analysis by using ANOVA (costat software).

Results

Shoot and root length

Shoot and root length in both genotypes significantly reduced with the increase in Cd concentration as compared to control (Fig.1). Maximum reduction was observed in both shoot and root length at 30 mM Cd concentration but more reduction in root length was observed than shoot length. GA_3 application alone reduced the shoot length than root length as compared to control while GA_3 in combination with Cd enhanced the shoot and root length at all levels of Cd. Maximum improvement was observed in shoot than root length in Haider as compared to Jou-17. Haider was found more tolerant than Jou-17.

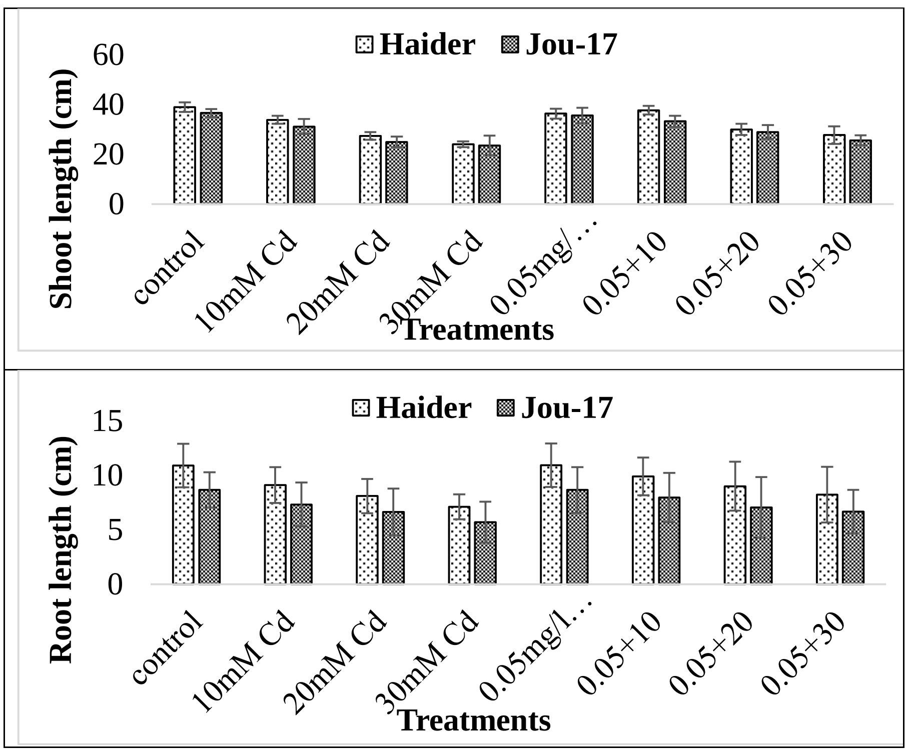


Fig. 1. Effect of GA3 alone and in combination with Cd on shoot length and root length in barley genotypes

Shoot and root fresh weight

Shoot and root fresh weight in both genotypes was gradually decreased as the concentration of Cd increased. Maximum reduction in root fresh weight at 30 mM Cd was observed than shoot fresh weight in both genotypes. Maximum reduction in root fresh weight was observed in Jou-17 as compared to Haider (Fig.2). Application of GA_3 alone showed not much improvement in shoot and root fresh weight as compared to control but in combination with Cd improved shoot and root fresh weight at all Cd levels. More improvement was shown in Haider than Jou-17. Haider showed more resistance against Cd stress than Jou-17.

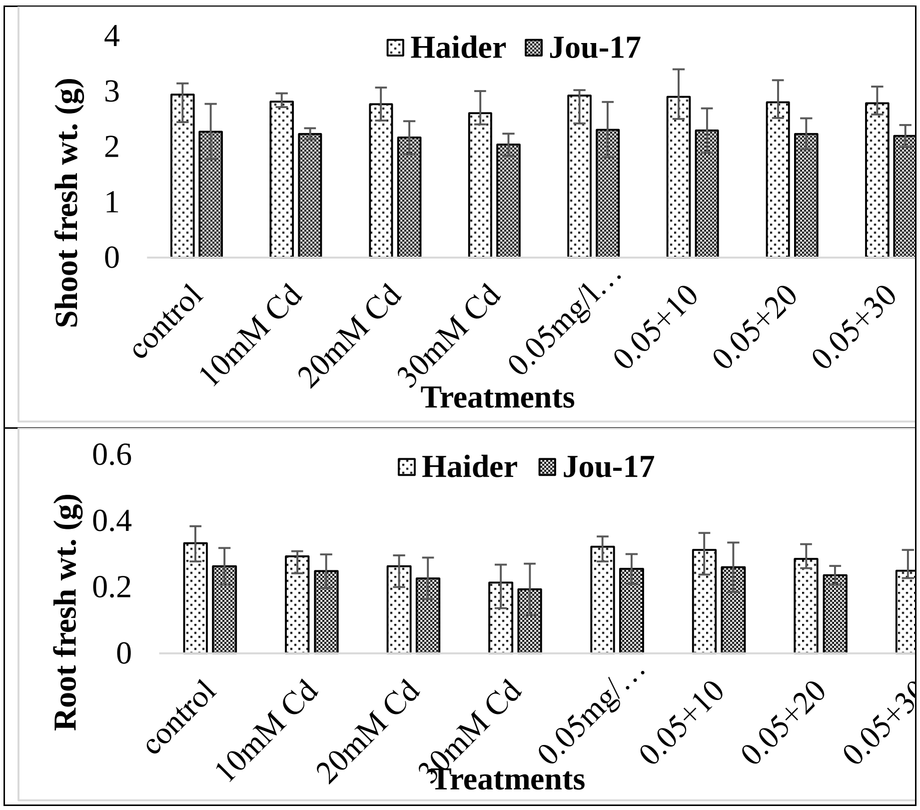


Fig. 2. Effect of GA3 alone and in combination with Cd on shoot fresh weight and root fresh weight in barley genotypes

Shoot and root dry weight

Significant reduction in shoot and root dry weight was found with the increase in Cd concentration. More reduction was observed in root dry weight as compared to shoot dry weight at 30 mM Cd in Jou-17 as compared to Haider. GA_3 reduced shoot and root dry weight as compared to control while in combination with Cd increased shoot and root dry weight at all Cd levels in both genotypes (Fig.3). Maximum increase in shoot dry weight was observed in Haider than Jou-17 at 10 mM Cd Jou-17 showed more sensitivity as compared to Haider.

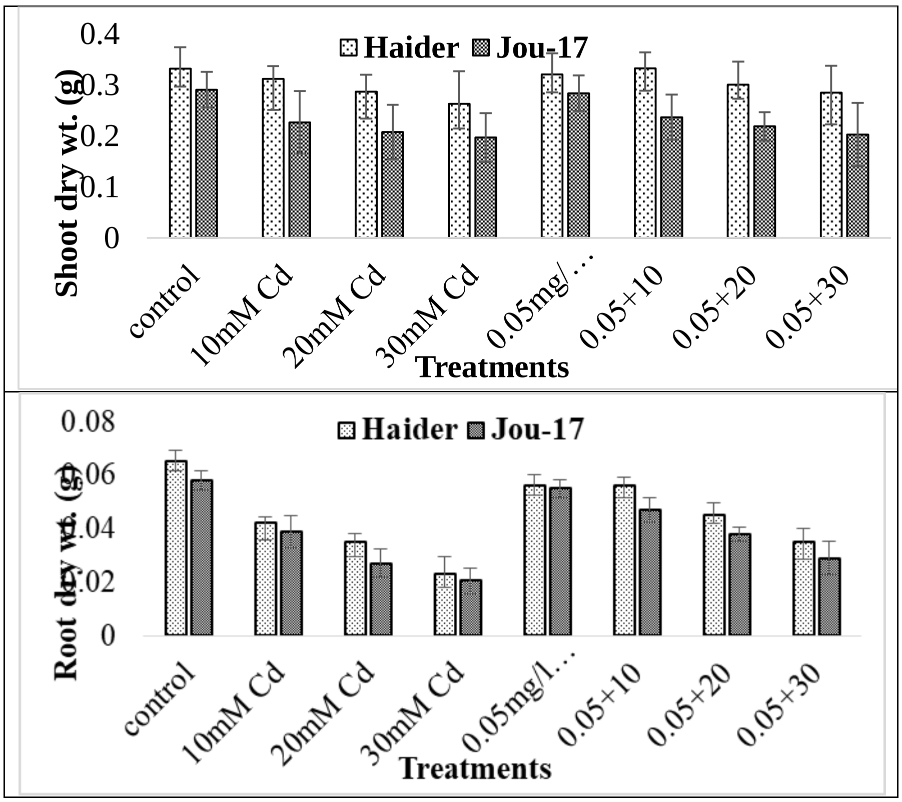


Fig. 3. Effect of GA3 alone and in combination with Cd on shoot dry weight and root dry weight in barley genotypes

Root anatomy

Upper epidermis and endodermis thickness decreased as the concentration of Cd increased. More reduction was observed at 30 mM as compared to 20 mM and 10 mM (Fig. 4). Cortical cells area and vascular bundle cell area of root was not affected by the addition of 10 mM Cd but as the concentration of Cd increased both cortical cell and bundle cell areas reduced but much reduction was observed at 30 mM. On the other hand, the metaxylem area and pith area was gradually decreased as the concentration of Cd increased (Fig.4). Application of GA_3 at concentration 0.05 mg/l showed maximum growth in both varieties alone and when applied in combination with Cd (Fig. 5). The application of GA_3 decline the adverse effect of Cd and improved root anatomy in both varieties but Haider showed better results as compared to Jou- 17.

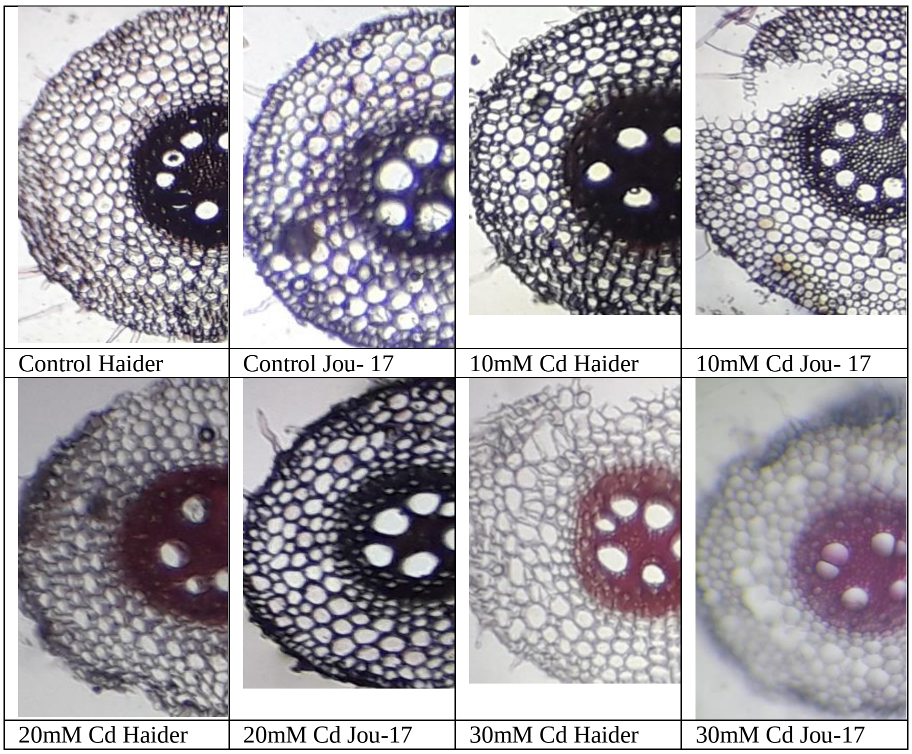
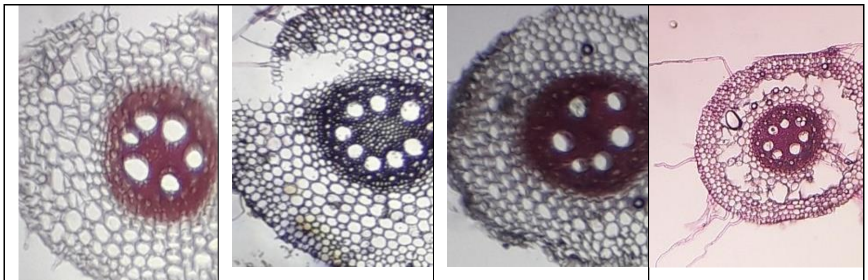


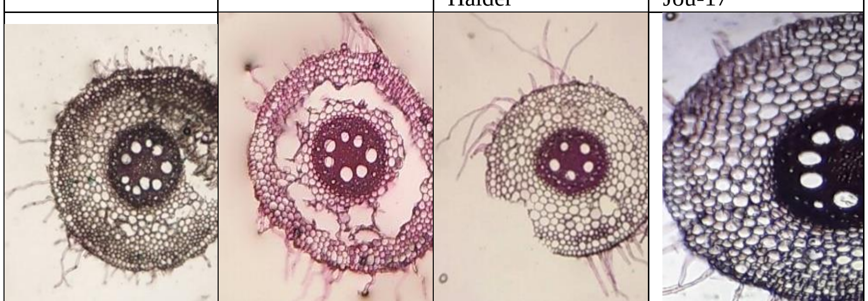
Fig. 4. Root anatomy of *Hordeum vulgare* L. of Haider and Jou- 17 at different levels of Cd.

Shoot Anatomy

Shoot epidermis thickness and cortex cell thickness was gradually decreased as the concentration of Cd increased at all levels 10 mM, 20 mM, and 30 mM. bundle cell area was increased at 10 mM Cd while it was reduced as the concentration increased but more reduction was observed at 30 mM (Fig. 6). application of GA_3 decline the adverse effect of Cd and developed the epidermis and cortex cell thickness alone and in combination with Cd. Maximum bundles cells area was observed at 0.05mg/l GA_3 (Fig. 7). It was observed that GA_3 reduced Cd adverse effect and maintain the bundles cells area. Both varieties were found affected as the concentration of Cd increased but Haider showed much tolerance than Jou- 17.



0.05mg/lGA_3 Haider	0.05mg/lGA_3 Jou-17	10mM Cd+0.05mg/lGA_3 Haider	10 mM Cd+0.05mg/lGA_3 Jou-17



Cd 20Mm+0.05 GA_3 Haider	Cd 20mM+0.05 Jou-17	Cd 30mM+0.05 GA_3 Haider	Cd 30mM+0.05 GA_3 Jou-17

Fig. 5. Root anatomy of *Hordeum vulgare* L. of Haider and Jou-17 at different levels of Cd and GA_3

Discussion

Cadmium (Cd) is a poisonous heavy metal known to affect human health and plant growth and development (Sanaei *et al*., 2020). It is the most active metal in the soil. Its excess disturbed metabolism and growth rate of plants. It also reduced yield in commercially important crops such as wheat, rice, maize and barley (Shanying et *al*., 2017). The present study revealed heavy metal stress decreased the shoot and root length, fresh and dry biomass in barley genotypes (*Hordeum vulgare* L.). Dealing with heavy metal stress, particularly for metals like cadmium (Cd) which can be toxic to plants, moreover, to overcome the toxic effect of metal GA3 can be applied (Rascio and Navari-Izzo, 2011) as this is focused in the present study. Cadmium is a toxic element in plants without any important physiological function (Baker and Whiting, 2002). The effect of GA_3 treatments on growth (shoot height, root length) of plants grown in Cd-contaminated soil is presented in Fig 1&2. The results demonstrated the effect of cadmium on the growth of plant and mitigate the effect of Cd when applying GA3. Similar effects of cadmium were reported by Haouari et al., 2012.

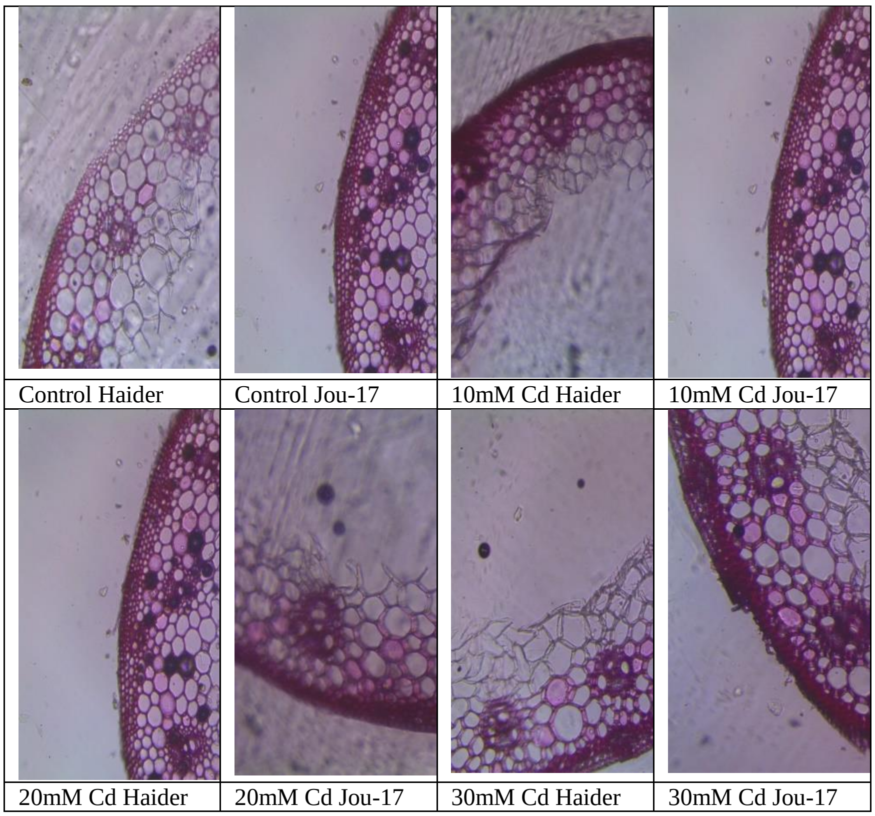


Fig. 6. Stem anatomy of *Hordeum vulgare* L. of Haider and Jou-17 at different levels of Cd

Our results showed that the root and shoot length were decreased as the concentration of Cd increased (Control >10mM >20mM >30mM). Maximum reduction was observed at 30mM Cd. Similar results were found that increased cadmium concentration also limit the growth in rice plants (Song *et al*., 2015). Reduction in shoot and root length might be due to reduced cell division in the cells (Kupper *et al*., 2002). Metal toxicity caused adverse effects on morphology of the plant also caused structural modification in the plants. This might be due to reduction in cell division and enlargement, nitrogen metabolism root elongation, damage of root tip and reduction in the formation of roots and in the uptake of mineral nutrients results in poor growth in plants and biomass (Gabbrielli *et al*., 1990).

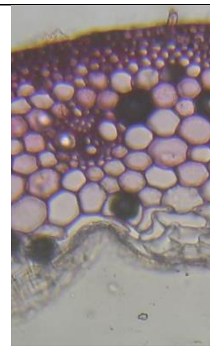
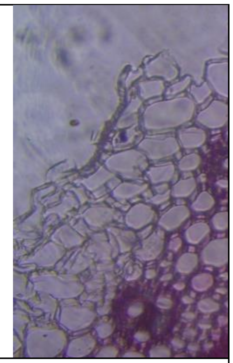
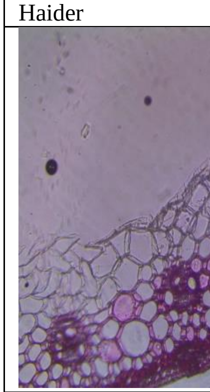
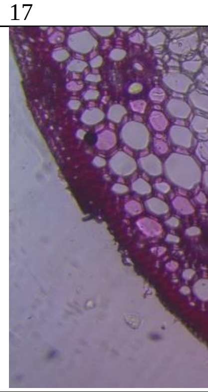
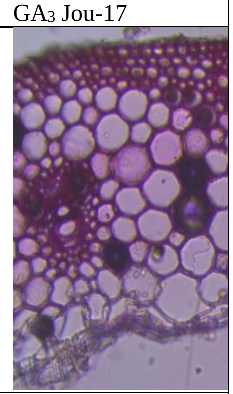
			
0.05mg/l GA_3 Haider	0.05mg/l GA_3 Jou-17	10mM +0.05mg/l GA_3 Haider	10mM+0.05mg/l GA_3 Jou-17
			
20mM +0.05mg/l GA_3 Haider	20mM+0.05mg/l GA_3 Jou-17	30mM +0.05mg/l GA_3 Haider	30mM+0.05mg/l GA_3 Jou-17

Fig. 7. Stem anatomy of *Hordeum vulgare* L. of Haider and Jou- 17 at different levels of Cd and GA_3

Application of GA_3 showed positive impact on plant growth and development under Cd stress. Similar findings were observed that application of GA_3 under Cd stress increased shoot and root length, and fresh and dry biomass in *Vigna radiata* L. plants (Hakla, *et al*., 2021). Both varieties showed different behavior under Cd stress, but more reduction was observed in Jou -17 as compared to Haider that is ultimately sensitive.

The results of recent study were found that the Cd had negative effect on barley shoot. Significant changes were observed in barley root and shoot (Zhao et al., 2000). The epidermis and cortical cell area was decreased as the concentration of Cd increased. Low concentration of Cd was remained in roots while the other was transferred into stem and others areal parts. Similar findings were observed by Sridar et al., 2007. Minimum Cd was retained in shoot of barley. Cd toxicity convert the normal cortical cells into the stellate cells. These cells are generally formed by the detachment of adjacent parenchymatous cells, which produced the air cavities. All levels of Cd decreased the size of metaxylem and phloem. Same was observed by Geitmann and Ortega in 2009.

The results of recent study was found that the cross sections of barley plants root. The epidermis and endodermis thickness of root was decreased as compared to the concentration of Cd was increased. Similar results were observed by Vaculik in 2012 in the two species of Salix. Epidermis is the first protection hurdle of the root against heavy metals entry into the plants. Endodermis is the second wall that protect the vascular bundles from toxic effect of heavy metals ions (Konotop *et al*., 2012). GA_3 improved epidermis and endodermis thickness in root. Endodermis thickness improved the water movement and nutrients uptake, which is most important processes that reduce the environment stress. Similar results were found in Maize plants (Zua et al., 2011). Cortical cell area was increased as the concentration of Cd increased in both varieties of barley (Haider, Jou- 17). The parenchyma cells produced more space that store water and nutrients. These parenchyma cells reduced the effect of Cd toxicity by dumping of metals ions (Gorigore and Toma., 2017). The lenght, width and area of vascular bundles was increased as the concentration of Cd increased. Vascular bundle area was increased that is the most important for the flow of water, as well as nutrient towards the other parts of plants (Baloch *et al.*, 1998).

Conclusion

The study reported that Gibberellic acid (GA) has a positive impact on barley (Hordeum vulgare L.) plants when exposed to cadmium-induced stress. The application of GA effectively ameliorates the adverse morphological and anatomical variations caused by cadmium toxicity. The research suggests that GA treatment could potentially serve as a promising strategy for mitigating the harmful effects of heavy metal stress on crop plants, such as barley, and enhancing their growth and development under such adverse conditions. However, further research is necessary to elaborate GA application on mechanisms involved in improvement of crop productivity environmental sustainability.

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