PERFORMANCE OF DIFFERENT BRASSICA SPECIES UNDER CADMIUM STRESS

SWEETY AKHTER

DEPARTMENT OF AGROFORESTRY AND ENVIRONMENTAL SCIENCE
SHER-E-BANGLA AGRICULURAL UNIVERSITY
DHAKA-1207

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BY

SWEETY AKHTER

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APPROVED BY:

Dr. Jubayer-Al-Mahmud
Associate Professor
Supervisor

Dr. Ferzana Islam
Professor
Co-supervisor

Dr. Jubayer-Al-Mahmud
Chairman
Examination committee

Dr. Jubayer-Al-Mahmud
Associate Professor
Department ofAgroforestry and
Environmental Science
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
Mobile: 01771-606735
E-mail: jamahmud_bd@yahoo.com

CERTIFICATE

*This is to certify that thesis entitled, **“Performance of different Brassica species under cadmium stress”**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** in**AGROFORESTRY AND ENVIRRONMENTAL SCIENCE**, embodies the result of a piece of bona fide research work carried out by **SweetyAkhter**, Registration No.:14-06061 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 been fully acknowledged by him.

Dated: June, 2021
Place: Dhaka, Bangladesh

(Dr. Jubayer-Al-Mahmud)
Associate Professor
Supervisor

DEDICATED

TO

MY BELOVED

FATHER-MAHTAB UDDIN

AND

MOTHER-SHAMSUN NAHER

LIST OF ABBREVIATIONS

Full form	Abbreviations	Full form	Abbreviations
Agro ecological zone	AEZ	Microgram	µ
Applied	App.	Milliequivalents	Meqs.
Agriculture	Agric.	Milligram(s)	Mg.
Bangladesh Agricultural Research Institute	BARI	Millimeter	Mm
Biology	Biol.	Metric ton	MT
Biotechnology	Biotechnol.	North	N
Cadmium	Cd	Review	Rev.
Cultivar	Cv.	Reactive oxygen species	ROS
Co –efficient of Variations	CV	Milli molar	Mm
Degrees of freedom	DF	Relative water content	RWC
Department	Dept.	Science	Sci.
Deoxyribo Nucleic Acid	DNA	Society	Soci.
East	E	Soil Plant Analysis Development	SPAD
Editors	Eds.	Soil Resource Development Institute	SRDI
Emulsifiable concentrate	EC	United states department of Agricuture	USDA
Environment	Environ.	Serial	Sl.
And others	et al.	Pollution	Pollut.
Food and Agriculture Organization	FAO	World Health Organization	WHO
International	Intl.	Water Use Efficiency	WUE
Journal	J.	Percentage	%
Kilogram	Kg.	Sum of Square	SS
Litre	L	Number	No.

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-Author

LIST OF CONTENTS

CONTENTS (CONT.)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

PERFORMANCE OF DIFFERENT BRASSICA SPECIES UNDER CADMIUM STRESS

ABSTRACT

Cadmium is a significant limiting factor in the production of oilseed crop in industrial area. A pot experiment was conducted in the Sher-e-Bangla Agricultural University from November 2019 to February 2020 to observe the performances of three *Brassica* species (*B. oleracea*, *B. campestris*, and *B. juncea*) seeds exposed to two levels of cadmium stress; mild and severe stress (2mM and 4mM $CdCl_2$) in a completely randomized design with three replications. The results revealed that cadmium had a significant negative impact on plant height, leaf number, siliqua length, seed per siliqua, SPAD value and seed weight of all tested Brassica species resulting in yield loss. Mild cadmium stress decreased yield of *B. oleraceae* (BARI Sharisha 17), *B. campestris* (BARI Sharisha 14) *and B. juncea* (BARI Sharisha 16) by 9.52%, 17.53% and 13.52%, respectively. Furthermore, severe cadmim stress decreased yield of *B. oleraceae*, *B. campestris and B. juncea* by 46.26%, 39.61% and 31.88%, respectively. So, under mild stress, BARI Sarisha 17 (*B. oleraceae*) is the best variety, while under severe stress conditions, BARI Sarisha 16 (*B. juncea*) is the best variety.

CHAPTER 1

INTRODUCTION

Heavy metal stress has been increase at an alarming rate due to industrialization and it causes serious environmental problems. Agricultural soils worldwide are slightly to moderately contaminated with toxic heavy metals that restrict the crop plants to reach their full genetic potential and cause significant loss by reducing the crop productivity (Yadav, 2010). Cadmium (Cd) is a hazardous metal that has become a major environmental contaminant due to its effects on plant growth and human health (Nouairi *et al*., 2009; Hasanuzzaman *et al*., 2013). Chlorosis, necrosis, leaf rolling, root growth inhibition, and stunted plant growth are all symptoms of plants cultivated in Cd-rich soil. Cd also influenced stomatal function, lowered water potential, cation efflux, membrane functions, photosynthesis suppression, metabolism, and the activity of several important enzymes, as well as causing death(Sharma and Dubey, 2007; Gill and Tetuja, 2011; Hasanuzzaman *and Fujita*, 2013).

Due to its enormous release as a byproduct from industry, cadmium is the most destructive soil contaminant of the various heavy metals. It's a non-essential and possibly hazardous metal that lowers dry matter and seed yields (Mediouni *et al*., 2006). Cadmium is the most harmful soil contaminant since it is released in large quantities as a consequence of industry. It is a nonessential and potentially toxic metal, into different plant parts (Epstein and Bloom, 2005). Increased cadmium levels harm photosynthetic systems severely.

The amount of Cd accumulated varies substantially between plant species and cultivars. The root accumulates more Cd than the shoot. Plants undergo significant physiological, metabolic, and genetic alterations as a result of Cd accumulation. Excess Cd generates free radicals and reactive oxygen species (ROS), which can damage proteins, lipids, DNA, and carbohydrates in plants, disrupting some physical and biological processes.

Mustard and rapeseed (*Brassica* spp) are one of the most important oil seed crops throughout the world after soybean and groundnut (FAO, 2004). In Bangladesh, there is a high need for edible oil. Mustard and rapeseed tops the list among the oil seed crops grown in this country in respect of both production and acreage (BBS, 2004).

Mustard and rapeseed contain antioxidants and other beneficial plant compounds thought to help protect our body against damage and disease. For instance it's a great source of glucotinase, a group of sulfur containing compounds found in all cruciferous vegetables, including broccoli, cabbage, mustard and oilseed crops.

Earlier studies revealed that mustard and rapeseed varieties are less resistant to Cd toxicity than cereals and grasses and encounter severe suppression of biomass production even at very low levels of Cd. Though, compared with other species, little information is available concerning the ability of tolerance and accumulation in mustard and rapeseed varieties under Cd stress. Therefore, the present work has been conducted to screen the mustard varieties under Cd stress.

The main objectives of the present experiment include:

(1) To understand the effect of Cd on growth performance of different mustard and rapeseed varieties,

(2) To understand the effect of Cd on the yield of different mustard and rapeseed varieties, and

(3) To screen Cd-tolerant and non-tolerant mustard and rapeseed variety (ies).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Mustard and Rapeseed

Mustard and rapeseed is belonging to the family Brassicacae (or Cruciferae) are important oil crops and currently ranked as the world's third important oil crop in terms of production and area. Among the species, *Brassica oleracea* and *Brassica campestris* are regarded as 'rapeseed' while *Brassica juncea* is regarded as 'mustard'. In Bangladesh, rapeseed and mustard are the most important among all oilseed crops. Total cultivated area under rapeseed and mustard cultivation is 0.234 million tonnes of oil per year (BARI, 2011). It is a good source of oil. The oil content in rapeseed and mustard is 40-44 and 40% and Oilcake of rapeseed and mustard contains 40% protein (Hasanuzzaman *et al.*, 2009). Globally, India account for 19.8 % and 9.8% of the total acreage and production (USDA). Increasing contamination and higher enrichment ratio of non-essential heavy metal cadmium (Cd) induce various toxic responses in plants when accumulated above the threshold level. These effects and growth responses are genotype and Cd level dependent (Irfan *et al.*,2014).

2.2 Abiotic stress

A lot of challenges is being faced by world agriculture like producing 70% more food for an additional 9.7 billion people in world by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change (Wilmoth, 2015). The productivity of different crops is not increasing with the food requirement. In most of the cases different abiotic stresses are responsible for the lower productivity. A major area of concern to cope with the increasing food requirements is reducing crop losses due to various environmental stresses (Shanker and Venkateswarlu, 2011). Gradual changes of global climatic conditions adversely affect our natural environment and produced different abiotic stress for crop production (Mittler and Blumwald, 2010). Various abiotic stress in plants caused by higher concentrated toxic substances. Sometimes much water (flood), shortage of water (drought) and too much fertilizer occured abiotic stress. Abiotic stresses change the plant metabolisms which are affect plant growth, development and productivity. Due to higher stress condition intolerable metabolic activities occur in plant cells and reducing plant growth, at extreme cases plants may die (Hasanuzzaman *et al.*, 2012a, b).

Besides reducing crop productivity abiotic stress influence the distribution of different plant species in different types of area and environment in worldwide (Araus *et al.*, 2002). The period of climate change, plants have continuously endured from environmental adversity which inhibits them from reaching and completing their full genetic potential and limits crop productivity worldwide (Hasanuzzaman *et al.*, 2009, 2010a, b; Hasanuzzaman and Fujita, 2013; Hasanuzzaman *et al.*, 2011a, b; 2012a–c; 2013a–d). Abiotic stress changes soil-plant atmosphere that reduced productivity of different major crop in various parts of the world (Ahmad and Prasad, 2012). Industrial waste materials are created abiotic stress by water and soil pollution with deposition of heavy metals. This heavy metal present in rivers, estuaries, near shore waters, and marine sediments because of the discharge in industrial activities (Mangal *et al.*, 2016). These stress produce harmful chemical compound in plants called reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), superoxide radical (O2-), hydroxyl radical (OH-), etc. (Choudhury *et al.*, 2013).

Abiotic stresses are a major decisive factor in crop and forage productivity (Boyer, 1982), and also influences the differential ordination of the plant species. (Chaves *et al.*, 2003). Now a-days climate change is a major problem which increases abiotic stress on a global scale, so adaptation strategies need to be established for crops to specific environments (Beebe *et al.*, 2011). Higher temperature also can create abiotic stress by accelerate mineralization of soil organic matter, making soil confines more intense, can limit root penetration into soil and plant development, further intensifying the up shots unfavorable climate (Beebe *et al*., 2013). Different stress factors inter relate with each other will probably increase damage to crop yields (Beebe, 2012; Yang *et al*., 2013).

Primary processes of plant such as photosynthesis, cell growth are affected by Abiotic stress. Abiotic stress such as water scarcity on carbon metabolism results in changes in the pool of sugars used for signaling cellular processes (Liu *et al*., 2013). Liu *et al*.(2004) reported that reduction of carbohydrate flux from leaves to pods, composed with reduced hexose to sucrose ratio in drought-stressed in pods of soybean are suggested as probable factors contributing to pod abortion. Mishra *et al.* (2011) reported that plants those are growing in environmental stresses condition rises lipid per-oxidation (degradation) and protein oxidation. Flexas and Medrano (2002) reported that in severe water deficit condition Ribulose-1,5- bisphosphate (RuBP) production and Rubisco carboxylation efficiency were both decreased.

Environmental stress such as energetic short wavelength ultraviolet (UV) photon which comes from sunlight is harmful for amino acids of essential proteins. Amassing of phytotoxic metals such as Cd, Zn, Cu are mass contaminants causing in retarded growth, chlorosis and necrosis (Oncel *et al.*, 2000). Heavy metals such as cadmium treatments in mung bean seedlings decline the levels of germinating by bringing of lipo oxgenase with the inhibition of the anti oxidative enzyme SOD and CAT (Somashekaraiah *et al.*, 1992).

Salinity stress is one of the major abiotic stresses that lessens the relative water content (RWC), at the rate of 100mM NaCl treatment in plants decrease RWC at 20% and also 10% chlorophyll content (Sheokand *et al.*, 2008). Weggler *et al.* (2000) found that when plants were grown in higher NaCl content soil, Cd uptake was increased. Muhling and Lauchli (2003) described that Cd and NaCl stress in combination results greater plasma membrane penetrability and increase the production of oxygen radicals and H_2O_2 in plants.

Many researchers estimated the crop reduces due to abiotic stress. According to Bray *et al.* (2000), In worldwide, abiotic stress reduced more than 50% yield on an average. According to report of Thakur *et al.*(2010) yield loss and lessen of biomass production of staple food crops up to 70%. It is challenging to understand the abiotic stress response in plants for its complexity, inter relationship, and variability of mechanisms (Patakas, 2012).

2.3 Cadmium stress

The heavy metal contamination in soil and water is a worldwide problem due to its harmful impacts on plants. (Nagajyoti *et al.*, 2012; Monteiro *et al.*, 2012). Cadmium is one of the most hazardous carcinogenic elements and it is produced by anthropogenic activity. It moves easily to the food chain through soil to plant root immersion and stores an appreciable amount in the living body. In terms of toxicity to plants and human, Cd is one of the most noxious heavy metals (Dong *et al.*, 2006; Li *et al.*, 2014) because of its high water solubility, relative mobility, and long half- life in living organisms (Juang *et al.*, 2012; Wu *et al.*, 2014). Atmospheric deposition is the major source of Cd in agricultural soils. Cadmium (Cd) is a toxic heavy metal usually present in rivers, estuaries, near shore waters, and marine sediments through the discharge of Cd compounds in industrial activities (Yuan *et al.*, 2004; Mangal *et al.*, 2016). The water-born cadmium level increases seriously may reach 1 mg/L (9 μM) (Ma *et al.*, 2008). It is well known that important heavy metals pretense threats to soil quality and human health. Cadmium is used for a wide range of industrial, urban, and agricultural applications. Higher concentration of heavy metal in may adversely affect crop growth. It may also affect physiological and biochemical activities of crop (Atafar *et al.*, 2010). Heavy metal may also alter the soil microbial community. In Bangladesh many rivers and land areas are polluted by heavy metals. Those rivers and land areas are present beside different industries (soap and detergent), garments, pharmaceuticals, dyeing, aluminum, carbide, match and ink manufacturing, textile pain, paper pulp and bar factories, steel workshop etc. (Rahman *et al.*, 2012). These types of industries are present beside some rivers such Turag, Buriganga, Sitalakkha, Dhaleshwari in Bangladesh.

Generally, the allocation of different heavy metals in soil is influenced by the nature of parent materials, climatic conditions and their relative mobility (Mohiuddin *et al.*,2011). Some important properties of soil such as pH and organic carbon governance the accumulation of heavy metals in soil. Highly toxic heavy metal pollutant Cadmium classified as human carcinogen (Henkel and Krebs, 2004). Excessive exposure to Cd^{2+} can lead to 'itai-itai' disease and it affects cardiovascular system (Shah and Nahakpam, 2012). The unceasing release of cadmium from different industries such as paint, batteries and jewelry and the low permissible limit (0.01 mg/L) state significant threat to the environment and human health (Nawrot *et al.*, 2010; Eichler *et al.*, 2014).

Various researches were conducted on availability of cadmium diverse area in Bangladesh was found 0.8 μg -7 μg per gram of soil. Cadmium concentration is increasing in our crop field gradually. Heavy metal pollution of aquatic system is increasing at an alarming rate due to anthropogenic activity (Malik *et al.*, 2010). Even at trace levels heavy metals like Cr, Pb, Cd, As etc. exhibit high toxicity. In Bangladesh most of the industries are present at the bank of the river, those industries are drainage their waste material in the river water. In 9th January 2017, daily newspaper ProthomAlo reported that riverside industries mainly leather industry deposits 11 items of different heavy metal in river. Rivers are a main pathway for metals transport in cultivable land (Miller *et al.*, 2003) .Cadmium outflow in soil depending on the source (Hasanuzzaman and Fujita, 2012a).

Cadmium is a toxic contaminant that can be taken up by plant roots and gathered into the xylem of the leaves. Cadmium inhibit the plant growth, changes the photosynthesis rate (Benavides *et al*., 2005). Cadmium can easily shifted to the food chain and emphasize a threat to human health (Clemens, 2006). The FAO/WHO mentioned maximum tolerable rate of Cd is 400–500 μg week−1 or 70 μg d^{-1}. Cadmium is a non-essential element for plants. It is the fifth most toxic metal to vertebrates, the fourth most toxic metal to vascular plants. It is supposed that the main reason for accumulation of Cd in crop field is successive given of fertilizers and agrochemicals for long period of time in agricultural land. Crop cultivation in cadmium polluted soil may also reduce water and nutrient uptake (Li *et al*., 2008), and causes chlorosis and necrosis of the leaves. The most target sites of cadmium is photosynthetic apparatus of crop and inhibits biosynthesis of photosynthetic pigments, can decrease electron transport efficiency, reduces photosynthetic carbon assimilation, and causes oxidative damage to sub-organelles (Maksymiec *et al*., 2007). Cadmium induced abnormal seed germination, reduced growth, disorganized development of reproductive organs and reduced yield (Gill and Tuteja, 2011). Also cadmium hindered the seed germination rate, root elongation, shoot elongation, and seedling growth of wheat (*Triticum aestivum*) (Chen *et al.*, 2010). Shekar *et al.* (2011) reported seedling survival percentage of beans was gradually reduced from the control as Cd stress levels increased. The survival percentage of seedlings in the control and in Cd 50, 100, 200, 300, and 400 mg kg^{-1} soil samples was 89.0 and 83.0, 76.0, 70.0, 62.0, and 54.0% respectively. Tomato plants growth were inhibited when there was 10 μM of cadmium solution in nutrient media. Therefore the main toxicity symptoms were chlorosis of leaves, reduced length and the browning of shoots. (Cherif *et al.*, 2011). Asgher *et al.* (2014) also stated that plant growth reduction was corelated to Cd-mediated reduction in the maximum photochemical efficiency of photosystem II (PS II), enhanced impairments in the net CO_2 assimilation rate and reduced ribulose 1,5-bisphosphate carboxylase (Rubisco) activity.

Gill and Tuteja (2010); Anjum *et al.* (2012) stated that metals/metalloids can be prompted the formation of ROS and an influential inducers of lipid per-oxidation in plants. Redox active metals (such as Cu, Cr, and Fe) can cause lipid peroxidation by producing ROS in redox cycling. However, redox inactive metals (such as Cd, As, Co, Hg, Al, Ni, Pb, Se, Zn etc.) fetch significant damage in antioxidant defense components such as thiol-containing antioxidants and enzymes. Many studies showed that by inducing lipid peroxidation Cd strongly altered the function of membranes and troubled in chloroplast metabolism through inhibiting chlorophyll biosynthesis and reducing the enzyme activity which is related in CO_2 fixation. (Cuypers *et al.*, 2011; Gallego *et al*., 2012; Gill *et al.*, 2013).Under Cd exposure different levels of lipid per-oxidation occur in different organs of the same plant. For example, Talukdar (2012) showed that MDA (a lipid per-oxidation product) accumulation was more marked in shoots than in roots of the Cd exposed lentil (*Lens culinaris*) seedlings. Stohs and Bagchi (1995) seen that some metals such as Cd, Pb, and Hg exhausted the protein bound thiol groups.

Bansal *et al.* (2002) stated that Cd also deter mitochondrial enzymes, such as α-keto-glutarate, iso-citrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase. Dias *et al.* (2013) stated that Cd-arbitrated disturbance in the coordination between carbon (C), nitrogen (N) and sulfur (S) metabolism in plant cells.

2.4 Effect of Cadmium on mustard and rapeseed varieties

Hassanuzzaman *et al.*(2019) showed that three *Brassica* species gathered Cd in their shoots and roots as a result of Cd exposure and the accumulation increased when stress level is increased. *Brassica juncea* gathered more Cd in its shoots and roots than *B. campestris*and *B. napus.* They also showed that Fresh weight and dry weight of all three *Brassica* species reduced under Cd stress in a dose-dependent manner.

Aidid and Okamoto (1993) showed that the reduction in the growth of *B. juncea* could be also due to the destruction of the elongation growth rate of cells, because of an unalterable inhibition applied by Cd on the proton pump responsible for the process. Ahmad *et al.*(2015) showed that the presence of heavy metals, like cadmium has been reported to decrease the amount of oil produced by *Brassica juncea.* Hernandez-Allica *et al.* (1997) made wide study regarding the heavy metal tolerance of different species (including several varieties of *B. campestris*, *B. rapa*, *B. napus*, *B. oleracea* and *B. carinata*, endorsing that they have high levels of tolerance mainly to Zn, and less to Pb and Cd.

Excessive Cd accumulation in soil plant interface resulted in its entry into the food chain. Mahmud showed that the increased Cd levels disturbed the plant metabolisms and reduced the key growth traits of *Brassica juncea* L. (Mahmud *et al*. 2019). The exposure of increasing Cd concentrations reported a reduction in biomass production, light harvesting pigments, leaf water levels, whereas induced the H_2O_2, MDA, proline, lipoxygenase activity, and MG contents in the tissues of *Brassica* species (*B. napus*, *B. campestris,* and *B. juncea*) in dose dependent manner (Mahmud *et al*.,2019). Previous findings also observed that excessive Cd levels stopped the root elongation, reduced the antioxidant defense system, Increased the oxidative stress induced by ROS and impaired the ultrastructure in root tip cells of *B. napus* L. (Ali *et al*.,2013).

Theriappan *et al.*(2011) showed that Heavy metals (Cd, Zn, and Hg) had been found to reduce the root and shoot lengths of *B. oleracea* var. Differences in growth performances were observed in 10 different cultivars of *B. juncea* grown under different Cd concentrations. (Qadir, 2003).

Few studies report yield increased with very low concentration of metals (Breckle *et al.*1991), a significant decline in biomass after exposure to metal stress was observed by Anjum *et al.* (2008) in *B. napus* (rapeseed) and *B.*

juncea (John *et al*.,2009) plants at different stages of growth. A noticeable reduction was observed in the number of siliqua per plant, number of seeds per siliqua, seeds per plant, and seed weight per plant in *B. napus* due to sewage water treatment containing Pb, Cd, and Cr (Ahmad *et al*.,2011).

2.5 Photosynthetic variation and yield attributes of mustard varieties against cadmium phytotoxicity

Brassica juncea[L] Czern. And Coss. (Family: Brassicaceae) an important oil crop is used as green vegetable and condiment. The species of *B. juncea* (mustard) are eminent heavy metal accumulators, particularly cadmium. Some research showed that the higher level of cadmium contamination makes toxic responses in mustards plants based on genotypic alterations in uptake and distribution (An,2004;Arao*et al.*, 2003; Page and Feller, 2015;Zhang *et al.*, 2009). To name amongst are root efficiency of cadmium preservation, cadmium-efflux rate, its necessary to extracellular matrix, cellular reclamation and complexation, and guideline of cadmium transport to photosynthetically lively aerial parts (Irfan *et al*., 2013; Marshner, 2012; Pérez-Chaca *et al*., 2014; Tanwir *et al*., 2015). The haphazard use of phosphate fertilizers, sewage slush wastes, and waste water in India has added adequately high level of cadmium to agricultural soil (Radha *et al*. 2014; Wuana and Okieimen, 2011). Cadmium contends against root immersion of nutrients; persuade secondary drought symptoms and cellular toxicity responses (Irfan *et al*., 2013; Tkalec *et al*., 2014). Cadmium-induced disease in mineral endorsement seriously affects the commotion of carbonic anhydrase (CA), chlorophyll content, and photosynthetic response. The plants, therefore, accumulate lesser dry mass of cultivar which results into decreased plant growth and suboptimal yield output.

Moreover, cadmium is an effective inhibitor of photosynthetic features (Krupa, 1999; Mohamed *et al*., 2012) as it stores in the aerial photosynthetic parts to interfere with chloroplast running (Babula *et al*, 2012) and Calvin cycle enzymes. Barcelo and Poschendrieder showed that stomatal resistance is induced by cadmium (Barceló and Poschenrieder, 1990) that bounds the internal CO_2 (López-Climent *et al.*, 2011) and carbon fixation to decrease net photosynthetic rate (Ekmekçi *et al.*,2008; Mohamed *et al.*, 2012). Cadmium and other heavy metals facilitated damage of photosynthetic apparatus includes light harvesting complex II and the damage of maximum quantum yield of PSII (Mysliwa-Kurdziel *et al.*,2012; Siedlecka *et al.*,1997). Ghani mentioned that reduced photosynthetic efficiency concurrently led to decline allocation of photosynthates toward sink to conciliation fruit yield (Ghani, 2010; Patel *et al.*,1980). Mohamed also showed that Cadmium also decreased the synthesis and level of photosynthetic pigments (Ekmekçi *et al.*, 2008; Mohamed *et al.*, 2012; Stobart *et al.*,1985). Stobart mentioned that Heavy metals like cadmium and lead also decrease the synthesis of 5-aminolavulinic acid and proto chlorophyllide reductase complex (Mysliwa-Kurdziel *et al.*,2012; Pérez-Chaca *et al.*, 2014; Stobart *et al.*, 1985). Therefore, multiple factors cumulatively reduce chlorophyll content (Gadallah, 1995; Ushaand Mukherji, 1992).

Escudero-Almanza *et al.*, (2012) also mentioned that Cadmium-induced inhibition of root Zn uptake seriously distresses photosynthesis and other physiological aspects of plant. Redox active metals at low concentrations escalate mitochondrial ROS production via Fenton and Haber–Weiss reactions and respiratory electron chain inhibition (Keunen *et al.*, 2011; Pérez-Chaca *et al.*, 2014).

CHAPTER 3

MATERIALS AND METHODS

This chapter shows a short description about experimental period, site description, climatic condition, crop or planting materials, treatments, experimental design and layout, crop growing procedure, fertilizer application, uprooting of seedlings, intercultural operations, data collection and statistical analysis.

3.1 Location

This experiment was conducted in the Field laboratory of Agroforestry and Environmental Science Department, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from October 2019 to February 2020. Location of the site is 23°74'N latitude and 90°35'E longitude with an elevation of 8 meter from sea level (Islam, 2014; Laylin, 2014) in Agro-ecological zone of "Madhupur Tract" (AEZ-28) (Anonymous, 1988). The experimental site is shown in the map of AEZ of Bangladesh in [Appendix 1].

3.2 Soil

The dirt in the test location came from the Modhupur tract (AEZ No. 28). It was a medium-high land with dark grey non-calciferous soil. The pH value of the soil was 5.7. The physical and chemical properties of the experimental soil have been shown in Appendix 3.

3.3 Climate

The experimental area has sub-tropical climate characterized by heavy rainfall during May to September and scantly rainfall during rest of the year. The annual precipitation of the site is 2152 mm and potential evapotranspiration is 1297 mm, the average maximum temperature is 30.3^0C and average minimum temperature is 21^0C. The average mean temperature is 25.8^0C. The experiment was carried out during rabi season, 2019-2020. Temperature during the cropping period ranged from 20^0C to 29.2^0C. The humidity varied from 61.72% to 70.45%. The day length was reduced to 10.5-11.0 hours only and there was no rainfall from the beginning of the experiment to harvesting. The monthly average temperature, humidity and rainfall of the site during the experimental work are enclosed in Appendix 2.

3.4 Materials

3.4.1 Plant materials

BARI Sarisha14 (*B. campestris*), BARI Sarisha 16(*B. juncea*), and, BARI Sarisha 17(*B. oleracea*) was used in the experiment. Feature of these varieties are given below:

BARI Sarisa 17(V_1): Short duration crop (duration 82-86 days), plant height 95-97 cm, plant don't lodge, pod/plant 60-65, seed/pod 28-30, flower and seed color yellow, because of yellow seed color comparatively 3-4% oil is greater than brown color seed usually. 1000 seed weight 3-3.4 g. Developed by Bangladesh Agricultural Research Institute.

BARI Sarisa 14(V_2): Short duration variety, plant height 75-85 cm, leaf light green, smooth, siliqua/plant 80-102, two chambers are present in pod but as like as four chambers. Developed by Bangladesh Agiricultural Research Institute.

BARI Sarisa 16(V_3): Late planting potential, plant height 175-195 cm, siliqua/plant 180-200, two chamber are present in pod, seed/siliqua 9-11, seed color pink, 1000 seed weight 4.7-4.9 g, crop duration 105-115 days. Developed by Bangladesh Agricultural Research Institute.

3.4.2 Earthen pot

Empty earthen pots with 18 inch depth were used for the experiment. Each container was filled with 12 kilograms of sun-dried soil. After that, pots were prepared for seed sowing.

3.5 Cadmium treatment

The cadmium treatments were mixed with the soil before seed sowing. There were three cadmium levels including control developed by adding respective amount $CdCl_2$ to the soil pot^{-1} as water dissolved solution. The cadmium levels were C (control), Cd (2mM) and Cd (4mM). When no cadmium added it termed as control (C).

3.6 Treatments

The experiment consisted of twofactor as mentioned below:

a) Total number of treatments: 03

i. Control (No cadmium)

ii. 2.00 mM $CdCl_2$

iii. 4.00 mM $CdCl_2$

b)Total number of variety: 03

- BARI Sarisa 17
- BARI Sarisa 14
- BARI Sarisa 16

c) Total number of replications: 03

d) Total number of treatments: 27

3.7 Design and layout

The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. There were all together 27 pots in the experiment. The layout is given below:

R_1	**R_2**	**R_3**
Cd_0V_1	**Cd_2V_1**	**Cd_4V_3**
Cd_2V_1	**Cd_0V_1**	**Cd_0V_3**
Cd_4V_1	**Cd_0V_2**	**Cd_2V_3**
Cd_0V_2	**Cd_4V_1**	**Cd_2V_2**
Cd_2V_2	**Cd_4V_2**	**Cd_4V_2**
Cd_4V_2	**Cd_2V_2**	**Cd_4V_1**
Cd_0V_3	**Cd_2V_3**	**Cd_0V_2**
Cd_2V_3	**Cd_0V_3**	**Cd_0V_1**
Cd_4V_3	**Cd_4V_3**	**Cd_2V_1**

3.8 Seed collection

Seeds of BARI Sarisa14, BARI Sarisa16, BARI Sarisa17 were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

Plate 1. Soil preparation

3.9 Pot Preparation

The collected soil was sun dried, creased sand sieved. The soil, cowdung and fertilizers were mixed well before placing the soils in the pots. Each pot was filled up with 14 kg soil. Pots were placed at the field of Sher-e-Bangla

Agricultural University. The pots were pre-labeled for each treatment. Finally, water was added to bring soil water level to field capacity.

3.10 Fertilizer Application

For pot experiment 27 pot requires 500gm of Triple superphosphate (TSP), 300gm of Muriate of Potus (MP), 500gm of Gypsum, 15gm of Zinc Sulphate, 30gm of Boric Acid, 10kg of Cowdung respectively. Full amount of TSP, MP, Gypsum, Zinc Sulphate, Boric Acid and Cowdung were applied at the time final pot preparation.

3.11 Sowing of seeds in seedbed

Seeds were sown three varieties BARI Sarisa-14, BARI Sarisa-16 and BARI Sarisa-17 on 11 November 2019 by hand as uniform as possible in the 27 pots. After sowing the seeds were covered with soil and slightly pressed by hand. Plant population was kept initially about 25-30 per pot.

3.12 Weeding and thinning

Weeds of different types were controlled physically for the first time and removed from the pot on 26th November 2019. At the same time first thinning was done. The final weeding and thinning were done after 24 days of sowing, on 5th December 2019. Care was taken to maintain continuous plant population per pot.

Plate 2. Weeding, Thinning and Tagging

3.13 Irrigation

Irrigation was done at every three days interval in all growth and reproductive stages.

3.14 Harvesting and threshing

The crop was harvested pot wise when 90% siliqua were matured. After collecting sample plants, harvesting was done on 10th February 2020. The harvested plants were tied into bundles and carried to the threshing floor. The plants were sun dried by spreading the bundles on the threshing floor. The seeds were separated from the stover by beating the bundles with bamboo sticks. Per pot yields of seed and straw were recorded after drying the plants in the sun followed by threshing and cleaning. At harvest, seed yield was recorded pot wise and expressed on hectare basis.

3.15 Collection of experimental data

Eight (8) plants from each pot were selected at random at harvest stage and were tagged for the data collection. The sample plants were displaced prior to harvest and dried accurately in the sun. The seed yield and stover yield per pot were recorded after cleaning and drying those accurately in the sun. Data were collected on the following parameters:

1) Plant height (cm)
2) Root length (cm)
3) Number of leaves plant^{-1}
4) Number of pods plant^{-1}
5) Number of seeds pod^{-1}
6) Pod length, weight
7) Weight of 1000 seeds (gm)

3.16 Statistical analysis

Collected data were statistically analyzed using Statistix 10 software. Mean for every treatments were calculated and analysis of variance and difference between treatments was assessed by Least Significant Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

Plate 3. Data collection

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Plant height

4.1.1 Plant height at 25 DAS

In this study maximum plant height at 25 DAS observed in V_3 variety (19.47cm) and minimum plant height observed in V_2 variety (13.00cm). Cadmium stress resulted in a considerable reduction in the height of all *Brassica* species studied as the cadmium level rises. In mild stress cadmium (Cd_2), plant height decreased the most in the V_1 variety (9.6%), which is significantly different from control, and the least in the V_3 variety (3.44%) compared to control, which showed non-significant variation from control. In severe stress cadmium (Cd_4), plant height decreased the most in the V_1 variety (23.62%) and the least in the V_3 variety (4.82%) when compared to control (Figure 2).

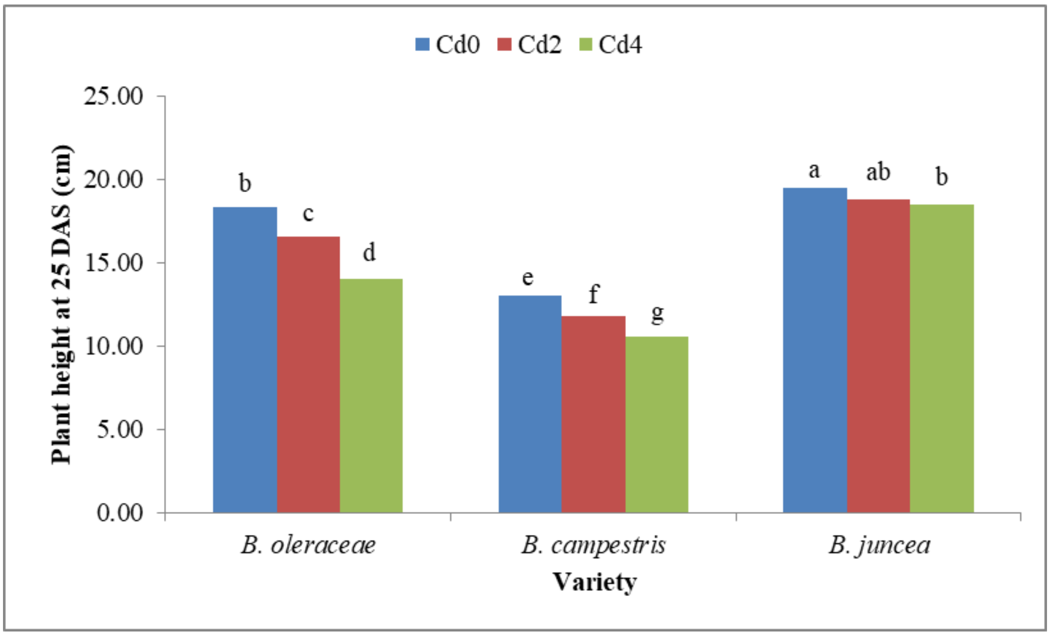

Figure 1. Effect of cadmium stress on plant heightof different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each

treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test.

4.1.2 Plant height at 35 DAS

In our research, the V_3 variety (50.33cm) had the highest plant height at 35 DAS, while the V_2 variety had the lowest plant height (39.50cm).Cadmium stress has showed a significant reduction in the height of all *Brassica* species tested as the cadmium levels increase. In mild stress cadmium (Cd_2), plant height decreased the most in the V_2 variety (15.62%), which is significantly different from control, and the least in the V_3 variety (4.62%) compared to control, which showed non-significant variation from control. In severe stress cadmium (Cd_4), plant height decreased the most in the V_2 variety (28.64%) and the least in the V_3 variety (6.95%) when compared to control (Figure 2).

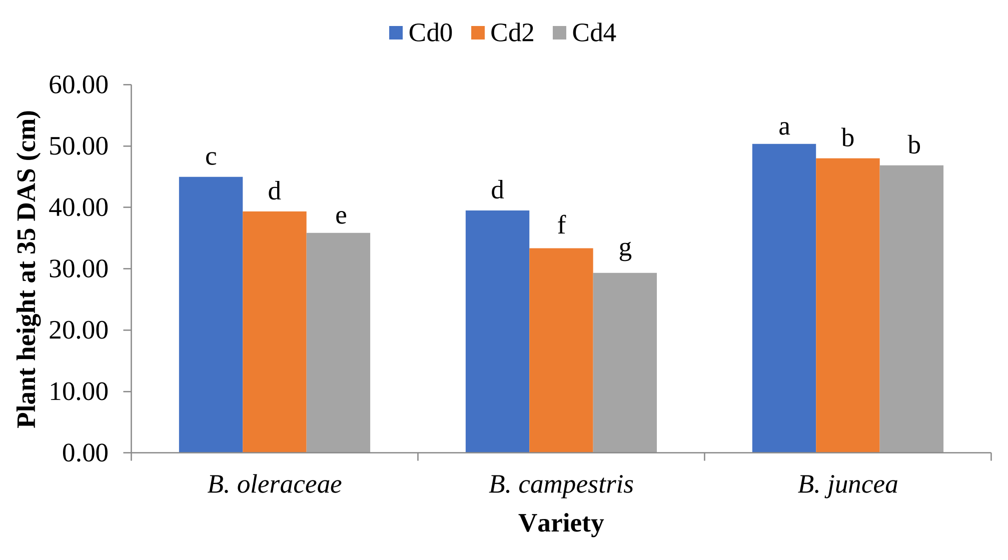

Figure 2. Effect of cadmium stress on plant heightof different Brassica species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM CdCl2, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test.

4.1.3 Plant height at 45 DAS

In our research, the V_3 variety (78.67cm) had the highest plant height at 45 DAS, whereas the V_2 variety had the lowest (59.33cm).Cadmium stress has resulted in a significant reduction in the height of all *Brassica* species tested as the cadmium level rises. In mild stress cadmium (Cd_2), plant height decreased the most in the V_2 variety (16.9%), which is significantly different from control, and the least in the V_3 variety (3.81%) compared to control, which showed non-significant variation from control. In severe stress cadmium (Cd_4), plant height decreased the most in the V_1 variety (34.55%) and the least in the V_3 variety (6.35%) when compared to control (Figure 3).

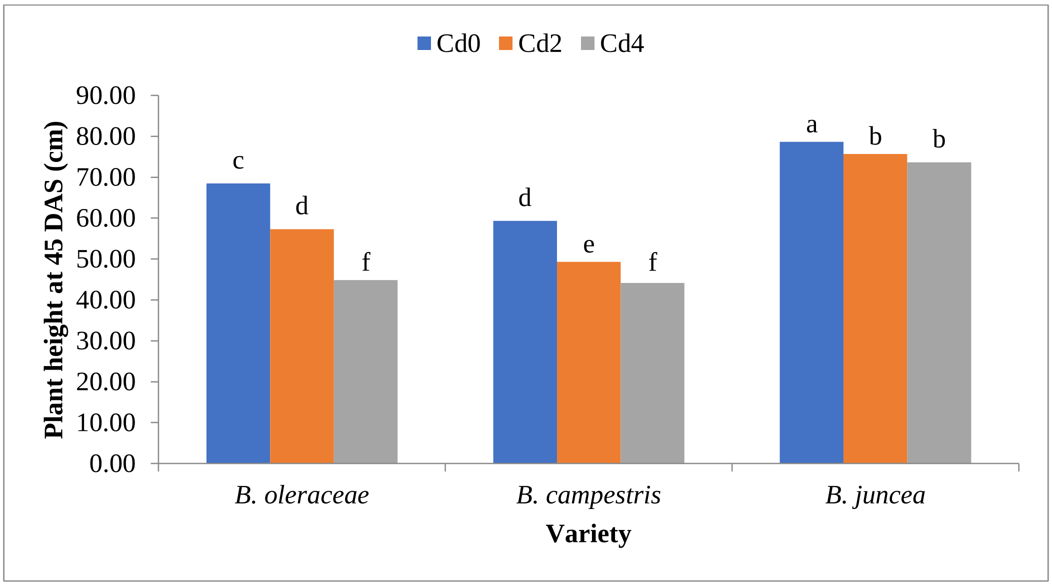

Figure 3. Effect of cadmium stress on plant height of different Brassica species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM CdCl2, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

Cadmium stress inhibits plant growth, development, and production by interfering with many physiological processes (Sanita di Toppi and Gabbrielli, 1999).Abiotic stress disrupts plant physiological functions (Conti *et al.*, 2019, Lisar*et al.*, 2012), resulting in a steady decrease in plant height as cadmium levels rise due to disruption in cell division and expansion. Khan *et al*. (2020) and Zhou *et al*. (2020) both found similar findings.

4.2 Root length

4.2.1 Root length at 25 DAS

In our study maximum root length at 25 DAS observed in V_3 variety (4.11cm) and minimum root length observed in V_2 variety (3.60cm). The root length in 25 DAS of all tested *Brassica* species was not significantly different. In the case of mild cadmium stress (Cd_2), root length was reduced the most among the varieties V_3 (11.27%), which differed from control, and the least among the V_1 varieties (4.63%), which did not differ significantly from control. Root length was significantly reduced in the V_1variety (23.70%) and significantly lower in the V_3 variety (11.99%) when exposed to severe cadmium (Cd_4) stress (Figure.4

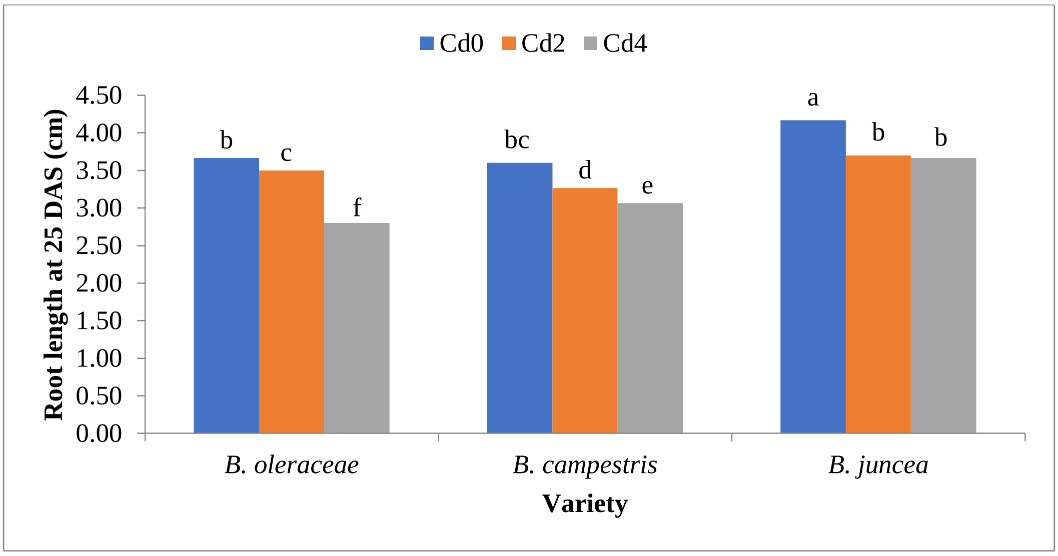

Figure 4. Effect of cadmium stress on root length of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.2.2 Root length at 35 DAS

In our study maximum root length at 35 DAS observed in V_3 variety (11.90cm) and minimum root length observed in V_2 variety (9.50 cm). The root length of all tested *Brassica* species decreased significantly in 35 DAS. In the presence of mild cadmium stress (Cd_2), root length decreased the most in the V_2 variety (22.10 %), compared to control, and the least in the V_3 variety (11.76 %). Root length was significantly reduced in the V_1 variety (33.79%) and the lowest in the V_3 variety (11.99%) in severe stress cadmium (Cd_4) compared to control (Figure5).

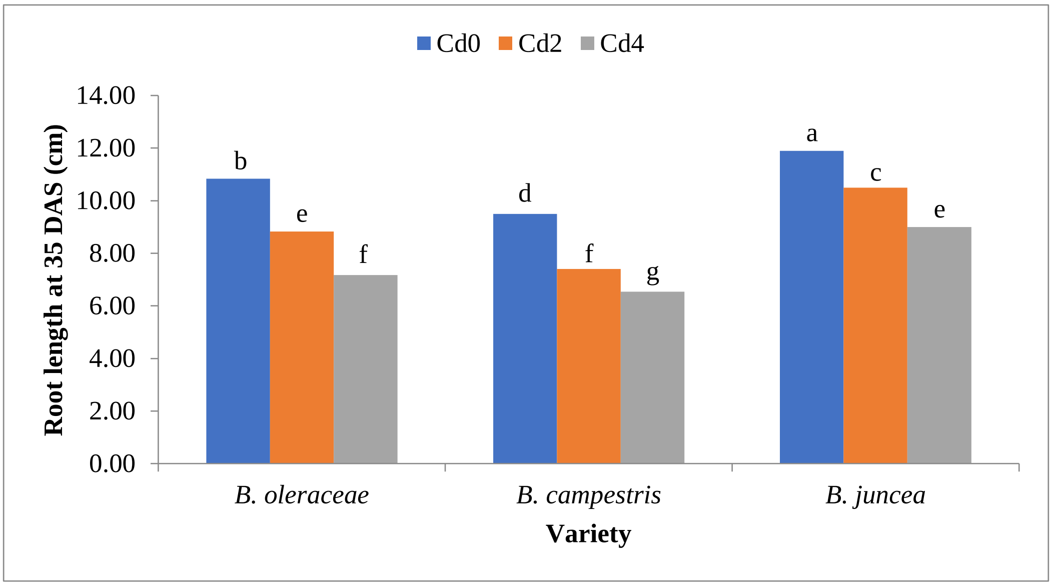

Figure 5. Effect of cadmium stress on root length of different Brassica species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM CdCl2, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at P≤0.05 applying Fisher's LSD test.

4.2.3 Root length at 45 DAS

In our study maximum root length at 45 DAS observed in V_3 variety (12.57cm) and minimum root length observed in V_2 variety (10.50cm). All of the *Brassica* species tested seemed to have shorter root length in 45 DAS. In the case of mild cadmium stress (Cd_2), root length was reduced in most of the V_1 varieties (19.16 %), which differed from control, and the V_3 variety (6.68 %), which showed a non-significant difference from control. When exposed to severe cadmium (Cd_4), root length was significantly reduced in the V_2 variety (24.09%) and the V_3 variety (17.82%), respectively, when compared to control (Figure 6)

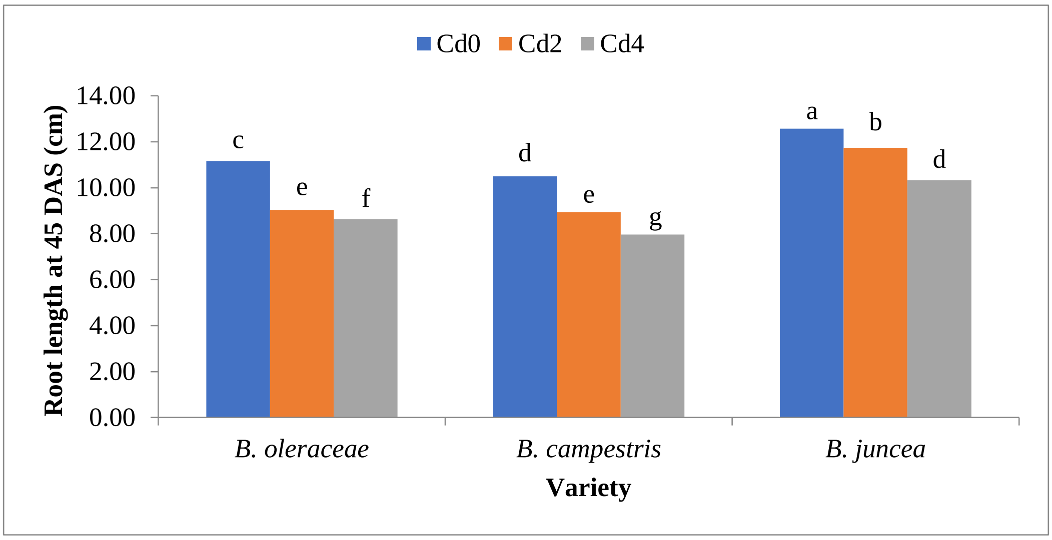

Figure 6. Effect of cadmium stress on root length of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

The roots of plants cultivated in heavy metal-contaminated media accumulated more metal than the shoots (Srivastava *et al*., 2014; Ahmad *et al*., 2015; Nahar *et al*., 2016; Rahman *et al*., 2016). Roots are the first organs in plants to come into contact with harmful metals, and they tend to accumulate more of the metal than shoots (john *et al*., 2009; Ahmad *et al*.,2011).

4.3 Leaf no. plant $^{-1}$

4.3.1 Leaf no. plant^{-1} at 25 DAS

In our study maximum leaf number plant^{-1} at 25 DAS observed in V_3 variety (5.00) and minimum leaf number plant^{-1} observed in V_2 variety (4.89). The number of leaves per plant in 25 DAS of all the tested *Brassica* species is not significantly different. When exposed to mild cadmium stress (Cd_2), the number of leaves was reduced the most in the V_3 variety (9.4%), and the least in the V_1 variety (0.64%), compared to control, which was almost identical. When exposed to high levels of cadmium (Cd_4), the number of leaves was reduced the most in the V_1 variety (13.62%) and the least in the V_3 variety (11.2%) (Figure 7).

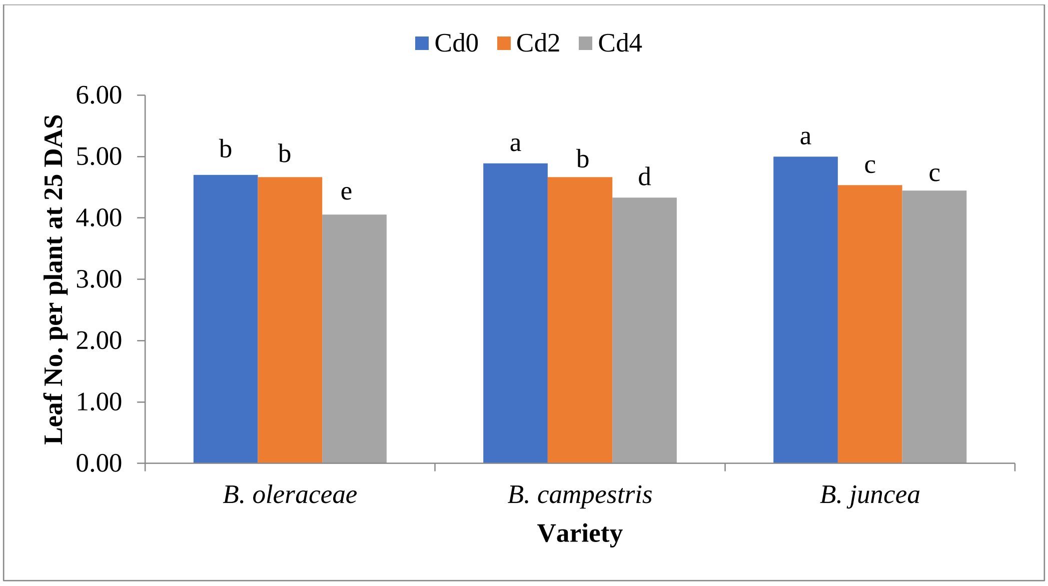

Figure 7. Effect of cadmium stress on leaf No. per plant of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.3.2 Leaf no. plant^{-1} at 35 DAS

In our study maximum leaf number plant^{-1} at 35 DAS observed in V_3 variety (6.00) and minimum leaf number plant^{-1} observed in V_1 variety (5.00). In 35 DAS of all tested *Brassica* species, the number of leaves per plant decreased as the cadmium level increased. When exposed to mild cadmium stress (Cd_2), the number of leaves decreased the most in the V_2 variety (10.8%) and the least in the V_1 variety (6.6%), compared to the control, which showed no significant difference. In severe stress cadmium (Cd_4), the number of leaves decreased the most in the V_1 variety (17.8%), and the least in the V_2 (14.26%) and V_3 (14.83%) varieties, compared to control (Figure 8).

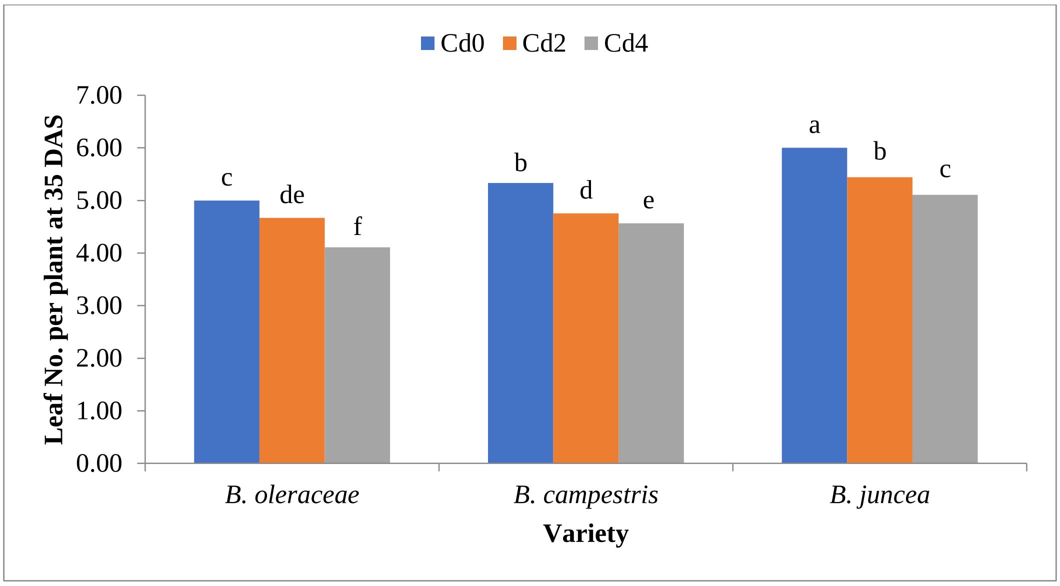

Figure 8. Effect of cadmium stress on leaf No. per plant of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.3.3 Leaf no. plant^{-1} at 45 DAS

In our study maximum leaf number plant^{-1} at 45 DAS observed in V_3 variety (7.50) and minimum leaf number plant^{-1} observed in V_1 variety (5.67). In 45 DAS there is a significant difference as cadmium levels rise. When exposed to mild cadmium stress (Cd_2), the number of leaves was reduced the most in the V_1 variety (19.57%) and the least in the V_3 variety (15.6%) compared to the control. When exposed to severe cadmium (Cd_4), the number of leaves was reduced the most in the V_1 variety (27.51%) and the least in the V_2, V_3 varieties (22.2%), compared to control (Figure 9).

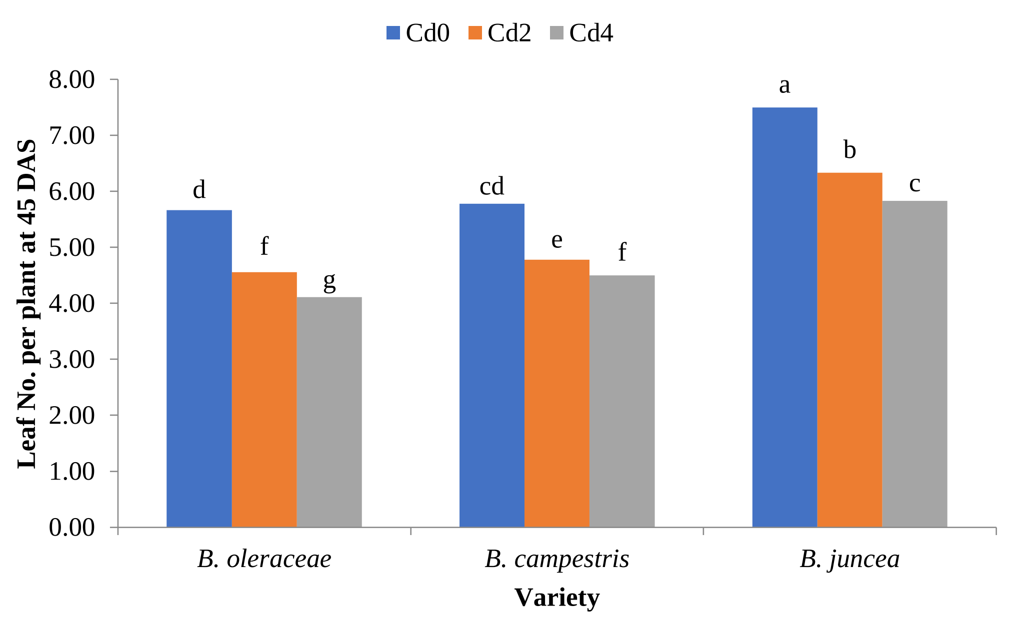

Figure 9. Effect of cadmium stress on leaf No. per plant of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

According to published research, Cd can impede plant growth by reducing soil microorganisms, damaging root tips, reducing nutrient and water intake by plants, and impairing photosynthesis (Sharma *et al*.,2006; Lag *et al*.,2010).As a result, during cadmium stress conditions, there were fewer leaves per plant.

4.4 SPAD value of leaf

In our study maximum SPAD value observed in V_3 variety (70.00) and minimum SPAD value observed in V_1 variety (65.67). Cadmium stress reduced the SPAD value of the leaves of all tested *Brassica* species as the cadmium level increased. The SPAD value of leaves decreased the most significantly (14.22 %) in mild cadmium stress (Cd_2), and the least significantly (8.1 %) in the V_3 variety. When exposed to severe cadmium stress (Cd_4), the SPAD value of the leaf decreased most significantly in the V_1 variety (36.04%) and the least significantly in the V_1 variety (21.43%) compared to control (Figure 10)

The suppression of proto chlorophyllide reduction and amino levulinic acid production causes a decrease in chlorophyll concentration (Stobart *et al*.1985). Cadmium stress impairs photosynthesis by changing photosystem II (Baszynski, 1986), reducing the quantity of plasto quinone in the chloroplast (Krupa *et al*. 1992), and disturbing the calvin cycle (Krupa *et al*., 1992). (Weigel, 1985).

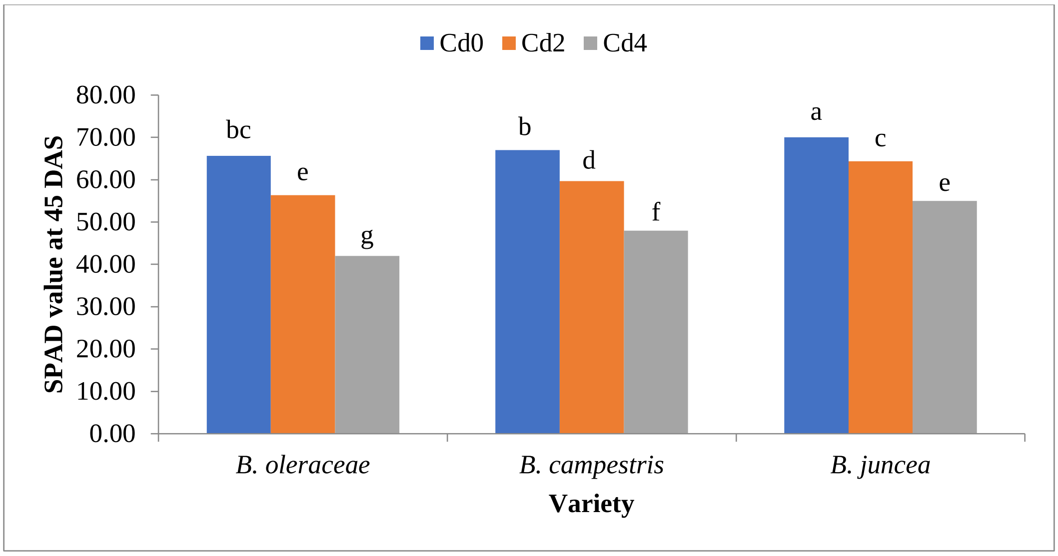

Figure 10. Effect of cadmium on SPAD value of leaf of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for eachtreatment. Bars with different letters are significantly different at P≤0.05 applying Fisher’s LSD test.

4.5 Length of siliqua

In our study maximum siliqua length observed in V_1 variety (4.81cm) and minimum siliqua length observed in V_3 variety (4.15 cm). The length of siliqua in all of the *Brassica* species studied did not change significantly as the cadmium level increased. The length of siliqua decreased the most (6.44 %) in mild cadmium stress (Cd_2), and the least non-significant reduction occurred in the V_3 variety (0.48 %), which is similar to control. In severe cadmium stress (Cd_4), the length of siliqua decreased the most (9.35%), while the least non-significant reduction (2.89%) occurred in the V_3 variety (Figure11).

Due to Pb, Cd, and Cr contamination, a significant reduction in the length and quantity of siliqua plant^{-1} was observed in Brassica species (Ahmad K *et al*. 2011). Different researchers have previously reported similar conclusions (Farid 2006; Kang *et al*. 2007; Khan *et al*. 2009).

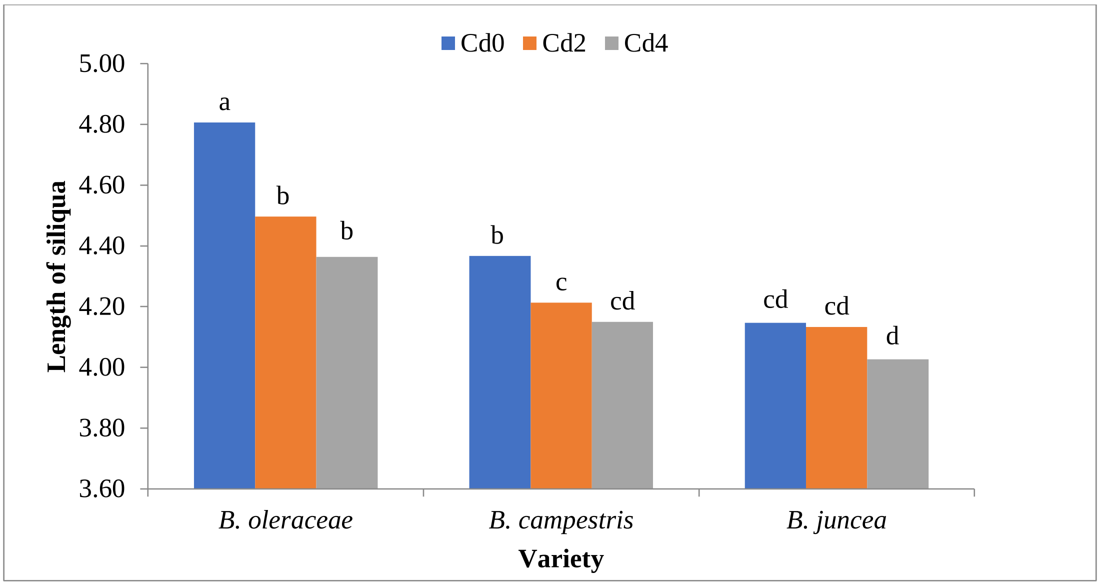

Figure 11. Effect of cadmium on length of siliqua of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.6 Number of siliqua plant^{-1}

In our study maximum number of siliqua plant^{-1} observed in V_3 variety (162.33) and minimum number of siliqua plant^{-1} observed in V_1 variety (55.33). As the cadmium level increased, the number of siliqua in all of the *Brassica* species tested decreased. When exposed to mild cadmium stress (Cd_2), the V_2 variety experienced the highest reduction (8.04%), while the V_1 and V_3 varieties experienced the least non-significant reduction (7.8%). When exposed to severe cadmium stress (Cd_4), the length of siliqua decreased the most in the V_2 variety (21.11 %) and the least in the V_3 variety (14.99 %) compared to control (Figure 12).

Photosynthetic cadmium toxicity restriction manifested itself in a genotype-dependent manner, resulting in considerable differences in growth and yield parameters, as demonstrated by Patel *et al*. (1980) and Ghani (2010). As a result of Pb, Cd, and Cr contamination a significant drop in the number of siliqua per plant in Brassica species was observed (Ahmad K *et al*. 2011)

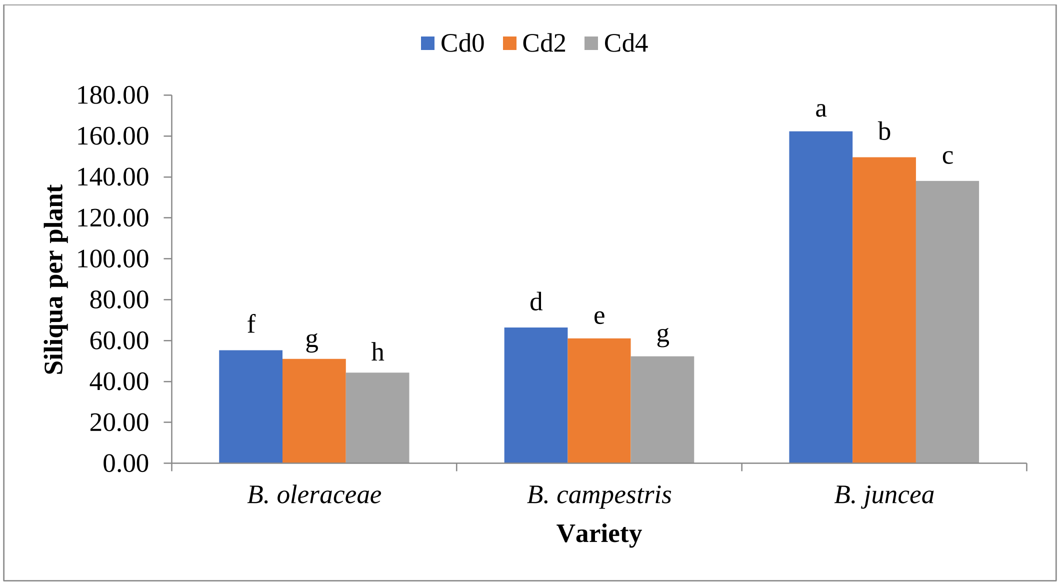

Figure 12. Effect of cadmium on siliqua per plant of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.7 No. of seeds siliqua^{-1}

In this study maximum number of seeds siliqua^{-1} observed in V_1 variety (26.33) and minimum number of seeds siliqua^{-1} observed in V_3 variety (9.00). The number of seeds siliqua^{-1} decreased as the cadmium level increased in all of the *Brassica* species studied. The number of seeds siliqua^{-1} reduced the most in the V_1 variety (8.85%) and the least in the V_3 variety (3.67%) under mild cadmium stress (Cd_2), compared to the control, which showed no significant decline. When exposed to severe cadmium stress (Cd_4), the number of seeds siliqua^{-1} reduced the most in the V_1 variety (17.70%) and the least in the V_2 variety (10.92%), compared to control (Figure 13).

Abiotic stress causes a large reduction in the number of flowers, which leads to a decrease in the number of siliqua production and, as a result, a decrease in marketable yield (Patel *et al*., 1980). Khan *et al*. also observed similar findings (2020).

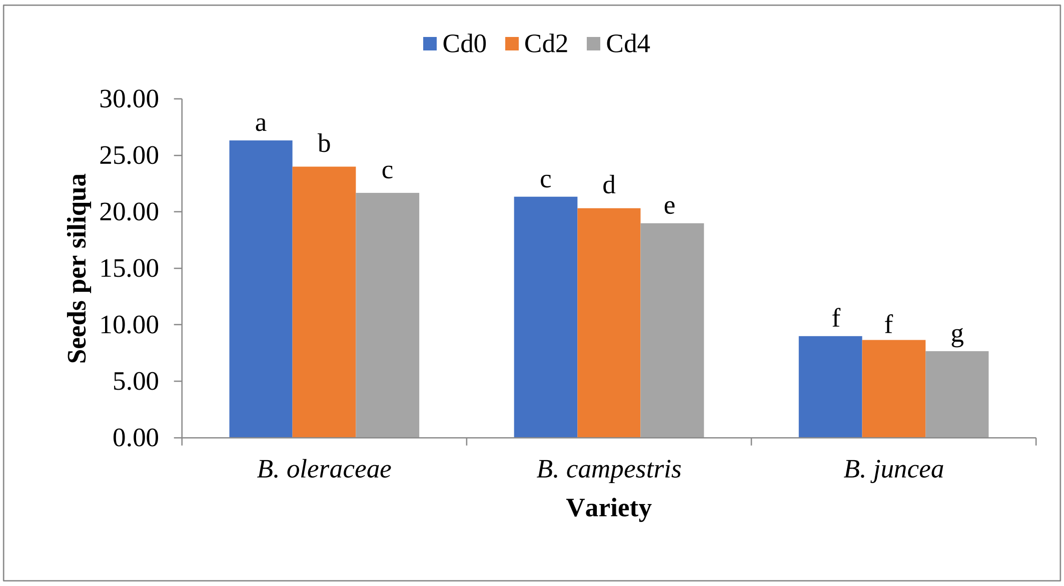

Figure 13. Effect of cadmium on seeds per siliqua of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM CdCl2, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.8 1000 seeds weight

In our study maximum weight of 1000 seeds observed in V_3 variety (4.71gm) and minimum weight of 1000 seeds observed in V_1 variety (3.34gm). As the cadmium level was increased, the 1000 seed weight of all tested *Brassica* species decreased. 1000 seed weight decreased significantly in the V_1 variety (8.08 %) in the mild cadmium stress (Cd_2) compared to the control, but not significantly in the V_3 variety (2.76 %). Under severe cadmium stress (Cd_4), 1000 seed weight reduced significantly in the V_1 variety (18.56%) compared to control, and the least significant decline occurred in the V_3 variety (6.58%) compared to control (Figure 14).

In response to cadmium toxicity, the yield parameters of mustard varieties (number of pods per plant, number of seeds per pod, weight of 100 seeds, and total seed yield per plant) decreased significantly (Irfan *et al*.,2015). Due to sewage water treatment containing Pb, Cd and Cr, a significant reduction in seed weight per plant was observed in Brassica species (Ahmad K *et al*. 2011). Different researchers have previously reported similar conclusions (Farid 2006; Kang *et al*. 2007; Khan *et al*. 2009).

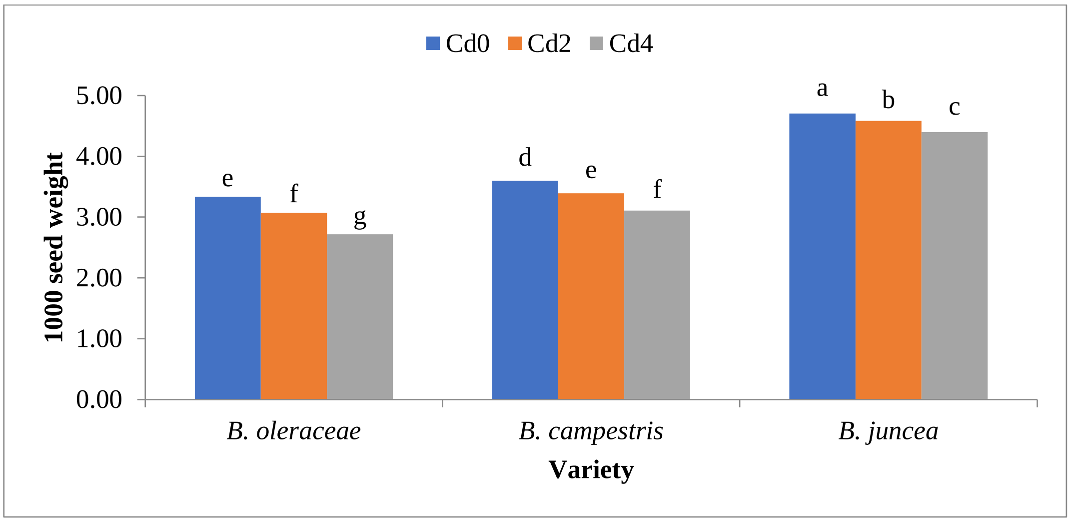

Figure 14. Effect of cadmium on 1000 seeds weight of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

4.9 Seed yield ha^{-1}

In our study maximum seed yield ha^{-1}observed in V_3 variety (2.07 tons) and minimum seed yield ha^{-1} observed in V_1 variety (1.47 tons). With an increase in cadmium level, cadmium stress significantly reduced seed yield per ha of all tested *Brassica* species. Seed yield per ha decreased significantly in mild cadmium stress (Cd_2), with the V_2 variety (17.53 %) having the greatest reduction compared to control, and the V_1 variety (9.52 %) having the lowest reduction. In severe cadmium stress (Cd_4), seed yield per ha decreased significantly in V_1 variety (46.26 %) compared to control, with the least reduction in V_3 variety (31.88 %) (Figure 15).

The parameters that determine the yield in response to cadmium toxicity, total seed production per ha of the two mustard kinds declined dramatically (Irfan *et al.*,2015). Due to sewage water treatment containing Pb, Cd, and Cr, a significant drop in seed weight per plant was observed in Brassica species (Ahmad K *et al.* 2011). Different researchers have previously reported similar conclusions (Farid 2006; Kang *et al.* 2007; Khan *et al.* 2009).

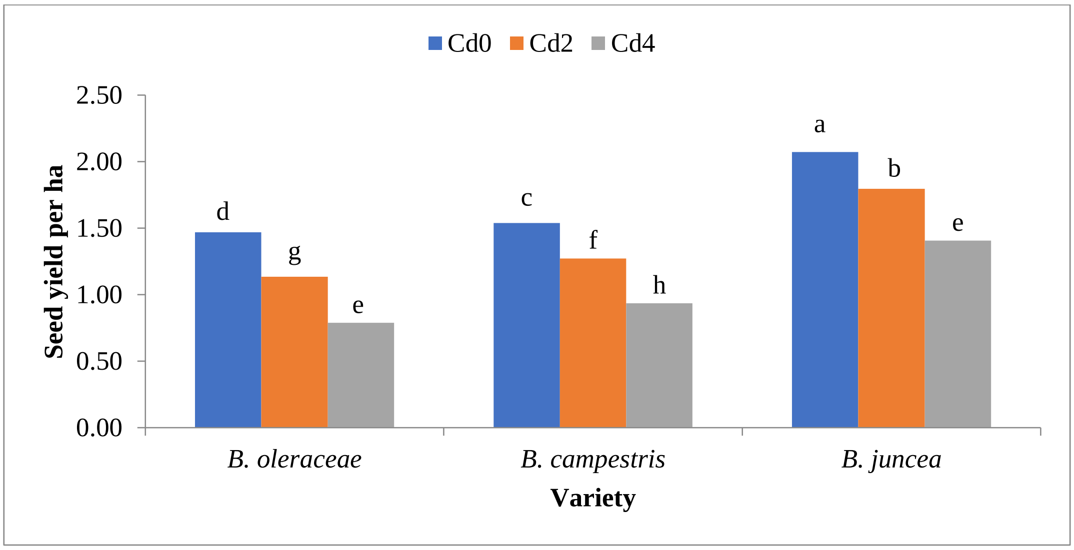

Figure 15. Effect of cadmium on seed yield of different *Brassica* species. Here, Cd0, Cd2 and Cd4 indicate 0 mM, 2 mM and 4 mM$CdCl_2$, respectively. Means (±SD) were calculated from three replications for each treatment. Barswith different letters are significantly different at P≤0.05 applying Fisher's LSD test.

CHAPTER 5

SUMMARY AND CONCLUSION

5.1 Summary

To discover the most cadmium-suited sarisa genotype, a pot experiment was undertaken to observe changes in growth and yield of three sarisa genotypes under three different cadmium treatments. During the months of November 2019 to February 2020, the experiment was conducted in the field of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. There were two factorial experiments with three sarisa types, namely BARI Sarisha17*(B.oleraceae)*, BARI Sarisha14 *(B. campestris)*, BARI Saisha16 *(B. juncea)*

The collected data was statistically processed to assess sarisa varieties under various cadmium conditions. In the case of sarisa varieties and cadmium treatments, the plant height at the 25 DAS was reduced the most in the V_1 variety (9.6%) compared to control in mild stress cadmium (Cd_2) and the least in the V_3 variety (3.44%) compared to control, which showed non-significant variation from control. In severe stress cadmium (Cd_4), plant height decreased the most in the V_1 variety (23.62%) and the least in the V_3 variety (4.82%) when compared to control. The plant height at the 35 DAS in mild stress cadmium (Cd_2) decreased the most in the V_2 variety (15.62%), which is significantly different from control, and the least in the V_3 variety (4.62%) compared to control, which showed non-significant variation from control. In severe stress cadmium (Cd_4), plant height decreased the most in the V_2 variety (28.64%) and the least in the V_3 variety (6.95%) when compared to control. The plant height at the 45 DAS in mild stress cadmium (Cd_2)decreased the most in the V_2 variety (16.9%), which is significantly different from control, and the least in the V_3 variety (3.81%) compared to control, which showed non-significant variation from control. In severe stress cadmium (Cd_4), plant height decreased the most in the V_1 variety (34.55%) and the least in the V_3 variety (6.35%) when compared to control. In the case of root length at the 25 DAS in mild cadmium stress (Cd_2), root length was reduced the most among the varieties V_3 (11.27%), which differed from control, and the least among the V_1 varieties (4.63%), which did not differ significantly from control. Root length was significantly reduced in the V_1variety (23.70%) and significantly lower in the V_3 variety (11.99%) when exposed to severe cadmium (Cd_4) stress. At 35 DAS, in the presence of mild cadmium stress (Cd_2), root length decreased the most in the V_2 variety (22.10%), compared to control, and the least in the V_3 variety (11.76%). Root length was significantly reduced in the V_1 variety (33.79%) and the lowest in the V_3 variety (11.99%) in severe stress cadmium (Cd_4) compared to control. At 45 DAS, in mild cadmium stress (Cd_2), root length was reduced in most of the V_1 varieties (19.16 %), which differed from control, and the V_3 variety (6.68 %), which showed a non-significant difference from control. When exposed to severe cadmium (Cd_4), root length was significantly reduced in the V_2 variety (24.09%) and the V_3 variety (17.82%), respectively, when compared to control. In the case of the number of leaves per plant in 25 DAS, all the tested *Brassica* species are not significantly different. When exposed to mild cadmium stress (Cd_2), the number of leaves was reduced the most in the V_3 variety (9.4%) and the least in the V_1 variety (0.64%), compared to control, which was almost identical. When exposed to high levels of cadmium (Cd_4), the number of leaves was reduced the most in the V_1 variety (13.62%) and the least in the V_3 variety (11.2%). At 35 DAS, under mild cadmium stress (Cd_2), the number of leaves decreased the most in the V_2 variety (10.8%) and the least in the V_1 variety (6.6%), compared to the control, which showed no significant difference. In severe stress cadmium (Cd_4), the number of leaves decreased the most in the V_1 variety (17.8%), and the least in the V_2 (14.26%) and V_3 (14.83%) varieties, compared to control. At 45 DAS, under mild cadmium stress (Cd_2), the number of leaves was reduced the most in the V_1 variety (19.57%) and the least in the V_3 variety (15.6%)

compared to the control. When exposed to severe cadmium (Cd_4), the number of leaves was reduced the most in the V_1 variety (27.51%) and the least in the V_2, and V_3 varieties (22.2%), compared to control. In the case of SPAD, the value of leaves decreased the most significantly (14.22%) in mild cadmium stress (Cd_2) and the least significantly (8.1%) in the V_3 variety. When exposed to severe cadmium stress (Cd_4), the SPAD value of the leaf decreased most significantly in the V_1 variety (36.04%) and the least significantly in the V_1 variety (21.43%) compared to control. In the case of the length of siliqua, it decreased the most (6.44%) in mild cadmium stress (Cd_2), and the least non-significant reduction occurred in the V_3 variety (0.48%), which is similar to control. In severe cadmium stress (Cd_4), the length of siliqua decreased the most (9.35%), while the least non-significant reduction (2.89%) occurred in the V_3 variety. In the case of per siliqua seed, under mild cadmium stress (Cd_2), the number of seeds per siliqua decreased the most in the V_1 variety (8.85%) and the least in the V_3 variety (3.67%), compared to the control, which exhibited no significant decline. In comparison to control, the quantity of seeds per siliqua was reduced the most in the V_1 variety (17.70%) and the least in the V_2 variety (10.92%) when exposed to severe cadmium stress (Cd_4).In the case of 1000 seeds, weight decreased significantly in the V_1 variety (8.08%) under mild cadmium stress (Cd_2) compared to the control, but not significantly in the V_3 variety (2.76%). Under severe cadmium stress (Cd_4), 1000 seed weight was reduced significantly in the V_1 variety (18.56%) compared to control, and the least significant decline occurred in the V_3 variety (6.58%) compared to control. In the case of seed yield per ha, it decreased significantly in mild cadmium stress (Cd_2), with the V_2 variety (17.53%) having the greatest reduction compared to control, and the V_3 variety (9.52%) having the lowest reduction. In severe cadmium stress (Cd_4), seed yield per ha decreased significantly in the V_1 variety (46.26%) compared to control, with the least reduction in the V_3 variety (31.88%).

5.2 Conclusion

The presence of Cd in the soil clearly impacts the mustard plant's growth, which is reflected in its yield properties, as evidenced by this pot experiment. All yield attributes are significantly reduced in plants cultivated in Cd-stressed soil. To address the cadmium problem, mustard varieties that are cadmium-tolerant must be chosen. Mustard plants are moderately tolerant of cadmium stress; however, the exact level of cadmium sensitivity varies with variety. Assessment followed by screening may be a more straightforward strategy for identifying cadmium-adaptive varieties. Cadmium stress disrupts plant physiological functions, which has a negative impact on mustard development and output. According to the results of the experiment, *Brassica campestris* is the best mustard variety for mild cadmium stress conditions, whereas *Brassicajuncea* is the best mustard variety for severe cadmium stress conditions. We conclude that, of the *Brassica species* studied, *B. juncea* is a relatively tolerant species to Cd toxicity based on physiological attributes. These *Brassica* species can also be grown in agroforestry systems with other plant species where cadmium stress is common.

RECOMMENDATIONS

- In the future, further growth and yield based study on this topic should be conducted to obtain more precise results in field condition.
- The physiological, pharmacological, and molecular basis of *Brassica* plants should be studied under cadmium stress.
- Performance of different *Brassica* species under other heavy metals stress (arsenic, lead, and mercury) should be conducted.

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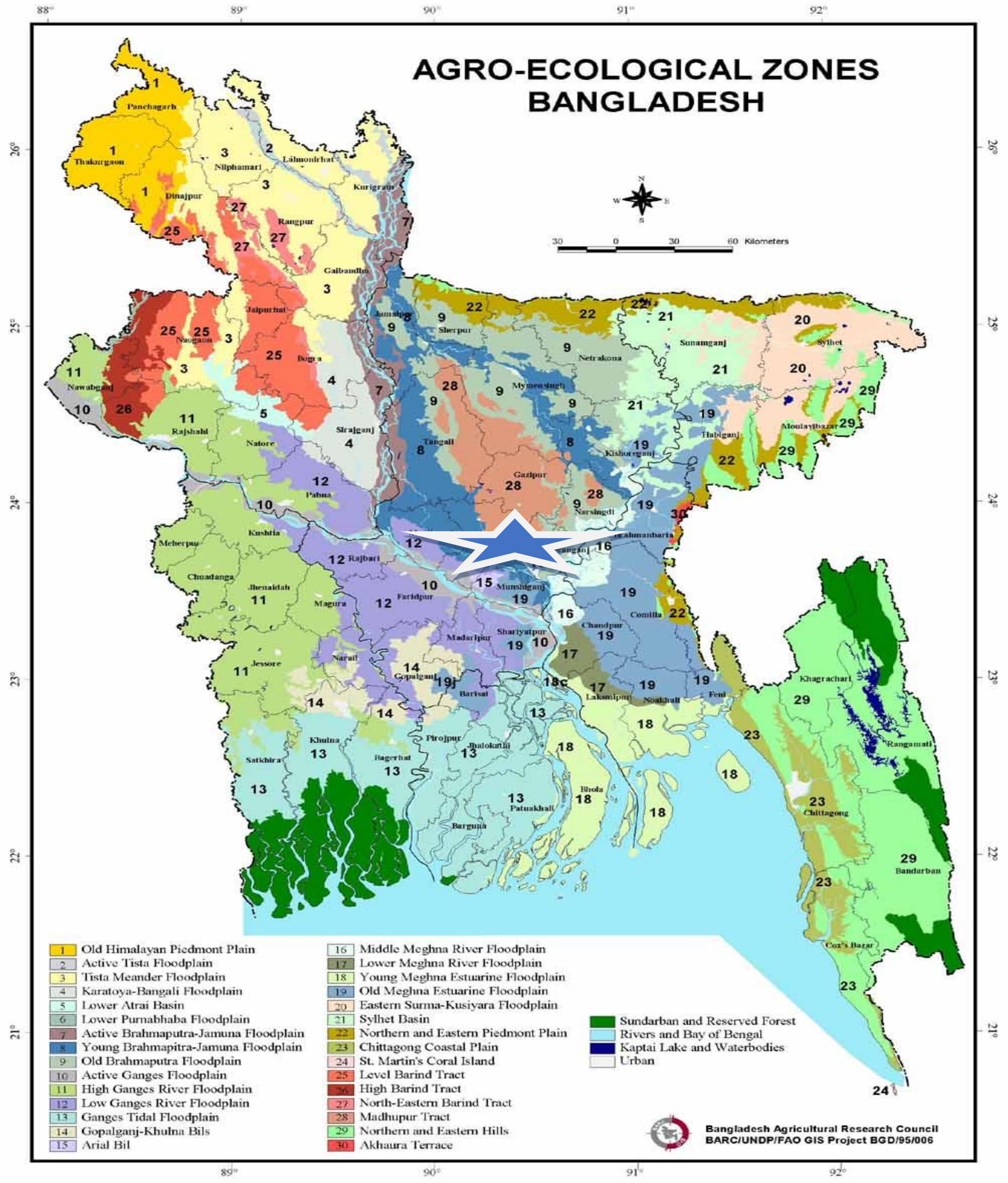
APPENDICES

Appendix 1. Map showing the experimental site under the study



★ Experimental site under study

Appendix 2. Monthly records of air temperature, relative humidity, rainfall and sunshine hours during the period from October 2019 to February 2020.

Month	Year	Monthly average air temperature (°C)			Average relative humidity (%)	Total rainfall (mm)	Total sunshine (hours)
		Maximum	Minimum	Mean			
Oct.	2019	36	21	28	69	Trace	219
Nov.	2019	31	18	24	63	Trace	216
Dec.	2019	28	16	22	61	Trace	212
Jan.	2020	27	13	20	57	Trace	198
Feb.	2020	29	18	23	70	3	225

Source: Bangladesh Meteorological Department (Climate division), Agargaon Dhaka-1212.

Appendix 3. The mechanical and chemical characteristics of soil of the experimental site as observed prior to experimentation (0 -15 cm depth).

Mechanical composition:

Particle size	Constitution
Texture	Loamy
Sand	40%
Silt	40%
Clay	20%

Chemical composition:

Soil characters	**Value**
Organic matter	1.44 %
Potassium	0.15 meq/100 g soil
Calcium	1.00 meq/100 g soil
Magnesium	1.00 meq/100 g soil
Total nitrogen	0.072
Phosphorus	22.08 µg/g soil
Sulphur	25.98 µg/g soil
Boron	0.48 µg/g soi
Copper	3.54 µg/g soil
Iron	262.6 µg/g soil
Manganese	164 µg/g soil
Zinc	3.32 µg/g soil

Source: Soil Resources Development Institute (SRDI), Khamarbari, Dhaka

Appendix 4. Mean values of different growth and yield contributing traits of three sarisha varieties under control and Cadmium stress treatment

	Plant height at 25 DAS (cm)	Plant height at 35 DAS (cm)	Plant height at 45 DAS (cm)	Root length at 25 DAS (cm)	Root length at 35 DAS (cm)	Root length at 45 DAS (cm)
V_1Cd_0	18.33	45.00	68.50	3.67	10.83	11.17
V_1Cd_2	16.57	39.33	57.33	3.50	8.83	9.03
V_1Cd_4	14.00	35.83	44.83	2.80	7.17	8.63
V_2Cd_0	13.00	39.50	59.33	3.60	9.50	10.50
V_2Cd_2	11.80	33.33	49.30	3.27	7.40	8.93
V_2Cd_4	10.60	29.33	44.17	3.07	6.53	7.97
V_3Cd_0	19.47	50.33	78.67	4.17	11.90	12.57
V_3Cd_2	18.80	48.00	75.67	3.70	10.50	11.73
V_3Cd_4	18.53	46.83	73.67	3.67	9.00	10.33

Cd_0: control; Cd_2: Mild Cadmium stress; Cd_4: Severe Cadmium stress

Appendix 4.Cont.

	Leaf No.per plant at 25 DAS (cm)	Leaf No.perplantat 35 DAS (cm)	Leaf No.per plant at 45 DAS (cm)	SPAD value at 45 DAS (cm)	Lenghth of siliqua (cm)	Siliqua per plant (cm)
V_1Cd_0	4.70	5.00	5.67	65.67	4.81	55.33
V_1Cd_2	4.67	4.67	4.56	56.33	4.50	51.00
V_1Cd_4	4.06	4.11	4.11	42.00	4.36	44.33
V_2Cd_0	4.89	5.33	5.78	67.00	4.37	66.33
V_2Cd_2	4.67	4.75	4.78	59.67	4.21	61.00
V_2Cd_4	4.33	4.57	4.50	48.00	4.15	52.33
V_3Cd_0	5.00	6.00	7.50	70.00	4.15	162.33
V_3Cd_2	4.53	5.44	6.33	64.33	4.13	149.67
V_3Cd_4	4.44	5.11	5.83	55.00	4.03	138.00

Cd_0: control; Cd_2: Mild Cadmium stress; Cd_4: Severe Cadmium stress

Appendix 4.Cont.

	Seeds per siliqua (cm)	1000 seed weight (cm)	Seed yield per ha (cm)
V_1Cd_0	26.33	3.34	1.47
V_1Cd_2	24.00	3.07	1.13
V_1Cd_4	21.67	2.72	0.79
V_2Cd_0	21.33	3.60	1.54
V_2Cd_2	20.33	3.39	1.27
V_2Cd_4	19.00	3.11	0.93
V_3Cd_0	9.00	4.71	2.07
V_3Cd_2	8.67	4.58	1.79
V_3Cd_4	7.67	4.40	1.41

Cd_0: control; Cd_2: Mild Cadmium stress; Cd_4: Severe Cadmium stress

Appendix 5. Factorial ANOVA Table for all the growth and yield parameters of three mustard and rapeseed varieties under control and cadmium stress treatment

5.1 Factorial ANOVA Table for plant height at 25 DAS

Source	DF	SS	MS	F	P
Replication	2	1.039	0.519		
Variety	2	234.728	117.364	784.97	0.0000
Treatment	2	29.423	14.712	98.40	0.0000
Variety*Treatment	4	9.070	2.268	15.17	0.0000
Error	16	2.392	0.150		
Total	26	276.652			

Grand Mean 15.681
CV 2.47

5.2 Factorial ANOVA Table for plant height at 35 DAS

Source	DF	SS	MS	F	P
Replication	2	0.72	0.361		
Variety	2	932.67	466.333	504.90	0.0000
Treatment	2	265.72	132.861	143.85	0.0000
Variety*Treatment	4	39.11	9.778	10.59	0.0002
Error	16	14.78	0.924		
Total	26	1253.00			

Grand Mean 40.833
CV 2.35

5.3 Factorial ANOVA Table for plant height at 45 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	9.48	4.74		
Variety	2	3087.12	1543.56	825.21	0.0000
Treatment	2	964.16	482.08	257.73	0.0000
Variety*Treatment	4	271.95	67.99	36.35	0.0000
Error	16	29.93	1.87		
Total	26	4362.63			

Grand Mean 61.274
CV 2.23

5.4 Factorial ANOVA Table for Root length at 25 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.02074	0.01037		
Variety	2	1.74296	0.87148	131.64	0.0000
Treatment	2	1.74296	0.87148	131.64	0.0000
Variety*Treatment	4	0.39704	0.09926	14.99	0.0000
Error	16	0.10593	0.00662		
Total	26	4.00963			

Grand Mean 3.4963
CV 2.33

5.5 Factorial ANOVA Table for Root length at 35 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.0030	0.0015		
Variety	2	31.9607	15.9804	633.35	0.0000
Treatment	2	45.8007	22.9004	907.61	0.0000
Variety*Treatment	4	1.0037	0.2509	9.94	0.0003
Error	16	0.4037	0.0252		
Total	26	79.1719			

Grand Mean 9.0741
CV 1.75

5.6 Factorial ANOVA Table for Root length at 45 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.0274	0.0137		
Variety	2	29.3385	14.6693	526.34	0.0000
Treatment	2	27.1652	13.5826	487.35	0.0000
Variety*Treatment	4	1.4126	0.3531	12.67	0.0001
Error	16	0.4459	0.0279		
Total	26	58.3896			

Grand Mean 10.096
CV 1.65

5.7 Factorial ANOVA Table for Leaf number per plant at 25 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.01512	0.00756		
Variety	2	0.18759	0.09379	13.27	0.0004
Treatment	2	1.56032	0.78016	110.39	0.0000
Variety*Treatment	4	0.32770	0.08193	11.59	0.0001
Error	16	0.11308	0.0707		
Total	26	2.20381			

Grand Mean 4.5919
CV 1.83

5.8 Factorial ANOVA Table for Leaf number per plant at 35 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.00690	0.00345		
Variety	2	4.02050	2.01025	274.82	0.0000
Treatment	2	3.26516	1.63258	223.19	0.0000
Variety*Treatment	4	0.10990	0.02748	3.76	0.0244
Error	16	0.11704	0.00731		
Total	26	7.51950			

Grand Mean 4.9996
CV 1.71

5.9 Factorial ANOVA Table for Leaf number per plant at 45 DAS

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.0081	0.00407		
Variety	2	16.7399	8.36996	1224.21	0.0000
Treatment	2	10.8231	5.41157	791.51	0.0000
Variety*Treatment	4	0.1222	0.03055	4.47	0.0129
Error	16	0.1094	0.00684		
Total	26	27.8028			

Grand Mean 5.4507
CV 1.52

5.10 Factorial ANOVA Table for SPAD Value

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	8.22	4.111		
Variety	2	323.56	161.778	85.02	0.0000
Treatment	2	1690.89	845.444	444.32	0.0000
Variety*Treatment	4	56.89	14.222	7.47	0.0014
Error	16	30.44	1.003		
Total	26	2110.00			

Grand Mean 58.667
CV 2.35

5.11 Factorial ANOVA Table for Length of Siliqua

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.01081	0.00540		
Variety	2	0.97383	0.48691	67.71	0.0000
Treatment	2	0.31201	0.15600	21.69	0.0000
Variety*Treatment	4	0.10388	0.02597	3.61	0.0279
Error	16	0.11506	0.00719		
Total	26	1.51559			

Grand Mean 4.3007
CV 1.97

5.12Factorial ANOVA Table for Siliqua per plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.3	0.1		
Variety	2	54507.2	27253.6	11565.38	0.0000
Treatment	2	1220.5	610.3	258.97	0.0000
Variety*Treatment	4	151.9	38.0	16.12	0.0000
Error	16	37.7	2.4		
Total	26	55917.6			

Grand Mean 86.704
CV 1.77

5.13 Factorial ANOVA Table for Seeds per siliqua

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.67	0.333		
Variety	2	1184.89	592.444	2472.81	0.0000
Treatment	2	34.89	17.444	72.81	0.0000
Variety*Treatment	4	8.89	2.222	9.28	0.0004
Error	16	3.83	0.240		
Total	26	1242.67			

Grand Mean 17.556
CV 2.79

5.14 Factorial ANOVA Table for 1000 seed weight

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	0.0088	0.00441		
Variety	2	11.5409	5.77043	3121.50	0.0000
Treatment	2	1.0178	0.50890	275.29	0.0000
Variety*Treatment	4	0.0729	0.01823	9.86	0.0003
Error	16	0.0296	0.00185		
Total	26	12.6700			

Grand Mean 3.6567
CV 1.18

5.15 Factorial ANOVA Table for Seed yield per ha

Source	DF	SS	MS	F	P
Replication	2	0.00076	0.00038		
Variety	2	2.02142	1.01071	2676.50	0.0000
Treatment	2	1.92197	0.96098	2544.82	0.0000
Variety*Treatment	4	0.00777	0.00194	5.15	0.0074
Error	16	0.00604	0.00038		
Total	26	3.95796			

Grand Mean 1.3803
CV 1.41