

Parvaiz Ahmad
Mohd Rafiq Wani
Mohamed Mahgoub Azooz
Lam-Son Phan Tran *Editors*

Improvement of Crops in the Era of Climatic Changes

Volume 2

 Springer

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Editors

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Preface

“Improvement of Crops in the Era of Climatic Changes” Volume 2

Climate change is an unprecedented threat to the food security for hundreds of millions of people who depend on small-scale agriculture for their livelihoods. Abiotic stress is the prime cause for deteriorating the average yield of major crops by more than 50%, which cause losses worth hundreds of millions of dollars each year. Plants are exposed to rapid, unpredicted, and diverse environmental disturbances, resulting in stressful conditions. Stress signal is first perceived by the receptors present on the membrane of plant cells. The signal information is then transduced downstream, resulting in an activation of various stress-responsive genes. The products of these stress genes, ultimately lead to stress tolerance response and enable the plant to survive and surpass the unfavourable conditions. Recent trends in population growth suggest that global food production is unlikely to gratify the future demands under predicted climate change scenarios, unless rates of crop improvement are accelerated or sweeping changes occur in patterns of human food consumption. The situation is generally more staid in less developed countries, where agro-ecosystems are already fragile, investment in agriculture is limited, and climate change is predicted to have its most devastating effects. Global climate change is likely to increase the problems of food insecurity, hunger and malnutrition for millions of people, particularly in south Asia and sub-Saharan Africa, and further exacerbate the problem by remarkably restricting the plant growth and development.

The potential yield of economically important crops is drastically coming down every year just because of abiotic stresses. It has been projected that global food production must increase by 70% by 2050 to meet the ever-increasing demand caused by burgeoning human population, increasing incomes and consumption. Several factors are contributing to high plant performance under different environmental conditions, therefore an effective and complementary use of all the available technological tools and resources is needed to meet the challenge. The mechanisms underlying endurance to environmental stress factors have long been the focus of intense research. The progress in biotechnology, genomic research, and molecular marker applications have brought to the forefront an interdisciplinary science that is revolutionizing twenty-first century crop improvement. Many novel genomics technologies like next generation sequencing and omics have emerged as powerful tools for understanding the genomic variation among crop species at different

molecular levels. Climate change is no more an illusion, its ruinous impact is globally witnessed and interventions must be highly addressed at international, regional and national levels. In this context, the book “*Improvement of Crops in the Era of Climatic Changes*” *Volume 2* will serve as avant-garde resource for researchers and students who are immersed in developing the improved crop cultivars and management methods. Written by a varied group of internationally distinguished experts, “*Improvement of Crops in the Era of Climatic Changes*” *Volume 2* is a concise, yet comprehensive resource for researchers, students and others seeking knowledge expansion in this burning area of research and will lead them to new pondering on the subjects of climate changes and crop improvement.

In this book, we present a collection of 14 chapters written by 51 reputed experts in the fields of plant abiotic stress tolerance, induced mutagenesis and crop improvement. It is a well-timed and painstakingly compiled contribution of the topics that are of vast scientific eminence. Chapter one (1) throws light on Brassicas: responses and tolerance to heavy metal stress. In this chapter, the authors stated that there is a great scope for understanding methodically the genetics and genomics of *Brassica* species and the mechanisms of actions underlying the metal-induced toxicities and the tolerances developed therein. Chapter two (2) addresses the recent advances in rapid and sensitive screening for abiotic stress tolerance, wherein the authors have elaborated the development and identification of molecular markers associated with tolerance response and their value in sensitive indirect selection among few crop species. Chapter three (3) is about transcriptomics of heat stress in plants. This chapter primarily provides the current understanding on the role of regulatory genes (transcription factors), heat shock protein genes, metabolic genes, signaling compounds, osmolytes, reactive oxygen species and role of miRNAs as well as small RNAs of plants under high temperature. In addition, it gives a brief account of various transcriptome approaches to study the expression profiling of genes during the heat stress. Chapter four (4) is about biotic stress and crop improvement, wherein the authors fussily tried to identify the most widespread plant viruses in Azerbaijan, using different molecular techniques and precisely evaluated some characteristics of plant responses to viral stress. Chapter five (5) is regarding the salt stress and sugar beet improvement: challenges and opportunities. In this chapter, the authors present the comprehensive discussion on the challenges and opportunities for improvement of salt tolerance in sugar beet and emphasized that future research should chiefly focus on physiological, molecular and metabolic dimensions to facilitate the development of such crops with inherent stress tolerance capacity.

Chapter six (6) describes the genotypic variation for drought tolerance in wheat plants. In this chapter, a rich gene pool, comprising of thousands of wheat genotypes with contrasting photosynthetic traits, productivity and tolerance to drought stress, and introduced from world gene pool, particularly, CIMMYT and ICARDA was created in Azerbaijan which could be of great help for monitoring the environmental stresses in field grown plants and in the selection of stress-resistant varieties. Chapter seven (7) deals with soil contaminants: sources, effects and approaches for remediation. Here, the authors authoritatively stated that the remediation of heavy

metal contaminated soils is necessary to reduce the associated risks, make the land resource available for agricultural production, enhance the food security, and scale down the land tenure problems arising from the changes in land use pattern. Chapter eight (8) describes the role of macronutrients in plant growth and acclimation: recent advances and future prospective. This chapter deals with the recent progress made in finding out the roles of macronutrients in plant growth and acclimation processes as well as future prospective of elemental research in plants. Chapter nine (9) is concerned with mutation breeding: a novel technique for genetic improvement of pulse crops particularly chickpea (*Cicer arietinum* L.). This chapter compresses various facets of contemporary knowledge for pulse crop varietal improvement, particularly chickpea, through induced mutagenesis with special thrust on qualitative as well as yield attributing traits. Chapter ten (10) deals with organic farming: the return to nature. In this chapter, the authors enumerated that organic foods have more plant secondary metabolites, higher micronutrient content and more conjugated fatty acids for better human health, including lower incidences of non-communicable diseases. Additionally, they stated that organic agriculture merges modernism, custom and science to manage the shared surroundings by encouraging the fair relationship and high quality of life for everyone involved. Chapter eleven (11) is about the role of cytological aberrations in crop improvement through induced mutagenesis. In this chapter, the authors scrupulously revealed the impact of mutagens on cytological behaviour and their overall role in crop improvement. Chapter twelve (12) deals with the wheat improvement: historical perspective and mutational approach—a review. This review enfolds various historical aspects, in addition to contemporary knowledge of wheat crop improvement programs through induced mutagenesis. Chapter thirteen (13) is about the cotton leaf curl virus disease predictive model based on environmental variables. This chapter was initiated to develop a disease predictive model to characterize the epidemiological factors conducive for disease spread and severity. The authors also envisaged that such models would be highly helpful in forecasting the diseases and subsequently help to decide the correct timing of pesticide applications.

Chapter fourteen (14) deals with transcription factors in abiotic stress responses—their potentials in crop improvement. In this chapter, the authors summarized contemporary understanding about TF activities in plants under adverse stress conditions and their use in crop improvement.

Chapters contributed in this book have been published keeping intact author's justifications, however suitable editorial changes were made, wherever considered necessary. In spite of our best efforts, there is a chance of some errors still creeping in the book, for which we seek reader's feedback. We wish to express abstemious appreciation to our well versed contributors, who readily accepted our invitation to write their chapters. Moreover, we would like to thank Springer Science+Business Media, LLC, New York, particularly Eric Stannard (Editor Botany, Springer), Andy Kwan (Assistant Editor, Springer), Kevin Wright (Developmental Editor, Springer), Flora Kim (Developmental Editor, Springer) and all the other staff members of Springer, New York who were directly or indirectly associated with us in the current

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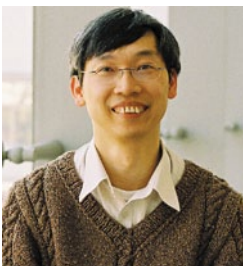
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Chapter 1

Brassicas: Responses and Tolerance to Heavy Metal Stress

Shaista Qadir, Asiya Hameed, Nahida Tun Nisa, MM Azooz, Mohd Rafiq Wani, Mirza Hasannuzaman, Alvina Gul Kazi and Parvaiz Ahmad

Abstract *Brassica* is considered as an important crop all over the world owing to its economically important products. *B.juncea* and *B.napus* are cultivated as oilseed crops globally. Heavy metal (HM) stress is one of the abiotic stresses that limit plant growth and development. Root and shoot lengths and fresh and dry weights have been observed to act as accumulators as well as indicators of metal toxicity in crops. *Brassica* has a potential to combat the metal-induced stress, thereby reducing

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the damage by undergoing various types of adaptations. However, cost-effective techniques are available in order to minimize the toxicity and to protect the surroundings from HM stress. Decrease in chlorophyll content confers to weak uptake of mineral ions due to the interference of HMs in plants. Nevertheless, low concentrations of some HMs demonstrate an efficient yield in some species. HMs disturb the composition of fatty acids and as a result lead to tremendous changes in lipid membrane that may ultimately cause lipid peroxidation. Proline accumulation enhances the tolerance level under osmotic stress and is known to regulate the water balance in crop plants. Increased glutathione (GSH) in *B. napus* and *B. juncea*, on exposure to HMs, has shown its active involvement in detoxification of free radicals either directly or through certain enzymes. Phytochelations are one of the important methods to reduce the phytotoxicity by binding complexes with high-affinity ligands in the vacuole, thereby keeping the released toxins away from the metal-sensitive metabolic centers in the cytoplasm. Ascorbate–GSH cycle plays an efficient role in reactive oxygen species (ROS) detoxification released through abiotic stress. Besides, ROS shows release of new isozymes of peroxidases. Genetic engineering has been established to enhance the plant's ability to endure and mitigate the environmental stress. This involves the insertion of foreign DNA into nuclear genome and genomic chloroplast. However, gene expression can be regulated by various promoters. Several transgenic approaches have been carried out successfully with enhanced accumulation of HMs in *B. juncea* cultivars. There is lot of scope to understand the mechanism of HM uptake as well as the capacity of plants to withstand the environmental stresses.

Keywords *Brassica* • Heavy metals • Growth • Osmotic stress • Oxidative stress • Antioxidants • Oil content • Metal uptake

1 Introduction

Brassica is an important genus of the family Brassicaceae, also called as mustard family. The family includes 375 genera and 3,200 species, along with a large number of cultivars and hybrids of cultivated origin. It is widely distributed throughout the world. Most species of the genus originated in Western Europe, the Mediterranean, and temperate regions of Asia. *Brassica* species are broadly considered as valuable source of dietary fiber. The oleiferous Brassicas are found within the species like *B. juncea*, *B. carinata*, *B. rapa*, *B. campestris*, and *B. napus*, and are jointly called as rapeseed–mustard oil. The plant is thus associated with great agricultural and horticultural importance because of the economically important products which the genus provides in the form of edible roots, leaves, stems, buds, flowers, seed, and oil (Hasanuzzaman 2008).

Among the family Brassicaceae, several wild relatives of *Brassica*, including *B. adpressa*, *B. fruticulosa*, *B. pinescens*, *B. oxyrrhina*, *B. barrelieri*, and *B. tournefortii*, are collectively referred as *Brassicacoenospecies*. These have useful

agronomic traits that can be introgressed into the cultivated Brassicas using the wide hybridization programs (Warwick 1993). In fact, Brassicas have been successfully crossed with members of *Brassicaceae* species and other wild relatives like *Sinapsis*, *Diploaxis*, *Erucastrum*, and *Raphanus* to obtain the wide hybrids (Nandakumar et al. 1988). There are mainly six species of *Brassica* that merit attention for their economic importance. Among the six species, three are diploid: *B. campestris* (AA, $2n=20$), *B. nigra* (BB, $2n=16$), and *B. oleracea* (CC, $2n=18$), and the other three are amphidiploids: *B. juncea* (AABB, $2n=4x=36$), *B. napus* (AACC, $2n=4x=34$), and *B. carinata* (BBCC, $2n=4x=34$). The amphidiploid species likely originated in nature from diploid ancestors through unidirectional hybridization followed by spontaneous chromosome doubling (Murphy 1994). The botanical or genomic relationship between these six species was established by UN (1935) and is represented in the form of a triangle, usually known as U's triangle. Three diploid species are orientated at the corners of the triangle. In the world, India ranks fourth in oil seeds economy. Mustard contributes 28.6% to the total oil seed production in India (Shekhawat et al. 2012). Heavy metal (HM) causes many direct and indirect effects on plants and animals. Exposure to HM stress leads to decrease in crop yield worldwide (Ahmad K et al. 2011; Ahmad et al. 2011a, 2012; Hasanuzzaman and Fujita 2012; Hasanuzzaman et al. 2013). Excessive amount of HMs causes disturbances in the mineral nutrition and carbohydrate metabolism of plants, thus strongly reducing the biomass production (John et al. 2009). HMs are reported to inhibit the metabolic processes such as nitrogen assimilation, photosynthesis, respiration, water uptake, and transcription (Boussama et al. 1999; John et al. 2009). HMs are responsible for oxidative stress in *Brassica* sp. because: (1) they generate reactive oxygen species (ROS), (2) they inhibit or stimulate the activities of antioxidant enzymes, and (3) HM stress also leads to lipid peroxidation (John et al. 2009; Ahmad et al. 2011a, 2012). This chapter focuses on the effect of HMs on growth, physiological, and biochemical aspects of *B. juncea*, besides discussing the metal tolerance through transgenic approaches.

2 Production in India

India is one of the major rapeseed–mustard-growing countries in the world, holding the top position in area under cultivation and second in production after China. From the past several decades, there has been a continuous increasing demand for edible oilseeds and other by-products in India as well as in other countries, because of which there has been a rapid increase in its production. *Brassica* contributes to 28.6% of the total oilseed crops in India, and occupies the second place among the most important edible oils after groundnut. The rapeseed/mustard oil has the lowest amount of saturated fatty acids as compared to other edible oils. However, the two essential fatty acids linoleic and linolenic acids are present in adequate amount as compared to other edible oils. Globally, 59.93 million t of *Brassica* oilseeds are produced from an area of 30.74 ha with an average yield of 1.9 t ha⁻¹ (USDA 2010).

However, India accounts for 10.7 and 21.7% of the yield and area, respectively. Owing to extensive population growth rate, the percapita oil expenditure is expected to increase from the present 13.4 to 23.1 kg/annum by 2030. This may be due to the change in the way of living, and around 102.3 mt of oil seeds will be needed to fulfill this demand. Regarding the present scenario of the rapeseed–mustard as compared to oilseed production in India, it has been predicted that more rapeseed–mustard need to be produced to fill the gap between demand and supply.

3 Effects of HMs on *Brassica*

HM toxicity is one of the major environmental health problems in modern era (Hasan et al. 2009; Alvarez-Ayuso 2008; Suzukiet al. 2001; Ahmad et al. 2011a, 2012). The term HMs is usually used for any element that has metallic properties (ductility, conductivity, density, stability, ligand specificity, etc.), atomic number greater than 20, and density five times higher than water. The release of HMs in nature is a consequence of anthropogenic activities, including industrial processes and damages to both man-made and natural ecosystems (Karimi and Zulkifili 2010; Nagajyoti et al. 2008; Tyler et al. 1989; Hasanuzzaman and Fujita 2012). These HMs do not vanish but in turn get accumulated in soil (Gisbert et al. 2003; Tangahu et al. 2011), which acts as a sink (Karami and Zulkifili 2010). Although a few of them have nutritional importance, e.g., iron and copper, most of them are cytotoxic. Some are toxic even at very low concentrations (Pehlivan et al. 2009; John et al. 2009; Ahmad et al. 2011a, 2012), while all are toxic at higher concentrations (Wuana and Okieimen 2010) and pose a threat to plants as well as human beings (Rausser and Meuwly 1995). As the concentration of metals is increasing day by day, techniques are required to reduce the release of toxins into the soil and water bodies. The HMs have great impact on morphological, physiological, and biochemical aspects of *Brassica* and are discussed below.

3.1 Growth

Plants, being essential component of our ecosystem, have contributed a lot for serving as biological monitors at the cost of forest dieback and decline, and have thus become a debatable topic. Deficiencies as well as surplus accessibility of an element also exhibit its negative effects. Exposure to HMs results in so many physiological breakdowns that it becomes nearly impossible to determine which effects are primary and which are secondary (Prasad 1995). Despite recent progress in understanding the individual aspects of metal toxicity and resistance mechanisms, little is known about the coordination of cellular sequestration mechanisms with adaptation of plant growth. Plant uptake of metals is dependent on a system that is metabolically mediated and also competitive with Zn and other metals.

Development of plant is considered as an essential mechanism that is formed as a result of coordination of the main processes (Vassilev et al. 1998). During this process, they are not found resistant to various stresses including those of HMs. These metals adversely affect the overall plant development, among which the most common are stunted growth (Fariduddin et al. 2009), leaf chlorosis, and variations in the activity of several essential enzymes of various metabolic pathways (Arduini et al. 1996; Godbold and Hutterman 1985). Parameters such as fresh weight, dry weight, and shoot as well as root lengths have been used as indicators of metal toxicity in plants (Baker and Walker 1989; Ahmad et al. 2011a, 2012; Mohamed et al. 2012; Shanmugaraj et al. 2013). The HMs obstruct the various plant physiological and growth processes. This association of HMs with different developmental processes of plant represents a dose–response curve. Various plants have been observed to act in different ways such as excluders, indicators, and accumulators (Ouzounidou et al. 1998). The same HM, if excessively present, demonstrates the decline in growth and yield (Raziuddin et al. 2011; Shafiq and Iqbal 2005; Shafi et al. 2009, 2010), blocks cell division and development and eventually causes death of the plant.

Raziuddin et al. (2011) observed a significant decrease in all growth parameters of *B. juncea* cv. NIFA-Raya and *B. napus* cv. Abasin on exposure to metal stress, although the former was less susceptible to toxic effects. Similarly, lead (Pb), one of the most important HMs, is frequently available in the environment (Shahid et al. 2011; Grover et al. 2010) and its most common sources are vehicles and automobiles (Wierzbicka and Antosiewicz 1993; Nicholson et al. 2003; Sezgin et al. 2003). This metal accumulates in the roots, stems, leaves, and seeds of the plant (Singh et al. 1998; Sekara et al. 2005; Yilmaz et al. 2009; John et al. 2009) and damages whole uptake system including chlorophyll formation and cell division (Gupta et al. 2010; Krzesłowska et al. 2010; Liu et al. 2008; Sharma and Dubey 2005a; John et al. 2009; Ahmad et al. 2011a, 2012). The severe reduction in root growth among *Brassica* (Canola) cultivars was attributed to Pb-induced impaired nutrient uptake and altered metabolism in plants (Ashraf et al. 2011). The transport of various ions like N, P, K, Ca, Mg, Zn, etc. was reported to get reduced in both roots and shoots of all the cultivars of *Brassica* due to the influence of Pb (Wensheng et al. 1997; Panda and Choudhary 2005; Fodor et al. 1998; Sinha et al. 2006; Gopal and Rizvi 2008), thereby hampering the various physiological processes. The reduction in absorption of nutrients in the presence of lead may result from their competition (e.g., those with atomic size similar to lead). According to Sharma and Dubey (2005a), the strong interaction of K^+ ions with Pb could result from their similar radii ($Pb^{2+} = 1.29 \text{ \AA}$ and $K^+ = 1.33 \text{ \AA}$). These two ions may compete for entry into the plant through the same potassium channels. Similarly, lead affects K^+ -ATPase and -SH groups of cell membrane proteins and causes an efflux of K^+ from roots. However, lead does not cause nitrogen efflux. Nitrate reductase acts as the rate-limiting step in the overall assimilation of nitrate (Xiong et al. 2006; Sengar et al. 2009). Xiong et al. (2006) demonstrated that lead induces a significant reduction in shoot nitrate content (70 and 80%), nitrate reductase activity (100 and 50%), and free amino acid content (81 and 82%) in *B. pekinensis*.

Lead also decreases the concentration of divalent cations (Zn, Mn, Mg, Ca, and Fe) and has been reported in *B. oleracea* (Sinha et al. 2006). Peško et al. (2011) also investigated the effect of seven HM ions (Cd(II), Cr(VI), Cu(II), Hg(II), Ni(II), Pb(II), and Zn(II)) on root growth of five cultivars of *B. napus* and observed that the toxicity of metal ions decreased in the following order $Cu > Cr > Hg > Cd > Pb > Ni > Zn$. Prasad et al. (1999) observed curtailed growth in *B. juncea* under Zn stress.

Mercury, another HM of concern, exhibits significant phytotoxicity in two cultivars of Indian mustard at elevated concentrations, and its uptake induces a significant reduction in both biomass and leaf relative water content (Shiyab et al. 2009). Different concentrations of HMs also influence the root volume. For example, in response to 20 ppm $CdCl_2$ in sand cultures, roots of saplings were 50% smaller in volume (Smith and Brennan 1984). Maize seedlings were found to be decreased at 25 μg Cd/g (Hasset et al. 1976). Root growth and weight also show a considerable reduction in American sycamore by Cd treatment (Carlsson and Bazzaz 1977). Sandalio et al. (2001) observed 20 and 90% decrease in growth rates on exposure to 5 and 50 μM Cd concentration, respectively. Root elongation has been observed to be totally stopped after 5 days of Cd treatment in *Platanus occidentalis* (Kahle 1993). Shoot growth, leaf length, as well as leaf area also reveal the same results against the stress except low doses of cadmium that show comparatively better growth and development of plant (Setia et al. 1993; John et al. 2009). Setia et al. (1993) demonstrated that 8 mM Cd^{2+} stress is responsible for decrease in diameter of new stems by 23% in wheat. Carlson and Bazaaz (1977) also reported reduced growth in American sycamore (*P. occidentalis*) on exposure to Pb–Cd interaction.

A gradual decrease of plant dry matter has been observed in response to HMs. Reduction of plant dry matter (root) was 10–30% in seedlings of American sycamore (*P. occidentalis*) at 10–100 ppm Cd in the nutrient media (Kahle 1993). Likewise, shoot dry weight shows 37–48% decrease with increasing concentrations of Cd and Pb in soybean (Haung et al. 1974). Bhattacharya and Choudhuri (1994) also demonstrated a marked 33.6% reduction in biomass of *Vigna* seedlings with Cd concentration of 10^{-5} M. Vassilev et al. (1998) observed a decrease in dry mass accumulation in barley plants grown in pots. The inhibitory effect was 32–35% at the first harvest (the stage of tillering in control), decreasing to 10–73% at the fourth harvest (the stage of full ripeness in control), with 45 mg Cd/Kg soil. Malan and Farrant (1998) observed a significant decrease in number of pods (83%) and seed mass (16%) with 0.05 mg/L Cd in soybean. HMs (Cd, Zn, and Hg) have been found to reduce the root and shoot lengths of *B. oleracea* var. Botrytis (Theriappan et al. 2011) and 10 different cultivars of *B. juncea* grown under different concentrations of Cd (Qadir 2003). The overall decrease arises due to the suppression of growth of plants owing to an irreversible inhibition by Cd on proton pump responsible for the process (Aidid and Okamoto 1993, 1992). Nevertheless, low concentration of Cd shows a good yield in tomato and eggplants (Khan and Khan 1983), tobacco cells (Hirt et al. 1989), and seedlings of alfalfa (Peralta et al. 2000).

3.2 Yield

The productivity of any crop is dependent on its inherent capacity for photosynthesis, photosynthetic area developed, and availability of photosynthetically active radiations within the canopy. Reduction in photosynthesis accompanied by biomass decrease against the metal stress appears to be an almost universal finding (Piotrowska et al. 2009; Islam et al. 2008; Ouzounidou et al. 1997; Dudka et al. 1996; John et al. 2009). While few studies report yield enhancement with very low concentration of metals (Breckle et al. 1991), a significant decrease 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 marked reduction was observed in the number of siliquae 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 K et al. 2011). Similar findings have been reported earlier by different workers (Bazai and Achakzai 2006; Farid 2006; Kang et al. 2007; Khan et al. 2009). Tamout-sidis et al. (2002) reported that the application of increasing doses of municipal wastewater reduces the overall yield of some vegetable crops, e.g., lettuce, endive, spinach, radish, carrot, and sugar beet. Sharma and Dubey (2005b) reported flower and pod senescence as a consequence of metal toxicity, which leads to the production of less number of viable pods and seeds and reduced yield under metal stress in rice crop. Kakar et al. (2010) and Raziuddin et al. (2011) reported that *B. napus* grown under the influence of HMs turns the soil saline, and as a result of which the plants are unable to take essential elements needed for their vegetative growth, which ultimately results in yield reduction. Ahmad K et al. (2011) proved that the wastewater having higher concentrations of HMs adversely affects the plant growth and development as well as yield. Prins et al. (2011) observed that *B. juncea* growing on seleniferous soils shows decreases in biomass, pollen germination, individual seed and total seed weight, number of seeds produced, and seed germination.

3.3 Photosynthesis

Photosynthetic inhibition is a well-known HM toxicity and has been reported by a number of workers (Xiong et al. 2006; Cenkci et al. 2010; Singh et al. 2010; Touiserkani and Haddad 2012). The decrease in photosynthetic rate may be explained on the basis of number of changes that occur in normal metabolic pathway on exposure to HMs. HMs have been found to inactivate the Photosystem II (PSII) activity along with gradual loss of granal stacks, thereby damaging the thylakoid acyl lipids, with subsequent formation of some polypeptides associated with oxygen-evolving complex and disorganization of light-harvesting complex II (LHC II) antenna system (Islam et al. 2007; Tukendorf and Baszynski 1991). PSII regulates the process of photosynthesis, because it undergoes water oxidation and

sustains the process of electron transport. It is formed of three parts, i.e., a core composed of reaction center proteins D1 and D2, cytochrome CP47, and 33-kDa Mn stabilizers, including various low molecular weight proteins, with an oxygen-evolving system (Barber et al. 1997). Chlorophyll as well as prosthetic groups are essential for charge separation and stabilization of proteins (i.e., both D1 and D2) (Nanba and Satoh 1987). HM involves the breakdown of PSII core proteins like D1 (Oquist and Hunner 1993). The root problem may be that the photosynthesis is coupled with a series of electron transport systems (ETS), and metal ions are suggested to block the electron transport pathway (Qufei and Fashui 2009), thus causing inactivation of PSII as a result of metal instability involved in sequences of reactions. Photosynthetic carbon fixation is the primary target of metal toxicity. Most of the products of this fixation produced by primary light reaction are utilized by carbon metabolism. Oxidized nicotinamide adenine dinucleotide phosphate (NADPH) constantly reinstates the terminal electron acceptor in order to balance the photochemical reaction centers. This also provides the coupling of electron transport with adenosine triphosphate (ATP) synthesis where proton motive forces carry out the feedback inhibition of electron flow, thereby providing a dissipation mechanism for excessive excitation energy, as the products of primary light reaction exceed its synthesis and simultaneously modify the rate of electron transport through PSII. It also affects the quantum yield of linear electron transport. Further, HM tends to accumulate in the chloroplasts and substitutes for essential divalent ions (Cenkeci et al. 2010; Gupta et al. 2009) leading to ion imbalance, thereby affecting the chloroplast activity which is believed to be the cause of PSII disassembly (Geiken et al. 1998). Decreased photosynthetic rate in *B. juncea* cv. NIFA-Raya and *B. napus* cv. Abasin, under the influence of HMs, can also be due to the interaction of metal ions with pigment systems of the plants (Raziuddin et al. 2011).

3.3.1 Influence on Pigments

Pigments are the most important part in plants (Hall and Rao 1999), and nutrient efficiency, plant development, and yield are the result of these pigments (Seyyedi et al. 1999). The chlorophyll consists of tetrapyrrole ring with Mg^{2+} as the central atom. Mn is a key element for photosynthesis as well as for regulation of enzyme synthesis (Doglanlar et al. 2012). A decrease in chlorophyll as a mark of metal toxicity has been reported by many workers (Kang et al. 2007; Sepehr and Ghorbanli 2006; Larsson et al. 1998; Skorzynska-Polit and Baszynski 1997; Rascio et al. 1993; Schlegel et al. 1987; John et al. 2009; Ahmad K et al. 2011; Ahmad et al. 2011a, 2012). Touiserkani and Haddad (2012) reported that the concentration of chlorophyll shows a gradual decrease on exposure to HMs, followed by chlorosis and necrosis (Skorzynska-Polit and Baszynski 1997). A significant decrease in chlorophyll content in *B. chinensis* and *B. pekinensis* (Liu et al. 2004), *B. juncea* cv. Vitasso and *B. napus* cv. Atlantic (Vatehova et al. 2012), and *B. juncea* (Mohamed et al. 2012) was also reported in response to

HM stress. Similarly, Raziuddin et al. (2011) observed a significant decrease in chlorophyll content of *B. juncea* cv. NIFA-Raya and *B. napus* cv. Abasin on exposure to metal stress. Touiserkani and Haddad (2012) observed a significant decrease in chlorophyll content of different *B. napus* cultivars grown under the influence of HMs.

δ -Aminolevulinic acid (ALA) is the first specific precursor in chlorophyll synthesis for ALA-dehydratase (a metal-sensitive enzyme) that is directly affected by HMs (Sasa and Sugahara 1976). The enzyme for the condensation of two molecules of ALA involves the presence of thiol group at the binding sites for both biosynthesis (Nandi and Shamim 1968) and formation of tetrapyrrole protochlorophyllide, and hence prevents the production of chlorophyll (Dahlin et al. 2000; Boddi et al. 1996; Masuda et al. 1996; McEven et al. 1996). The HM stresses have been reported to increase the chlorophyllase activity and subsequent enzymatic degradation, which leads to the deletion of chlorophyll molecules. Reduction may also confer to poor uptake of mineral ions owing to the presence of HM in growth medium. Horvath et al. (1996) reported a reduction in chlorophyll content on exposure to Cd. Ouzounidou et al. (1997) observed the same in leaves of wheat when subjected to 1 mM Cd, which reflects the indirect effect of cadmium on the content of essential nutrients. Erdei et al. (2002) also demonstrated a gradual reduction in barley seedlings against varying concentrations of cadmium. Chatterjee and Chatterjee (2000) reported a decline in chlorophyll content in *B. oleracea* var. Botrytis and cv. Maghi on exposure to copper, cobalt, and chromium.

Apart from inhibition of biosynthetic enzymes for chlorophyll formation, the increased levels of free radicals of fatty acids produced from polyunsaturated fatty acids due to the higher activity of lipoxygenase may also contribute to the decreased level of chlorophyll with HM treatments (Somashékaraiah et al. 1992; Klein et al. 1984; Hildebrand and Hymowitz 1982). The decrease in chlorophyll content may also be correlated with the adverse toxicity of metals on their uptake and accumulation of essential nutrients in plants, viz., Fe, Mg, Ca, and K as reported in wheat (Ouzounidou et al. 1997; Greger and Ogrer 1991; Greger and Lindberg 1987). Earlier reports suggest that the change in Fe to Zn ratio may be responsible for the reduced chlorophyll content in plants (Root et al. 1975). Recent studies, however, suggest that the formation of LHC is disturbed in HM-treated leaves (Horvath et al. 1996), and as a result, the LHC protein synthesis stops at the transcriptional level (Tzivelka et al. 1999). The product formed during stress also causes photoxidative breakdown, as demonstrated in barley/oat leaves (Luna et al. 1994). Similar results were reported by Hegedus et al. (2001) in barley, where the high quantity of chlorophyll was disintegrated after the initial Cd treatment. Mg, being an important component of chlorophyll molecule, gets removed by an enzyme called Mg dechelataase; subsequently, a ring opens, and as a result of dioxygenase activity, the binding protein is set free for degradation. The rest of the chlorophyll catabolites are carried to the vacuole where further metabolism occurs (Buchanan-Wollaston 1997). Liu et al. (2008) reported an increase in chlorophyllase activity upon Pb stress in *Sedum alfredii*.

3.3.2 Carotenoids

Carotenoids (accessory pigments) are present in the thylakoid membrane of chloroplasts (Collins 2001). They form the diverse set of pigments found in nature and are produced by all photosynthetic and many non-photosynthetic organisms (Nishio 2000). Due to chlorophyll degradation, leaves appear yellow, but this is seen merely when the plant reaches to senescence naturally or under the stress conditions. Carotenoids have an essential role in photoprotection and in scavenging ROS (Young and Britton 1990). During this mechanism, the transfer of excited chlorophyll and singlet oxygen to carotenoid takes place, which quenches as well as dissipates them without undergoing any chemical change (Young and Britton 1990; Bartley and Scolnik 1994). They accumulate primarily in photosynthetic membranes in association with LHC and reaction center complex. Carotenoid content is least affected (Clijsters and Van Assche 1985) or usually enhanced under the stress conditions (Ralph and Burchelt 1998; Foyer and Harbinson 1994). The plant's potential to adjust the levels of accessory pigments is significantly essential to endure the abiotic stress and, thereby, enhance the tolerance, as they shield the photosynthetic tissues against photosensitization. The carotenoids are responsible for de-epoxidation of singlet oxygen and oxygenated state of carotenoids. The xanthophylls undergo interconversion from one form to another in a cyclic way, leading to the non-photochemical quenching of the excessive excitation energy (Demming-Adams and Adams III 1992). While an enhancement in non-photochemical absorption of this excitation energy has been observed in land plants against HMs (Krupa et al. 1993a), the use of xanthophyll cycle in such dissipation has not been reported so far. The ability of HMs to stimulate the production of carotenoids is supposed to restore the damaged pigments during their interaction with excited chlorophyll molecules.

3.4 Oil Content

In the global oilseeds context, rapeseed–mustard occupies an important place, and in India these are next to groundnut, contributing about 32% of the total oilseed production. Rapeseed–mustard showed a remarkable progress in terms of production and productivity during the last decade, but the data from National Productivity reflect that despite a major gain in realized yield, substantial breakthrough in terms of potential yield remains to be achieved. Abiotic stresses, including drought, heat, cold, salinity, and HMs, are the increasing problems not only affecting the plant yield but also altering the composition of fatty acids of membrane lipids.

Fatty acids are free carboxylic groups with a biological chain of commonly occurring 16–18 carbon atoms. Some of the chains are either saturated or unbranched and few are unsaturated and branched containing three carbon rings or hydroxyl groups. Components of phospholipids, glycolipids, hormones, and intracellular messengers are essential groups of fatty acids synthesized in cytosol. Synthe-

sis and breakdown of citrate carrying an acetyl group occur from mitochondria to cytoplasm. NADPH is required for this process, which is produced by pentose phosphate pathway as well as during the movement of reducing equivalents from mitochondria by malate–pyruvate shuttle. Fatty acids are triacylglycerols (neutral fat) present in adipose tissue, which can be activated to acyl CoA by the hydroxylic action of lipases. They are then translocated to inner mitochondrial membrane by carnitine, and simultaneously destroyed in the mitochondrial matrix. They are elongated and desaturated by enzyme systems in the endoplasmic reticulum membrane. Besides nicotinamide adenine dinucleotide (NADH) and O_2 , the complex consists of a flavoprotein, a cytochrome, and a nonheme iron protein required for carrying out this process.

Sinha et al. (2010) showed that *Brassica juncea* cultivated on Cu, Cr (VI), As(VIII), As(V) contaminated soils, non-significant decrease in oil yield was observed, except for Cr and higher concentrations of As(V). Both As (V) and Cr showed a decrease in oil content, but the maximum reduction has been found in Cr-treated plants (Sinha et al. 2010). Adverse climatic changes like temperature (Pleines et al. 1987; Tremolieres et al. 1982), salinity (Allakhverdiev et al. 1999; Elenkov et al. 1996), and HMs (Howlett and Avery 1997; Fodor et al. 1995; Frostegard et al. 1993; Krupa and Baszynski 1989; Hameed et al. 2012) change the composition of fatty acids in plants. High temperature leads to tremendous enhancement of $C_{18:1}$ content and a decrease in $C_{18:3}$ content. Low temperature increases $C_{18:1}$ and $C_{18:2}$ desaturation, resulting in higher $C_{18:3}$ content (Pleines et al. 1987; Tremolieres et al. 1982). In a study carried out by Ouarti et al. (1997), cadmium enhances the proportion of $C_{16:0}$ and lowers the proportion of $C_{18:2}$ and $C_{18:3}$ in 17-day-old tomato seedlings. The result of this study suggests that metal stress induces changes in the unsaturated fatty acids. In addition, the buildup of $C_{16:0}$ rather than $C_{18:0}$ signifies a change in the ratio of products from fatty acid synthase. Likewise, Krupa and Baszynski (1989) reported the similar findings with thylakoids and demonstrated the lowering of all individual glycolipids and phospholipids, and the maximum reduction was found in phosphatidylcholine content. Composition of acyl lipids removed from thylakoids was illustrated by a considerable decrease in the trans- δ -3-hexadecanoic acid with an exception of linolenic acid that tends to fall among all lipids.

4 Responses of *Brassica* Towards HM Stress

4.1 Osmotic Stress

Water is considered as a major factor in the regulation of plant growth. Plant water relation is strongly disrupted by metal ion toxicity (Brunet et al. 2009). This effect is primarily dependent on the chemical property of metals like its valence state, ionic radii, and potential of forming organic system. Beyond the limits, all

metal ions in plants cause alteration of water relations (Haug and Caldwell 1985), alteration in turgor pressure (Qureshi et al. 2007), and alteration in plasma membrane properties, along with inhibition of root growth (Hagemeyer and Breckle 2002). These actions in turn give rise to secondary impacts like hormonal imbalances, deficiency of essential nutrients, changes in photoassimilate translocation, and alteration of water relations. Increased interest in plant water relations under metal toxicity stress also derives from the fact that plants in metal-enriched soil frequently suffer from osmotic stress, mainly because of poor physical soil conditions as well as shallow root system. Osmotic stress involves some alterations in the solute concentration around a cell, causing rapid modifications in the water across its cell membrane. When the concentration of salts is high outside the cell, exosmosis of water takes place. Salt stress also prevents the mobilization of essential ions, substrates, and cofactors from the cell and results in “cell shock.” Likewise, at low concentrations, water is drawn into the cell in greater quantity, thus causing it to enlarge, rupture, or undergo apoptosis. HM-induced drought conditions threaten the cells with dehydration, and the productivity and quality of many commercially grown agronomical and horticultural crops are often adversely affected. To overcome this stress, plants are equipped with many beneficial molecules like osmolytes and osmoprotectants.

4.2 Osmolytes and Osmoprotectants

4.2.1 Proline

For a long time, proline was considered as an inert compatible osmolyte that protects the subcellular structures and macromolecules under osmotic stress (Mehta and Gaur 1999; Kavi-Kishor et al. 2005; Ahmad et al. 2008, 2010c, 2011a, 2014a, b; Rasool et al. 2013; Katare et al. 2012). However, proline accumulation can influence the stress tolerance in multiple ways (Szabados and Savoure 2010). Other than plants, proline accumulations have been widely distributed in eubacteria, protozoa, and algae (Rasool et al. 2013). A common response of plants to HM stress is the accumulation of proline (Muneer et al. 2011; John et al. 2009; Qureshi et al. 2007; Ahmad et al. 2011a, 2012; Parmar et al. 2013), other organic acids, etc. So far, research has been carried out to study the impact of HM-induced ion toxicities on different plants (Zhang et al. 2000; Schat et al. 1997; Bassi and Sharma 1993), including cultivars of *B. juncea* (Buddh and Singh 2012; Sharma et al. 2010; Qadir 2003; Saradhi et al. 1993; Ahmad et al. 2011a, 2012), and maximum studies revealed the accumulation of proline contents under HM stress. An experiment conducted on *B. oleraceavar. Botrytis* under the influence of Cd, Zn, and Hg reported an increase in proline content (Theriappan et al. 2011). Proline gets accumulated in plants under different stresses like mineral nutrient deficiencies (Possingharri 1956; Ahmad et al. 2014a), high salinity (Arshi et al. 2002; Thomas and Bohnert 1993; Katare et al. 2012; Rasool et al. 2013), osmotic stress (Voet-

berg and Sharp 1991), and oxidative stress (Ahmad et al. 2010b, 2011b; Ahmad and Umar 2011; Ahmad 2014). Proline accumulation has been shown to increase the resistance of plants to various stresses (Aspinall and Paleg 1981) by acting as a solute that protects them against enzyme denaturation (Sharma and Dubey (2005a, b); Paleg et al. 1984, 1981; Nikolopoulos and Manettas 1991), a reservoir of energy sources like carbon (C) and nitrogen (N), a solute that alleviates the protein synthesis mechanism, a means of reducing the cell acidity, a sink for energy to regulate the redox potentials, and a shuttle for reducing the power among cell components (Forlani et al. 2000). Kishor et al. (1995) also demonstrated that accumulated proline showed the tolerance capacity against the osmotic stress. Accumulation of proline played a great role in maintenance of water balance in plant tissues (Costa and Morel 1994) and scavenging of free radicals from the cells (Smirnoff and Cumbes 1989). It also acts as a part of nonenzymatic free radical detoxification similar to the mechanism of glutathione (GSH), α -tocopherol, and glucose (Alia et al. 1995). It is very difficult to speculate the mechanism that accounts for the accumulation of proline under extremely varying conditions (Bassi and Sharma 1993; Alia and Saradhi 1991; Saradhi and Saradhi 1991). The movement of ETS has been reported to reduce in plants or its parts against the stress. As a result, there is a buildup of NADH^+H^+ . In addition, the enhancement of NADH to NAD^+ ratio under water stress has been already demonstrated. Such an increase in NADH might affect the substrate despite metabolic reactions requiring NAD^+ . Furthermore, buildup of organic acids causes a reduction in cytosolic pH. The accumulation of citrate is attributed to declining NAD^+ as well as synthesis of malate and lactate (by oxidizing NADH). Hence, proline synthesis from glutamic acid is regarded as an adaptive mechanism in order to decrease the buildup of NADH and also to lessen the acidity, whereby two of NADH^+H^+ are used for combining each molecule of proline with glutamic acid. Boussama et al. (1999) suggested that the induction of NADH glutamate-dehydrogenase activity under Cd stress might provide the glutamate required for enhancing the synthesis of proline. These findings strikingly show that proline has a key role in nonenzymatic free radical detoxification processes.

4.3 Oxidative Stress and Antioxidants

Aerobic metabolism provides marvelous energy benefits to the organisms, but at the same time a constant hazard is the oxidative damage arising from by-products of respiration and photosynthesis, such as superoxide and hydrogen peroxidocellular macromolecules such as proteins, lipids, and DNA. The phenomenon is known as oxidative stress. HMs were found to induce oxidative stress in plants (Yadav 2010; Singh et al. 2010; Grover et al. 2010; Liu et al. 2008; Pourret et al. 2008; Okamoto et al. 2001; Piqueras et al. 1999; Hendry et al. 1992; Somashekaraiah et al. 1992; John et al. 2009; Ahmad et al. 2011a, 2012; Shanmugaraj et al. 2013; Parmar et al. 2013). Oxidative stress is induced by the generation of ROS in the cells