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## RESEARCH ARTICLE

### OMICS TECHNOLOGIES FOR HEAVY METAL STRESS TOLERANCE IN PLANTS.

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#### Abstract

Heavy metal pollution has become a major constraint to crop yield and quality in the current times. These toxic metals amend various biochemical and physiological pathways and thus impose harmful effects on the growth and development of the plants. Nowadays, this situation has increased at an alarming rate and ultimately leads to food crisis in near future. To counter balance the heavy metal toxicity in plants, an in-depth understanding ranging from morphological to metabolome level is an essential step to improve crop yield. Recent advances in the omics technologies have assisted to unravel various metabolites/genes, stress proteins, ions and transcription factors and could be used as novel candidates to engineer plants tolerant to heavy metal stresses. Here we discuss the plant-heavy metal dynamic interactions in the light of omics era.

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#### Introduction:-

From the last few decades, heavy metal toxicity possess a great threat to the environment due to indiscriminate usage of synthetic fertilizers, mal-agricultural practices, industrialization and other anthropogenic activities which in turn leads to deterioration of soil quality thus affects the crop yield (Piscopo et al., 2016; Pirzadah et al., 2018). This situation creates an urgency to focus the research on assessing the impact and magnitude of HMs on plants and other living creatures. The toxicity of HMs depends upon various factors viz., pH, dosage, oxidation state and time of exposure. However, it is described that HMs at low concentration possess beneficial effects and the phenomenon is known a hormetic effect while as at high concentration they exhibit reversal effect (Morkunas et al., 2018). Many of the HMs known as biometals play a pivotal role in various metabolic pathways and form an important constituent of metallo-enzymes, but excess dosage of both essential as well as non-essential HMs in the substratum leads to toxicity and thus halts various processes ranging from morphological, genomic, proteomic and metabolomic level (Shahzada et al., 2018). The negative impact of HMs on plants involves halted growth, reduction in biomass, chlorophyll and protein degradation, restrain water channel proteins, inhibit nutrient uptake, inactivation of enzyme defense machinery and eventually death of the plant (Fidalgo et al., 2013; Singh et al., 2016; Pirzadah et al. 2018). Thus, HM pollution is one of the important limiting factors to economic growth and it is predicted that it reduces the production yield by 70% worldwide and therefore are considered as major constraint to crop yield (Jewell et al., 2010; Wang et al., 2015; Singh et al., 2016). Further, if this situation persists at the current pace it will ultimately disturb the equilibrium and makes the soil inadequate for crop production. Therefore, it is imperative to better understand the plant-HM dynamic interaction and searching novel tools for crop improvement programmes. Presently, the arrival of omics technologies (genomics, proteomics, metabolomics, ionomics and transcriptomics) possess a great thrust in stress biology to unravel various regulatory elements involved in plant-HM interactions. The novel metabolites obtained during omics analysis will be helpful to impart HM tolerance in commercialized

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crops besides being used to engineer the plants with desired agronomic traits (Atkinson and Urwin, 2012). Keeping in view the above facts, the major goal of the current supplement is to unravel the plant-HM dynamic interactions and HM stress tolerance in the light of omics era.

### **Harmful effects of HM on plants**

Various heavy metal possess a great threat to agricultural sector when their level in the soil increases beyond threshold values (Xiong et al., 2014; Pierart et al., 2015). However, the degree of toxicity of HMs in plants varies depending upon distinct factors viz., genotype, exposure time and HM concentration in the soil. HM toxicity affects the plant by disrupting various physiological and metabolomic pathways. These obnoxious chemicals are responsible to cause various abnormalities ranging from morphological to molecular level and thus leads to the reduction in crop yield (Amari et al., 2017). Moreover, the toxicity of these HMs generate free radicals (ROS) that disturbs the equilibrium of redox homeostasis and is considered to be the main cause of HM stress in plants (Huang and Wang, 2010; Gill and Tuteja, 2010; Pourrut et al., 2011; DalCorso et al., 2013; Iqbal et al., 2015; Shahid et al., 2016; Abozeid et al., 2017). Usually, plants upon HM induced stress resulted in shunted growth (Sharma and Dubey, 2007), inhibit photosynthesis, restrain aquaporins, oxidative damage, and disturb carbohydrates metabolism, breakdown of osmolytes, inactivation of enzymes and ultimate apoptosis (Dalcorso et al., 2010). Wang et al., (2013) reported that excess concentration of copper (Cu) not only causes reduction of biomass but also disrupt electron transport chain (ETC) by impregnating plastocyanin protein of photosynthesis. Excessive Cu concentration alters morphology and impedes root length by regulating plant hormone (auxins) which antagonizes nitric oxide activity (Peto et al., 2011). Bardiya-Bhurat et al., (2017) observed chlorophyll degradation in *Tagetes erecta* subjected to nickel (Ni) and lead (Pb) induced stress. Besides, it is reported that Pb causes oxidative damage, impairment in seedling growth, transpiration, seed germination, chlorophyll biosynthesis and enzyme inactivation by reacting with their sulfhydryl (-SH) group and thus disrupts their structure and function (Pourrut et al., 2011; Kumar et al., 2017; Hakeem et al., 2018). Zhou et al., (2018) revealed that Pb toxicity halts the growth of privet plants and decline their photosynthetic efficiency as evident from various photosynthetic indices by causing chlorophyll degradation. HMs are also involved to suppress plant growth by inhibiting mitosis thus disrupts cell division and elongation as proclaimed by various researchers (Thounaojam et al., 2012; Singh et al., 2013; Wani et al., 2018; Pirzadah et al., 2018). Similarly, cadmium (Cd) is involved to alter various physiological processes by inhibiting mineral uptake, restrains aquaporins, and thus affects the crop yield (Asgher et al., 2015; Rizwan et al., 2016).

### **HM stress tolerance in plants in the light of omics era**

To address the challenges of HM toxicity and better understanding of plant-HM dynamic interactions, the novel approaches of omics technologies play a significant role to unravel the dynamic regulatory elements that help to impart stress tolerance in plants besides; the genes and metabolites could be used as novel candidates in crop improvement programmes to engineer plants in enhancing tolerance to HM stress and developing crops with novel and desired agronomic traits. Current omics tools which involve genomics, proteomics, ionomics, transcriptomics and metabolomics helps us to provide a comprehensive picture of plant-HM stress tolerance mechanisms.

### **Genomics**

Plants adapted to HM stress are having well-regulated gene expression and signalling system. Our understanding of these factors and the key mechanisms involving a wide array of genes and their expression is still in its early stages. In the past few decades, several molecular mechanisms were identified, which are particularly related to HM transport and tolerance. Moreover, expression profiles of several major genes associated with HM stress were elucidated and provided vital insights to our understanding of HM stress in plants. Therefore, the identification and characterization of such novel candidate genes is of paramount importance to give a comprehensive view of HM tolerant genes. However, the lack of knowledge in relation to transport, chelation and sequestration of HM ions and expression of genes during this process is limited. Studying the molecular genetics of model organisms has made significant contribution in understanding the HM sequestration metabolism in plants (Hossain et al., 2012). Such studies related to constitutive gene expression can help to a significant level in applying the molecular genetics that can help in plant genetic transformation and phytoremediation. A recent development through genetic manipulation offers a promising tool for HM stress tolerance in plants. Reports revealed that genes responsible for glutathione which includes  $\gamma$ -glutamylcysteine synthetase (GSH1), glutathione synthetase (GSH2), cystathionine synthase (CTS), ATP sulfurylase (APS), serine acetyltransferase (SAT), glutathione reductase (GR) and PCs which include phytochelatin synthase (PCS) glyoxalases (glyoxalase I and II) synthesis provides tolerance against HMs by regulating glutathione and therefore induces resistance against various HMs. Hossain et al., (2012) reported that overexpression of HM induced genes resulted in tolerance and hyper-accumulation of HMs in several plants. The

expression level of GSH and PCs genes were studied in *Medicago* seedlings subjected to Cd and Hg stress. It was observed that the expression of genes like *ECS/GS/hGS/GR1/GR2/GPX* and *PCS* using semi-quantitative RT-PCR were analysed and the results clearly revealed the change in the expression level of genes with respect to the duration of exposure to Cd and Hg stress (Ortega-Villasante et al., 2007). In the same way, the rice roots were subjected to Cd stress, which leads increased expression of GST and APX gene family (Lee et al. 2010). GSTs have been found to be directly associated with the sequestration of Cd<sup>2+</sup> by the formation of GSH-Cd complexes (Adamis et. al., 2004). Similarly, gene expression level was studied in hydroponically grown ryegrass plants, which were exposed to variable concentration of Si and Al stress.

Pontigo et al., (2017) reported that the Si transporter genes (*Lsi1* and *Lsi2*) in the roots of ryegrass showed down-regulation under Si and Al stress alone but the same genes exhibits up-regulation upon Si application to Al stressed plants thus indicating that Si has the ability to overcome Al stress by modulating the corresponding gene expression levels. Xu et al., (2018) reported that *JrVHAG1* gene plays a significant role in Cd tolerance through ABA-signal pathway and besides involve the regulation of MYB transcription factors. It clearly indicated that there is a strong interaction at molecular level to combat the negative impacts of HM toxicity. Based on the above mentioned studies it can be suggested that the gene expression related to glutathione, phytochelatins and metallothioneins are the ideal candidates in the HM detoxification mechanism so as to impart HM tolerance in plants. With the evolution of CRISPR/Cas9-(Clustered Regularly Interspaced Short Palindromic Repeats)-a genome editing tool, it becomes possible to edit the genome of an organism with more accuracy and this technology has gained a rapid momentum in the current era due to its simplicity and high efficiency, thus revolutionizes the genomics field. CRISPR technology is a novel promising genome editing tool that could be employed to develop HM resistant plants. CRISPR/Cas9 was recently used to develop a novel *indica* rice lines, which possess low Cd levels in grains by knocking out the metal transporter gene *OsNramp5* without compromising the yield production (Tang et al., 2017). This technique can be used for the manipulation of important genomic sequences in plants and thus helps in the identification and characterization of key genetic elements that takes part in phytoremediation processes. Sequence analysis of plants helps us to develop CRISPR systems for imparting HM tolerance in plants by targeted engineering involved in different HM detoxification pathways viz., hyper-accumulation, complexation, compartmentation, chelation, volatilization and degradation. CRISPR/Cas9 technology could be used to modify the expression of HM-induced genes/metabolites/proteins to generate HM resistant plants thus boost the phyto-technology and enhance production yield. Modifying genes of interest, their expression, signalling pathway and redox homeostasis networks that support hyper-accumulation, tolerance or sequestration, can be a revolutionary approach for rejuvenating the HM contaminated soils via phyto-engineering and concurrently, recovering elements of economic interest via phytomining besides generate energy through biomass production.

### Proteomics

From the last few decades, a comprehensive analysis on plants-HM interactions has been conducted to unravel the HM stress tolerance mechanism. Although, genomics have been a valuable tool in addressing plant-HM interactions (Bohnert et al. 2006), but modifications in gene expression at transcript level have not always been reflected at protein level (Gygi et al. 1999; Hossain and Komatsu, 2013). Therefore, an exhaustive proteomic analysis offers a novel platform to analyze specific proteins that earnestly involved in the detoxification of HMs and to study complex biological reactions. Although, the plant-HM interaction mechanisms has been elucidated extensively over the previous decades (Hossain et al. 2012). However, the proteomic approach robustly illustrated the involvement of defense proteins/enzymes that take part in several metabolic pathways (POD, CAT, GSH, GSTs, Gly I, Prx, Trx) and therefore aids in mitigating HM oxidative stress by either direct sequestration of HMs or scavenging of ROS generated during oxidative stress. The previous proteomic study reported that there exhibits a positive correlation among stress induced proteins and the magnitude of stress. Proteins being an important osmolytes act directly in the HM detoxification mechanism and thus proteomic knowledge is of paramount importance for in-depth analysis. Besides, the proteomic tool is an excellent approach to elucidate the potential link among proteins abundance and plant stress modifications. Moreover, it provide a thorough knowledge to better understand various physiological processes under HM stress viz., recognition of stress biomarkers, signaling cascades that in turn leads to the modifications in the expression of various novel genes/metabolites at transcriptional level that could be utilized for an acquisition of increased HM tolerance in plants (Kosová et al., 2011; Singh et al., 2016). Several proteomic studies in plants subjected to HM stress revealed over-expression of defense proteins for quenching ROS besides, the molecular chaperones play an essential role in maintaining the conformation of functional proteins involved in HM stress tolerance in plant in order to retain the redox homeostasis (Wang et al., 2015). The first and foremost reaction of plants towards HM toxicity involves the stress sensing and then transduces the signal into the cell to

stimulate genes and generate signaling cascades (Hossain et al., 2012; Singh et al., 2016). Therefore, to decode the language of protein signatures involved in imparting HM stress tolerance in plants, a comprehensive study of molecular mechanism is an essential step to better understand the plant-HM interactions.

### Metabolomics

The metabolomics science is essential to decode various low molecular weight metabolites that play a fundamental role in HM stress tolerance in plants (Arbona et al., 2013). The function of organic acids to impart tolerance to plants against HM toxicity has been widely studied over the last few decades. The secretion of organic acids viz., citric acid, malic acid, oxalic acid in response to HM stress in plants helps to sequester these toxic metals inside the vacuoles (Osmolovskaya et al., 2018). The fundamental mechanism involved in sequestration of HM by organic acids is their capability to form strong bonds using carboxyl group with HM ions through metal chelation or compartmentation of free ions (Ma et al., 1997; Pirzadah et al., 2014; Osmolovskaya et al., 2018). Current research has unravelled the role of certain organic acids to detoxify the  $Al^{3+}$  by either forming chelating complexes inside cytosol or at the root-soil interface or rhizosphere. Several plants viz., amaranth, spinach and tomato are reported to secrete organic acids (oxalates) when subjected to  $Al^{3+}$  stresses (Ryan et al., 1993; Ma et al., 1997; Yang et al., 2008; Zhu et al., 2011; Osmolovskaya et al., 2018). Though, citric acid possesses strong affinity for iron (Fe), Cd and Ni but it is mainly involved in chelating Fe (Cataldo et al., 1988). Similarly, malic acid is involved to chelate zinc (Zn) especially cytosolic ions (Mathys, 1977).

Glutathione is an essential low molecular weight water-soluble tripeptide (c-Glu-Cys-Gly) and plays a pivotal role in the HM tolerance mechanism (Pietrini et al., 2003). It either acts directly to scavenge HMs or is used as a precursor to generate powerful antioxidants that act on ROS generated during HM-induced stress in plants (Noctor and Foyer, 1998; Halliwell, 2006; Talukdar and Talukdar, 2014). Besides, GSH is involved in the biosynthesis of phytochelatins (PCs) that plays a crucial role in sequestration of HMs and thus maintains a steady state level. Various authors reported enhancement of glutathione level in response to Cd, Cr and Al stress in *Pisum sativum* (Metwally et al. 2005), *Vigna radiata* (Karuppanapandian et al. 2006) and *Vigna mungo* (Karuppanapandian and Manoharan, 2008).

### Ionomics

This omics approach involves the role of macronutrients (Ca, N, K, P, S and Mg) and micronutrients (Zn, Cu, Fe, Mg, Co, Mo and Mn) to alleviate HM stress in plants. These elements play an important role in various physiological processes such as; growth and development but excessive concentration beyond threshold level results in the negative impact on plants. Although toxic to plants at high concentration, but under optimal level they act as strong alleviating agents to combat HM stress.

Calcium (Ca) plays a pivotal role in regulating the activities of various enzymes and thus acts as a defense agent to alleviate HM stress in plants. Reports revealed that Ca mitigates Cd toxicity in plants as both compete for the same Ca-channels (Nelson et al., 1986; Lauer Júnior et al., 2008). Due to some unique features among Ca and Cd mineral elements such as; chemical similarity (ionic radii), same ion channels and intracellular Ca-binding sites, Cd replaces Ca-ions and thus affects the growth attributes but application of Ca causes reversal effects by alleviating the Cd toxicity (Sarwar et al., 2010; Farzadfar et al., 2013; Choong et al., 2014). Various authors proclaimed the alleviation effect of Ca in several plants such as; *Arabidopsis*, *Lactuca sativa*, *Brassica juncea* (Suzuki, 2005; Zhenyan et al., 2005; Ahmad et al., 2015; Siddiqui et al., 2012; Zhao et al., 2015; Srivastava et al., 2015; Gong et al., 2016). The mitigation effect of Ca to reduce the Cd toxicity in plants could be due to the negatively charged plasma membrane and high level of intracellular Ca which neutralizes the membrane surface and thus reduces the Cd toxicity or it could be due to the enhanced pH level in the substratum that immobilizes the metal ions and thus makes them unavailable to plants (Tlustos et al., 2006; Singh et al., 2016).

Magnesium (Mg) is one of the main constituent of chlorophyll and is also involved to activate more than 300 enzymes viz., glutathione synthase, protein kinases, carboxylases etc), besides plays an important function in regulating ion transport and maintain cationic equilibrium in plants (Hailes et al., 1997; Bose et al., 2011). Various studies have been revealed the alleviation efficiency of  $Mg^{2+}$  to mitigate HM toxicity ( $Al^{3+}$  and  $Cd^{2+}$ ) (Kashem and Kawai, 2007; Bose et al., 2011; Shen et al., 2016). Application of  $Mg^{2+}$  to the nutrient medium mitigates the  $Cd^{2+}$  toxicity in *Brassica rapa* as reported by Kashem and Kawai, (2007). Shen et al., (2016) reported that application of  $Mg^{2+}$  mitigates the  $Pb^{2+}$  toxicity as evident by enhanced biomass accumulation and photosynthetic efficiency.

Sulphur (S) being an important structural component of various coenzymes and vitamins plays also an essential role in protein biosynthesis. Sulphur mitigates the Cd toxicity due to the formation of hydrogen sulphide (H<sub>2</sub>S) in the soil substrate through vulcanization which in turn leads to the generation of insoluble cadmium sulphide (CdS) and thus makes it unavailable to plants (Dong et al., 2007). Moreover, S is also involved in phytochelatin biosynthesis, regulates ethylene signaling and therefore alleviates the HM toxicity in plants (Masood et al., 2012).

Micronutrients such as; Zn, Fe, Cu, Mn, Mo and Co although take part in various metabolic processes but excessive level of these elements possesses hazardous effects to plants by halting numerous biochemical pathways. The importance of these micronutrients either is due to their role in redox reactions or acts co-factors of various enzymes (Sanita di Toppi and Gabbriellini, 1999). Besides their role in various biological systems, these are also reported to play an essential role in mitigating HM stress by either act directly to reduce HM solubility in soil (Matusik et al., 2008), compete for the identical membrane transporters and sequestration of HM in their plant vacuoles or indirectly by means of dilution effect and enhance the antioxidant enzyme defense machinery (Salt and Rauser, 1995; Zaccheo et al., 2006; Jalloh et al., 2009). Moreover, the application of silicon (Si) to rice seedlings alleviates the Cu/Cd toxicity by regulating P-type HM ATPases as reported by Kim et al., (2014). Another important mitigating trace element involves selenium (Se) that has positive impact on plant growth and development and enhances the production yield (Pukacka et al., 2011; Pezzarossa et al., 2012). Currently, there are various reports to alleviate HM toxicity using Se as an alleviating agent (Kumar et al., 2012; Mozafariyan et al., 2014; Han et al., 2015). An in-depth analysis was established to study the mitigating effect of Se against various HMs such as; Cd/Cr/As and Pb (Feng et al., 2013; Saidi et al., 2014; Qing et al., 2015). The underlying mechanism involves the inhibition of Cd/Pb uptake by direct sequestration or formation of non-toxic Se complex compounds and inhibits their translocation to the aboveground part besides reduction in the generation of free radicals (Lin et al., 2012; Saidi et al., 2014; Qing et al., 2015).

### Conclusion:-

HM pollution is currently a major challenge due to agricultural practices, industrialization and population explosion as it directly affects the production yield and thus causes food crisis in near future. Although, plants possess an innate ability to detoxify these toxic metals by regulating various metabolic pathways but as the concentration of these toxic metals is increased day by day, many commercially important crops are not going to cope up the HM toxicity and ultimately it will reduce the food basket. As plants face multiple stresses, therefore an interdisciplinary approach is needed to unravel different HM regulatory pathways to better understand the plant-HM dynamic interaction. The current review concludes that the omics technologies have the potential to revolutionize the stress physiology by providing a comprehensive elucidation of the genes/metabolites/proteins or ions that play a pivotal role in HM stress tolerance.

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