PERSPECTIVE

A thorough assessment of biotechnological options for improving heavy metal tolerance in neglected and underused legume crops

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ABSTRACT

Heavy metal contamination of agricultural land and water as a result of growing industrialization and urbanization, as well as natural processes, has become one of the most significant limitations to crop growth and production. Plants should be able to manipulate numerous physiological, biochemical, and molecular processes to increase their growth and development under heavy metal stress, according to a number of studies. It is now feasible to customize legumes and other plants to overexpress stress-induced genes, transcription factors, proteins, and metabolites that are directly implicated in heavy metal stress resistance thanks to contemporary biotechnological tools and procedures. This article gives a comprehensive overview of several biotechnological techniques and/or tactics for increasing heavy metal detoxification by boosting phytoremediation processes. Synthetic biology methods utilized in the engineering of legume and other crop plants to withstand heavy metal stress are also reviewed, as well as some pioneering examples of synthetic biology tools being used to change plants for specific features. CRISPR-based plant genetic engineering, including their involvement in altering the expression of numerous genes/transcription factors to increase abiotic stress tolerance and phytoremediation capacity utilizing knockdown and knockout techniques, has also been critically examined.

Key Words: *Legume crops; Biotechnology; Heavy metals; CRISPR*

INTRODUCTION

Because plants are sessile by nature, they are subjected to a variety of demanding environmental circumstances throughout their lives that can negatively impact their growth and development. Because heavy metals accumulate in many sections of agricultural plants, they limit plant growth/ productivity and pose serious health risks to humans. Various metals and metalloids, such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co), aluminum (Al), and chromium (Cr), cause significant toxicity when they reach the soil agro-ecosystem by natural or manmade processes. Preposterous anthropogenic actions have resulted in an expanded pool of heavy metals, which has raised the amount of metals existing in the earth's crust. As a result, it is critical to distinguish between naturally occurring metals and those that have built up as a result of human activities. Weathering of rocks, soil erosion, forest fires, and volcanic eruptions are natural causes of heavy metal pollution, whereas human sources include extensive mining, metal smelting, chemical fertilizer application, industrial/sewage discharge, and coal combustion. The usual geochemical cycle of metals/elements has been disrupted by rapid industrialization and technological breakthroughs, which has increased their accumulation in soil horizons. Increased heavy metal bioaccumulation over the threshold level has been found to have a severe influence on the natural food chain and microbial flora, and is now being viewed as a serious danger to the ecosystem and environment.

Plants require tiny amounts of specific elements as growth regulators or cofactors in cellular/biochemical processes, and they are also found in a variety of proteins/biomolecules. These heavy metals may have a harmful effect on plants at greater concentrations, affecting their cellular, biochemical, and molecular functioning. To counteract the harmful effects of heavy metals, plants have evolved intricate systems for metal absorption, storage, and detoxification. Plants use a variety of enzymatic and non-enzymatic antioxidants, as well as the synthesis of suitable solutes and the formation of low molecular weight thiols/phytochelatins, to counteract the harmful effects of heavy metals. Underutilized legumes have made a substantial contribution to rural dietary needs, particularly in the face of unfavorable weather conditions such as drought and famine. These crops are lifesavers for millions of people, especially in indigenous subsistence agricultural operations, where ensuring food and nutritional security is a huge concern. Psophocarpus tetragonolobus, Horsegram pp, Phaseolus lunatus, Vicia faba, and Lablab purpureus, for example, are known underutilized legumes that serve as a potential source of protein/fibers when compared to other crop legumes like Glycine max, Vigna unguiculata, Cicer arentinum, Phaseolus vulgaris, Pisum sativum, and Arachis hypogaea. These neglected legumes are high in proteins/necessary amino acids, Polyunsaturated Fatty Acids (PUFAs), vitamins, dietary fiber, and critical minerals, and are also thought to be a medicinal legume that can help cure a variety of chronic conditions. Apart from that, they're noted for their ability to adapt to a broad range of climatic circumstances, earning them the moniker "climate smart crops." However, little is known about the adaptation processes used by these underused legumes in the face of heavy metal exposure and how they are able to colonize them in order to reclaim land by restoring soil fertility. As a result, the current study aims to assess new findings and successes in decoding various mechanisms/strategies that underused legume plants might use to tolerate/hyper accumulate heavy metals stress. Furthermore, the current review will highlight physiological, biochemical, and molecular responses associated with heavy metal tolerance, as well as advances in various system/synthetic biology tools that can be used to improve the growth and productivity of underutilized legume plants in metal-prone soils.

Heavy metals, when present at low quantities, stimulate plant growth and development by serving as cofactors for a variety of enzymes engaged in numerous physiological and metabolic pathways. However, when their levels above a certain point, they pose a serious threat to plant growth and development, which has become a global problem. Leguminosae, one of the largest and most varied plant families with over 700 genera and 20,000 species, is frequently employed for phytoremediation of heavy metal cause toxicity in soils. Glycine max, Phaseolus vulgaris, and Lablab purpureus are the most often utilized legumes for phytoextraction/phyto-stabilization, owing to their capacity to colonize metal-enriched soils and restore fertility, promoting crop growth and production. Lablab purpureus L., often known as the hyacinth bean or Indian bean, has been shown to be resistant to heavy metals such as Cd, Hg, Pb, Zn, P, and Cr.

Heavy metal contamination of agricultural soils has become a serious concern all over the world, since it has significant negative effects on plant development and productivity, as well as on animals that eat them. Plants require a little amount of heavy metals such as Cd, Hg, As, Cr, Ni, Zn, and other metals. At higher concentrations, they disrupt various physiological, biochemical, and molecular processes in plants, causing membrane damage, photosynthetic rate reduction, ion homeostasis disruption, nutrient uptake, and ultimately nucleic acid and protein synthesis disruption, resulting in

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growth arrest and death. Because all underutilized legumes are still considered "orphan crops" due to their genome revolution, and only a few reports have demonstrated their heavy metal stress tolerance, it is critical to understand potential heavy metal toxicity mechanisms in these crops.

The management and maintenance of ion and osmotic balance in plant cells is a critical physiological condition for plant and agricultural sustainability. Plants that thrive in heavy metal-stressed environments must adapt/acclimate to escape the severe impacts of metal-induced toxicity at all three levels, physiology, biochemistry, and molecular. The ways by which plants deal with heavy metal stress may be genotype specific or controlled to some extent by environmental factors. Researchers in the field of plant science are still attempting to understand the complicated methods by which plants may endure heavy metal stress while maintaining their growth and development without showing signs of toxicity. This section briefly summarizes some recent developments in synthetic biology tools, the role of microRNA (miRNA), Plant Growth Promoting Rhizobacteria (PGPR), and recent advances made in the regulation of various aspects of heavy metal stress tolerance in plants using salicylic acid and nitric oxide.

CRISPR and CRISPR associated protein 9 (Cas9) are sophisticated RNA-guided genome editing mechanisms initially discovered in Streptococcus pyogenes as part of an adaptive immune response to invading nucleic acid. CRISPR/Cas and related technologies have completely changed the way genomes are modified in the modern era. The CRISPR/Cas systems comprise a set of flanking sequences known as "spacer sequences," which are transformed to precursor CRISPR RNA during transcription (crRNA). Furthermore, when this crRNA joins with trans activating crRNA (tracrRNA), it produces a mature crRNA/tracrRNA complex that is directed by the spacer sequence and binds to the protospacer-adjacent motif in the presence of Cas nuclease (PAM). The binding causes a Double-Strand Break (DSB) in the targeted DNA sequence, which is then exploited for the progressive change of genes in various tissues and organs using the CRISPR edited DNA template. CRISPR/Cas-related technologies are now being employed in eukaryotes to edit genomes of different agricultural plants against various abiotic stressors, although tailoring plants against heavy metal stress using the CRISPR/Cas system is still in the experimental stage. By permitting strong, dynamic, and efficient change of template DNA sequences, this editing tool has had a tremendous influence on the area of advanced molecular biology.

CONCLUSION

Rapidly changing environmental circumstances as a result of increased anthropogenic activities have an impact on plant agriculture production, which in turn has an impact on the world population demand-supply imbalance. As a result, agricultural plants must be engineered, which entails using advanced biotechnological techniques to target genes involved in signaling and regulatory networks. By targeting certain genes, genome editing using site-specific nucleases can be utilized to increase abiotic stress resistance, including heavy metal stress. The CRISPR/Cas system has emerged as a game-changing technique for genome editing in plants in this context. Although the CRISPR/Cas9 technology has been widely used to modify plant species against abiotic and biotic stressors, its use in improving heavy metal tolerance is still restricted. However, some research has shown that Genetically Modified (GM) crops might cause allergies, inflammation, and injury to humans and animals. Nonetheless, when these studies were re-evaluated for dependability, they revealed serious faults in their design, execution, and analysis. Foods generated from GM crops have been widely consumed in affluent nations for decades with no negative consequences. GM crops are being utilized to generate recombinant DNA medications, antibodies, biofuels, and polymers, and might help relieve food and medical supply concerns in the future. Furthermore, plant scientists are working hard to create synthetic biology tools including synthetic promoters, riboswitches, and other gene regulatory tools for effective genetic modification of plants in the face of heavy metal stress. Overall, heavy metal sensitive genes, transcription factors, and metal proteins/ligands have been genetically manipulated to increase plant tolerance to heavy metal stress as well as their phytoremediation capabilities. However, their full potential must be explored by creating futuristic experiments that use a multi-disciplinary approach, including both system and synthetic biology techniques, to generate superior crop varieties that are more resistant to abiotic stressors.