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Mini-Review

Chemical mutagenesis for improving potential of plants to remediate environments with heavy metal contaminants

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ABSTRACT

Toxic levels of heavy metals are increasingly posing threats to all organisms on earth. One possible remediation strategy involves the use of plants to remove toxic metals from contaminated soils. It is widely recognized that genetic improvement of plants with increased metal tolerance and uptake capacity would be required to enhance the practical prospect of this phytotechnology. In this paper, a summary of the studies using chemical mutagenesis as a breeding approach to obtain mutants with altered response to toxic metals is presented and discussed briefly. It is concluded that chemical mutagenesis probably deserves more attention as it is a valuable alternative to transgenic plant technology as far as generating plants with improved potential for phytoremediation of heavy metals in contaminated soils or for studying mechanisms of metal tolerance and uptake is concerned.

KEYWORDS: abiotic stress tolerance, heavy metal toxicity, mutation breeding, ethyl methane sulfonate (EMS), phytoremediation, phytotechnology

INTRODUCTION

The use of plants to aid removal of toxic metal contaminants from soil and water or phytoextraction is a sub-cateogory of the technology known as phytoremeditaion [1]. An important pre-requisite for the practical use of plants in this way is the ability of plants to accumulate and tolerate increased amounts of toxic metal contaminants. Genes associated with metal tolerance could be transferred to boost metal tolerance of plants selected for phytoextraction purposes. Another powerful approach in obtaining plants with enhanced heavy metal tolerance and accumulation is through mutagenesis. Unlike plant transformation to generate metal-accumulating plants for phyto-extraction purposes, mutagenesis breeding being a non-GMO approach is not going to attract the same level of adverse public concern particularly in Europe [2]. This also allows mutant plants to be tested directly under real field conditions for improved metal extraction potential [1, 3].

Mutagenesis produces mutants with heritable alterations in the genomes, phenotypes and physiological responses, which are critical for determining the biological functions of genes in plants. Various approaches for mutagenesis involving chemical, physical (e.g. x-ray, UV and gamma-ray irradiation), and biological (e.g. introduction of T-DNA and heterologous transposons) methods have been developed [4]. Each has advantages and disadvantages for the study of gene function. Here, the emphasis is on the studies using chemical mutagenesis to obtain plants with altered response to metal exposure.

Ethyl methane sulfonate as a mutagen

High mutation rates in organisms have been obtained via chemical mutagenesis using methyl

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methanesulfonic acid (MMS), nitrosomethylurea (NMU), and diepoxybutane (DEB) as mutagens. Mutagenesis in *Arabidopsis* using ethyl methane sulfonate (EMS) is the most extensively applied mutageneis technique. EMS is an alkylating agent that donates an ethyl group to nucleic acid, leading to base mispairing. An alkylated guanine pairs with a thymine base, and thus produces essentially GC $\rightarrow$ AT transitions, which causes an amino acid change or deletion [4, 5]. The popularity of this technique is mainly because EMS is highly mutagenic, causes low mortality, and can be conducted in any laboratory with a fume hood [4, 5]. In addition, EMS generates (a) irreversible genome mutations in bulk, allowing mutagenesis process without the need to screen a large number of individual mutants, and (b) mutants that have lost their function or exhibited novel phenotypes including dominant or functional proteins due to alterations of specific amino acids.

EMS mutagenized *Arabidopsis thaliana*

A. thaliana is ideal for conducting mutation experiments mainly because it is a small plant with a short life cycle and has a natural tendency to self-fertilize producing a large quantity of small seeds. M_1 generation refers to individuals that are treated directly with a mutagen, whereas M_2 generation refers to progeny that are derived from self-fertilization of M_1 populations thereby producing homozygous recessive mutations. Hence, M_2 generation is mainly used in mutant screening [4]. Lehle Seeds (USA) is a commercial supplier of EMS mutagenized *Arabidopsis* seeds. Although purchasing EMS mutated M_2 seeds from a commercial source allows limited control of the initial genotype used and the way seeds are pooled, it is safer for researchers because EMS is a highly volatile carcinogen.

Over the past 20 years, several thousands identified *Arabidopsis* mutants defective in various processes of plant growth and development are available as genetic stocks [6]. These mutations interfere with basic metabolism (e.g. amino acid, lipid, mineral uptake), cellular and physiological processes (e.g. photosynthesis, light perception and chloroplast differentiation), developmental processes (e.g. root growth, gametogenesis, seed formation, flowering, senescence), metabolic and signal transduction pathways (e.g. response to hormone, pathogens, environmental signals), structural genes, and mechanisms controlling genetic regulation (e.g. transcription factors, DNA binding sites) [6, 7]. The elucidation of physiological, biochemical, genetic and molecular attributes of *Arabidopsis* mutants has yielded valuable insights into all areas of plant biology.

Mutants and phytoremediation studies

Mutagenesis treatments have been used successfully to generate mutants with enhanced tolerance to various abiotic stresses. Novel genes with potential applications in genetic improvement of metal bioaccumulation characteristics have been identified. Based on the phenotypic performance in growth media containing metals in comparison to the wild-type, new mutant variants of *A. thaliana* [8, 9, 10-12, 13, 14, 15], *Brassica juncea* [16], barley [17, 18], legumes [19], peas [20], and sunflowers [2, 3], have been isolated (Table 1). The subsequent characterization and genetic analysis of these mutants should provide a better understanding of the mechanisms that govern heavy metal toxicity, tolerance, accumulation, stress signalling, and antioxidative defence in plants.

In Pb-related studies, Chen *et al.* [8] initiated a research program to screen EMS-mutagenized *Arabidopsis* M_2 populations to identify mutants with increasing Pb accumulation and tolerance. More than 500,000 seedlings were screened, using root length as an indicator. Three mutants, APb2, APb7 and APb8 were isolated. These mutants were able to accumulate levels of Pb in the shoots more than twice of that in wild-type plants. The mutant plants also accumulated elevated levels of Mn, Cu, Mg, Zn and S. A possible mutation in the *man1* gene controlling the regulation of metal-ion and uptake or homeostasis has been suggested. Schulman *et al*. [16] has developed a new screening method by incubating *B. juncea* seedlings in a solution containing radioisotopes of the investigated metals. Subsequent visualization of metal accumulation in the tissue was detected with a phosphorimager. Twenty one Pb-accumulating mutants were isolated from the screening of 50,000 M_2 seedlings. Subsequent characterization

Table 1. Application of chemical mutagenesis for isolation of mutant plants with altered response to heavy metals.

Mutant line	Phenotype	Plant	Uptake of metals by mutant plants in comparison to wild-type plants	Reference
man1	Manganese accumulator	*Arabidopsis thaliana*	Accumulated 7.5 times Mn, 4.6 times Cu, 2.8 times Zn, 1.8 times Mg, 2.7-fold S higher in leaves, and 10-fold more Fe in roots, when grown in soil.	[9]
Als	Aluminium sensitive	*A. thaliana*	Root growth was inhibited in the presence subtoxic level of $AlCl_3$ (0.75 mM). Root growth was inhibited by 36% in the ecotypes of Col-0 and Ws-0, while 64% in La-0 when grown in gel with 1.0 mM $AlCl_3$.	[13]
cdht1, cdht4	Cadmium hyper-tolerant	*A. thaliana*	Root growth was longer than 2 mm upon exposure to 200 µM $CdCl_2$, and accumulated 56% less total Cd.	[14]
cad1	Cadmium sensitive, phytochelatin synthase deficient	*A. thaliana*	Leaf growth was inhibited by 2- to 3-fold over 3 d in 6 µM $CdSO_4$; Cd accumulation remained the same over that period. Further experiment with *cad1-1* on 0.8% nutrient agar containing 3 µM $CdSO_4$ resulted in the progressive production of chlorotic leaves and discrete brown pigment on roots. Also sensitive to Hg.	[10-12]
cup1	Copper sensitive	*A. thaliana*	Growth was inhibited on medium containing 5 µM $CuSO_4$; most sensitive to Cu, slightly sensitive to Cd, and less sensitive to Hg. The leaves accumulated 2- to 3-fold more Cu in the presence of 5 or 20 µM $CuSO_4$, respectively, and 2- to 4- fold more Cd in the presence of 3 µM $CdSO_4$.	[15]
cup2	Copper sensitive	*A. thaliana*	Grown poorly on medium containing 50 µM $CuSO_4$; appeared stunted with short roots and fewer initiated roots when grown on higher $CuSO_4$ concentrations.	[22]
APb2, APb7, APb8	Pb accumulator	*A. thaliana*	Longer root growth, lower shoot dry weight, and 3-fold higher Pb concentrations in shoots when grown in hydroponic culture with 4.1 mg/L Pb. Also accumulated higher Mn, Cu, Mg, Zn and S levels when grown on the same medium.	[8]
7/15	Pb accumulator	*Brassica juncea*	Roots accumulated 3.6 times higher Pb, stunted root growth (shorter and thicker), decreased root cell size (cells failed to elongate), stunted and thickened hypocotyl, 37% more cell-wall material in roots.	[16]
RL819/2, RL820/6	Aluminium tolerance	*Hordeum vulgare*	Longer roots and higher root tolerance index. Roots exhibited no hematoxylin stainability at the 0.03 mM Al^{3+}, no or partial stainability at 0.06 mM Al^{3+}, and partial stainability at 0.09 mM Al^{3+}.	[17]
M_2, M_{10}, M_{25}	Aluminium tolerance	*H. vulgare*	Relative cell growth was > 26% higher in modified MS treated with 30 µM Al, Al accumulation was significantly lower.	[18]

Table 1 continued..

raz	Require additional Zn	*Medicato truncatula*	Leaf necrosis was evident. Higher concentrations of Zn, Mn and Cu were found in most tissues, while a higher Fe concentration was observed in roots. Reduction of general growth, and leaf loss was 48% higher in medium containing low levels of nutrients at 3 µM Zn and 0.2 µM Mn. Leaf necrosis was reduced when plants contained high levels of Zn.	[19]
SGECd^t	Cd tolerance and accumulation	*Pisum sativum*	Grew normally without exhibiting any stress symptoms in the presence of 3 µM $CdCl_2$; accumulated 3- and 3.5-fold more Cd in roots and shoots, respectively. Higher Mg and Ca contents in roots, and Mg, Ca, Zn, Mn and B in shoots.	[20]
M_2 14/185/04	Giant mutant	*Helianthus annuus L.*	Increased concentrations of Cd, Zn, and Pb by 1.8-, 2.7-, and 3.0-fold, respectively. Significantly enhanced metal extraction ability in the above ground parts: 7.5-fold for Cd, 9.2-fold for Zn, and 8.2-fold for Pb.	[2]
17/67-35-190-04 (line B), 6/15-35-190-04 (line E)	Zn and Cd tolerance	*H. annuus L.*	Improved growth and enhanced metal uptake capacity. Carotenoids were increased significantly in mutant lines, to counteract against Cd- and Zn-induced oxidative stress. Increased antioxidative enzyme activities were found in mutant line E (5.3 times for *DHAR, 2.3 times for GR, 2.5-fold for APOX, and 3.8-fold for GST) and mutant line B (2.5-fold GST) grown in metal-contaminated soil.	[3]

*DHAR: dehydroascorbate reductase, GR: glutathione reductase, APOX: ascorbate peroxidase, GST: glutathione S-transferase.

of mutant 7/15-1 suggested Pb accumulation was due to the enhanced cell wall binding and precipitation in the roots. The eventual characterization of such genes may provide tools for genetic engineering to develop or genetic screening of plant germplasm to identify plants with enhanced Pb phytoremediation potential.

Using M_5 population (the 5^{th} generation) of sunflower lines developed from EMS-mutagenized seeds, enhanced tolerance (increased biomass) and accumulation capacity for Zn, Cd and Cu were confirmed to be heritable [3]. The mutant lines have been proposed to be useful for phytoextraction of these metals from contaminated soils. Moreover, these plants were used to aid investigations into the relationship between oxidative stress and tolerance to these metals in sunflower plants. It was found that mutant plants grown on a metal contaminated soil contained more carotenoid, an antioxidant pigment, than on control soil (not contaminated with any metal). Furthermore, the activity of some antioxidant enzymes was more elevated in the mutants than wild-type plants. This suggests the importance of elevated protective antioxidative defence mechanism in mutant plants underpinning their increased metal tolerance. This is also consistent with many studies showing correlations between increased antioxidative defence and protective treatment against toxicity of heavy metals with a nitric oxide donor such as sodium nitroprusside [21].

CONCLUDING PERSPECTIVE

It is clear from a summary of the findings of those studies in Table 1 and the above-briefly discussed studies that chemical mutagenesis is a useful technique to generate mutant plants with improved metal uptake and tolerance. The mutant plants could be useful in multiple ways including for improved phytoremediation of soils contaminated with toxic levels of heavy metals. Also, in light of the ongoing public concerns of genetic engineering, it is, therefore, surprising that only relatively few mutant plants with altered response to toxic metals have been reported and characterized further.

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