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Enhanced translocation of uranium, strontium and cesium by transgenic *Sesbania* and *Arabidopsis* plants harboring the rabbit *CYP2E1* gene

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Genetically modified *S. grandiflora* and *A. thaliana* confer P450 2EI enzyme were examined for accumulating Cs, Sr and U. The absorption of these radionuclides (^{137}Cs, ^{90}Sr and ^{238}U) by the generated transgenic plants was analyzed through quantitative gamma assay. Both transgenic plants which derived from homozygous T3 were grown in soil containing ^{137}Cs 17600 Bq/g, ^{90}Sr 147±7 mg/kg and supplemented with uranium at 25 mM. Transgenic plants with a high *CYP2E1* expression level as proved by Western Blot analysis that the absorption of radioactive nuclides increased significantly in comparison with wild type plants or *GUS* transgenic plants (control). *Arabidopsis* and *Sesbania* transgenic plants were able to accumulate 80% and 70%, respectively of the available ^{238}U versus control plants and thus, ^{238}U concentration was significantly decreased in the soil by 50% and 60%, respectively versus the soils cultivated with control or wild type plants. Similarly, ^{90}Sr accumulation levels recorded 70% and 60% increase in transgenic *Arabidopsis* and *Sesbania* plants, respectively which led to an important decrease in ^{90}Sr by 50%. Similarly, 70% and 60% increases in ^{137}Cs levels were recorded in transgenic plants, respectively leading to 40% and 30% decreases in ^{137}Cs in correlated soils. The CYP2E1 gene improves plants ability to resist radioactive pollutants and may open a new avenue for resisting other pollutants such as U, Cs and Sr through the utilization and deployment of transgenic flowering plants. However, the mechanism by which *CYP2E1* enhances the uptake of these radioactive nuclides still to be investigated and examined.

Capsule Summary: Genetically modified *S. grandiflora* and *A. thaliana* were examined for accumulating Cs, Sr and U, which accumulate the radiative elements and the CYP2E1 gene improve the plants ability to resist and grow in polluted soil.

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INTRODUCTION

Toxic metals interfere with biochemical and homeostatic processes in the cell through the production of free radicals (Dushenkov, 2003). The last few decades of wars in Kosovo, Iraq, and some other places have resulted in a depleted uranium (DU) contamination in addition to and other toxic

heavy metals (IAEA, 2010). This illustrates the need for remediation of such contaminations in order to prevent the inevitable long-term effects not only in the contaminated regions but also on the surrounding countries (Djedidi et al., 2015; Zhu et al., 2002).

Many remediation techniques are used in soils contaminated with radionuclides such as surface acid extraction (Fujimura et al., 2015), long-term mobility of radiometers (Robinson et al., 2000), distribution of radionuclides (Lokas et al., 2014), removing clay particle (Brooks, 1998), application of fertilizers (Hattori et al., 2006), and phytoremediation by plants (Broadley et al., 2001), and their rhizospheric associated organisms (Delvaux et al., 2000) such as mycorrhizal fungi (Dighton and Horrilla, 1998), fungi (Entry et al., 1999), *Hyalella azteca* (Entry et al., 1997), in addition to Pseudomonas and Bacillus sp bacterial biomass (Newman and Reynolds, 2005). Different phytoremediation approaches are used such as phytoextraction with *Catharanthus roseus* (Fulekar et al., 2010), *Chamaecyparis obtusa* (Yadav and Kumar, 2019), sunflowers (*Helianthus annuus*) (Favas et al., 2014), *Gammarus pulex* (Baudin and Garnier-Laplace, 1994), phytoextraction (Dickinson and Pulford, 2004), also known as phytoacumulation and phytoabsortion (Yadav and Kumar, 2019) or phytosequestration technique (Ebbs et al., 1997), which was developed specifically for inorganic pollutants such as Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, and Zn, metalloids (As, Se), in addition to radionuclides (^{90}Sr, ^{137}Cs, ^{243}U, and ^{238}U) and nonmetals (Ebbs et al., 1997). In this, the removal of contaminants is achieved through the root network and the accumulation potential into the plant biomass, after sequestration, the biomass is harvested to complete the extraction of contaminants (Schnoor et al., 1995).

Despite that many plants decompose and may purify pollutants; however, this is not always work, thus genetic modification may be required (Hu et al., 2005; Van Aken, 2008). The enzyme P450 2E1 has the potential to compost organic pollutants in animals and even in plants (Lee et al., 1996). The cytochrome P450 2E1 gene has been reported firstly by Doty and co-workers used tobacco and poplar plants as models in which both expressed decomposing of many hydrocarbons and even gaseous pollutants (Banerjee et al., 2002; Doty et al., 2000; Doty et al., 2007; Doty, 2008; James et al., 2008; Shang et al., 2001; Van Aken and Doty, 2010). More recently, transgenic *Arabidopsis thaliana* and *Sesbania grandiflora* harboring the expressing *CYP2E1* were developed; and it was found that both transgenic plants were efficiently and significantly capable of removing broad spectrum of pollutants from the environment such as trichloroethylene (TCE) and dichlorodiphenyltrichloroethane (DDT) (Mouhamad et al., 2012) in addition to various heavy metal contaminants (Mouhamad et al., 2014). Therefore, the aim of the current study is to explore and exploit the capability of the previously generated T3 *A. thaliana* and *S. grandiflora* plants in removing radionuclides such as Uranium and Strontium, and Cesium from contaminated soils.

MATERIAL AND METHODS

Plant material

Transgenic plants of *A. thaliana* L. and locally grown *S. grandiflora* L. harboring CYP450 2E1 enzyme containing the vector pSLD50-6 were previously generated [38], and thus T2 and T3 generations of transgenic Arabidopsis plants, T3 generation of transgenic Sesbania, in addition to the control with no vector. Transgenic plants of both types (as a control) were used in the current study.

Radioactive nuclides translocation experiment

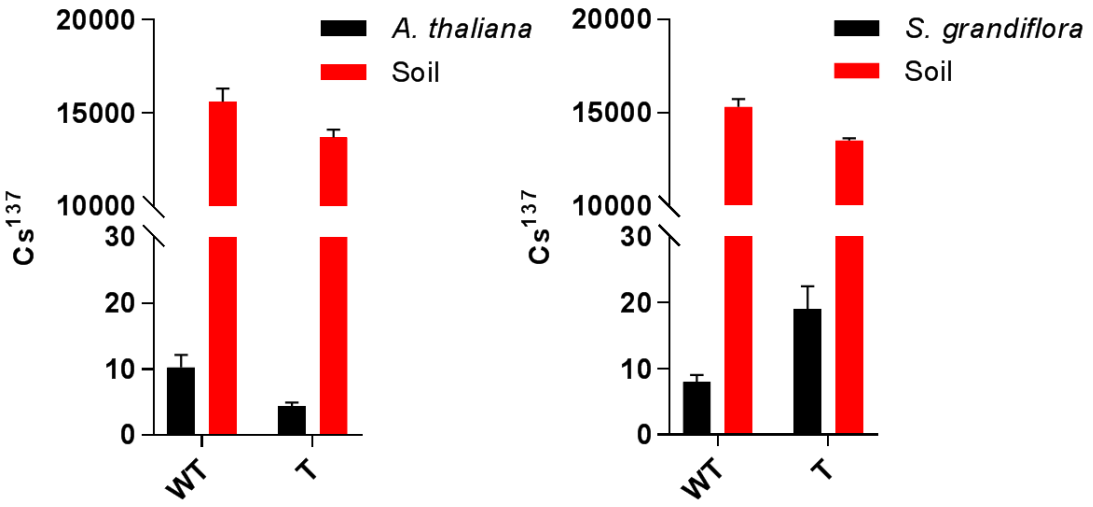

Fig. 1: Translocation of Cs^{137} (Bq/g) in transgenic *A. thaliana* (A) and *S. grandiflora* plants and (WT) wild type plants transformed with an empty vector; (T) transgenic plants with the expressing gene *P450 2E1.*

Soils contaminated with ^{137}Cesium 17600 Bq/g and ^{90}Strontium 147±7 ppm were used in the *Arabidopsis* and *Sesbania* transgenic plants experiment. Also, F1 and F2 transgenic *A. thaliana* were tested for their U^{238} translocation ability by growing them in soils contaminated with 527 Bq g^{-1} dose of U^{238} (25 mM) contamination. Plants under investigation were grown individually in pots filled with soil:peat with three replicates, and each replicate included three pots. Wild type plants that transformed with empty vector of *A. thaliana* and *S. grandiflora* were treated as the transgenic, treated plants were grown in a sterilized greenhouse conditions (25°C for 16/8 hrs. light/dark photoperiod using florescent light with an intensity of 3000 lux) for 12 days.

RESULTS AND DISCUSSION

Translocation of ^{137}Cesium

Results showed 70% increased translocation of ^{137}Cs in transgenic *A. thaliana* compared with non-transgenic with a decline of 30% in ^{137}Cs in control plants grown in soil (Figure 1). On the other hand, *S. grandiflora* transgenic lines illustrated a 60% higher translocation compared to wild type plants with 40% decrease in ^{137}Cs concentration in soils planted with transgenic *S. grandiflora* compared to wild type plants.

Cesium accumulation in leaves might disturb their basic physiological functions. The first observed reaction in cesium-treated plants was a decrease in stomata opening. The control function of stomata in respect to photosynthetic is CO_2 assimilation and transpiration that were found to be modified by the presence of cesium. Decreased stomata opening limits transpiration and the uptake of water by roots, however, photosynthetic CO_2 assimilation is not changed during short-term exposure to CsCl. Stomata closure in the presence of cesium may be a result of a decrease in osmotic potential due to ^{137}Cs accumulation in the vacuoles (Dräxl et al., 2013; Gupta and Walther, 2016).

Moreover, (Zhu et al., 2002) reported that ^{137}Cs possibly inhibits the channels responsible for K transportation into the guard cells, which might lead to an impaired stomata opening and restricts production of assimilates important to dry mass accumulation in *A. thaliana*. Although photosynthesis and transpiration were affected by cesium, photosynthetic water utilization efficiency was rather stable. The lowering of chlorophyll fluorescence seems to be a secondary effect of a disturbed biosynthesis. In a greenhouse study, ammonium nitrate and ammonium chloride increased the uptake of ^{137}Cs in cabbage, Tepary beans, Indian mustard (*Brassica juncea*), and reed canary grass. Moreover, rhizofiltration, a way of removing radionuclides from contaminated soils, was found to be most effective when plant roots are large and radionuclides are more available for plant uptake (Entry et al., 1999). Two plants that have proven the most successful at rhizofiltration are sunflower (*Helianthus annuus*) and water hyacinth (*Eichornia crassippes*). Both have been found to accumulate significant percentages of radionuclides (^{137}Cs, U, ^{90}Sr) within a few hrs to a few days (Tettey-Larbi et al., 2013). The current results exhibit that transgenic *A. thaliana* is not suitable for remediating polluted soils with ^{137}Cs since it behaved similarly to the

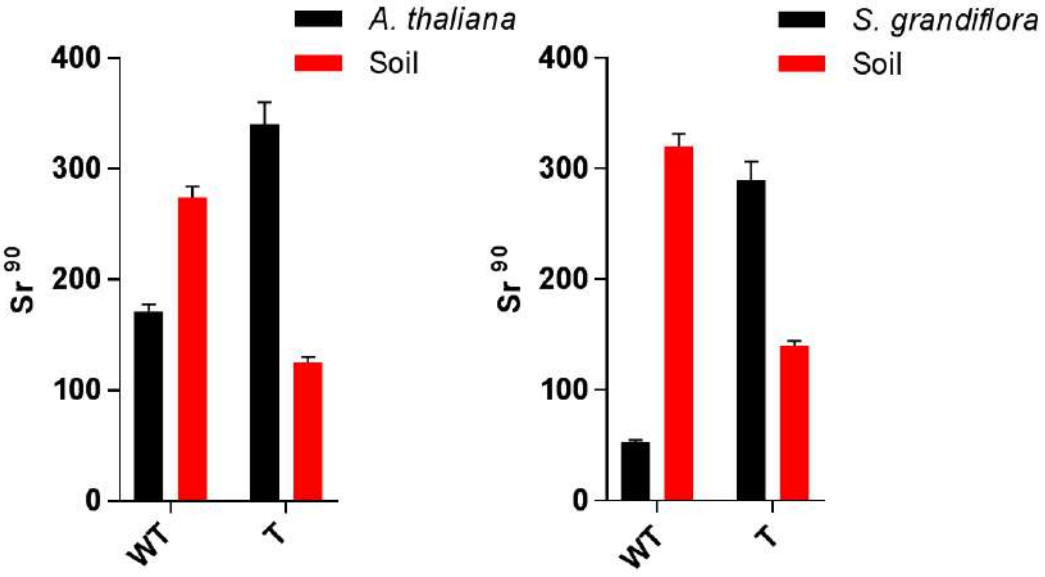

Fig. 2: Translocation of ^{90}Sr (ppm) in transgenic *A. thaliana* (A) and *S. grandiflora* plants. (WT) wild type plants transformed with an empty vector; (T) transgenic plants expressing gene *P450 2E1.*

wild type. On the other hand, transgenic Sesbania translocate ^{137}Cs better than the wild type and thus, can be used for remediating soils polluted with ^{137}Cs, probably in combination with other previously reported plants such as *Amaranthus retroflexus* (Lasat et al., 1998), *Calendula alata* (Borghei et al., 2011), and *Calotropis gigantean* (Eapen et al., 2011).

Translocation of ^{90}Sr

A. thaliana transgenic lines showed increased accumulation reached 70% in comparison to wild type plants (Figure 2). Results showed an increased accumulation of ^{90}Sr in transgenic *S. grandiflora* reached 60% compared with wild type plants. Furthermore, a decrease in ^{90}Sr concentration was observed in soils planted with transgenic *A. thaliana* and *S. grandiflora* amounted 50% compared with wild type plants in both cases. It is clear from the above presented data that *A. thaliana* and *S. grandiflora* transgenic plants have developed mechanisms that inactivate or chelate the metal ion upon its entry into the plant cytosol. This system prevents the metal from inactivating or activating structural proteins, whilst at the same time allowing elements essential for the plant's metabolic function to be taken up and transformed into forms that are tolerable to the plant. Such mechanisms were supported by (Dushenkov, 2003), who reported that the toxic effects of metals are assessed by inhibition of the primary root length increment following 24 and 48 hrs incubations as compared to the roots grown in water or in 3 mM $Ca(NO_3)_2$ solution. The same author also reported that in the absence of $Ca(NO_3)_2$, metals were found in both the meristem and in the root cap. Pb and ^{90}Sr were revealed primarily in the cell walls and it was suggested that inhibition of divisions in the root cap upper layer cells and a decrease in the sloughing off its cells can stimulate the quiescent center cell divisions.

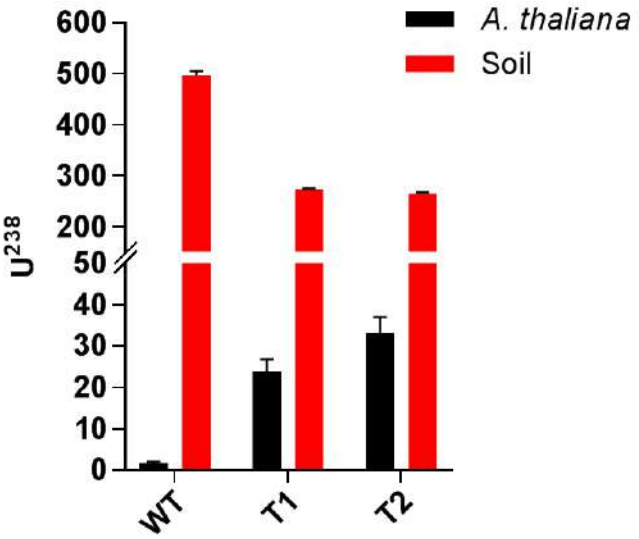

Fig. 3: Translocation of ^{238}U (Bq/g) in transgenic *A. thaliana*. (WT) wild type plants transformed with an empty vector; (T1) first generation of the transgenic plants expressing the gene *P450 2E;* (T2) second generation of the transgenic plants expressing the gene *P450 2E1*.

Accumulation of uranium

A. thaliana transgenic lines showed increased translocation of ^{238}U compared with non-transgenic plants (control). F1 and F2 transgenic plants were able to translocate 80 and 90% of ^{238}U respectively. Additionally, ^{238U} was decreased by 40 and 50% in soils planted with *A. thaliana* F1 and F2 plants respectively compared with soils planted with wild type plants (Figure 3).

Previously, it was reported that cytotoxicity of ^{238}U was prevented by reactive oxygen species (ROS) scavengers, antioxidants, in addition to glutamine (ATP generator). Additionally, the in hibition of hepatocyte dichlorofluorescein oxidation was mediated by mannitol which is a hydroxyl radical scavenger, butylated hydroxyanisole, and butylated hydroxytoluene which are considered strong antioxidants. Glutathione depleted hepatocytes were also resistant to the toxic effects of ^{238}U with minimum observed dichlorofluorescein oxidation (Ning and Grant, 2000). *In vitro* reduction effect of glutathione or cysteine was also accompanied by the uptake of oxygen with an inhibition effect of Calcium or Uranium. The cytotoxicity induced by U(VI) and ROS formation was stopped by Ca(II), this suggests ROS formation which is mediated by U(IV) and U(VI) in isolated hepatocytes. The reduction mechanism of U(VI) required for toxicity has not been fully investigated. Cytochrome P450 inhibitors, particularly CYP2E1 inhibitors also prevented cytotoxicity, except for DT diaphorase or glutathione reductase inhibitors. This suggests that P450 reductase and reduced cytochrome P450 might be contributing to U(VI) reduction to U(IV) and the bottom line is that the cytotoxicity of U(VI) is associated with mitochondrial/lysosomal toxicity mediated by the reduced biological metabolites and ROS (Dushenkov, 2003; Reed et al., 2019; Veith et al., 2018).

CONCLUSIONS

This study is the first to report the enhanced translocation of radionuclide by *CYP2E1* transgenic plants. However, further investigation is required, in order to understand how the *CYP2E1* gene improves plant phytoremediation and translocation capacity. It is proposed that certain specific electron transfer enzymes are produced in *Arabidopsis* and *Sesbania*, so as to facilitate the CYP2E1 role in decomposing radionuclide pollutants, as occurs with the rabbit CYP2E1 enzyme, which requires oxidoreductase NADPH-P450 and cytochrome b5 for electron transfer.

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