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OVEREXPRESSION OF ARABIDOPSIS PHYTOCHELATIN SYNTHASE GENE IN TOBACCO AND IMPACT OF PLANT GROWTH UNDER CADMIUM AND ARSENIC

Environmental Science

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ABSTRACT

The overexpression of *Arabidopsis phytochelatin synthase1* gene in transgenic tobacco plants and tolerance to cadmium and arsenic were evaluated in homozygous *Arabidopsis thaliana phytochelatin synthase1* transgenic tobacco lines as well as wild type control plants. Initially, no significant differences were observed in root length between wild type control and transgenic rice lines in the absence of cd/as. The effect was more prominent in root compared to shoot because these are the primary site of defence in tobacco. It was concluded that the transgenic tobacco plants showed significant enhancement in tolerance at different concentrations of cadmium and arsenic hence these transgenic tobacco lines could be cultivated under heavy metals (cd/as) and also reduce the concentration of heavy metals (cd/as) in the soil.

KEYWORDS

INTRODUCTION

Heavy metal pollution is currently a major threat for entire living organism throughout the world. These heavy metals are usually derived from anthropogenic activities in various fields, such as agriculture, industry, livestock and medicine, which then produce waste disposal in soil, water and atmosphere. (Tchounwou *et al.,* 2012; Briffa *et al.,* 2011). Although some heavy metals are essential organisms, many of them can be dangerous to high concentration due to the complex composition of the compounds in the cells. One of the most important things about heavy metals such as inanimate pollutants is that once they are in the environment, they cannot rot. They persist and accumulate in the environment, affecting and contaminating the food chain, caused by the toxicity of these heavy metals, various health problems arise in humans (Mohammed *et al.,* 2011). The use of heavy metals in the agricultural area has become a second source of pollution, such as the use of pesticides, fertilizers, and more. Natural causes may also increase heavy metal contaminations such as volcanic activity, corrosion of iron and water and soil restructuring soil erosion and local climate evaporation of soil. Heavy polluted areas often indicate the simultaneous presence of various toxins, e.g. As, Cd, Pb, Cu, Zn and Cr (Kim *et al.* 2003; Loska *et al.* 2004). Semimetal iron, arsenic (As) and cadmium (Cd) are toxic to all forms of life on the earth. Because of their origin in both human activities and natural resources, these elements are present in the soil throughout the world, even at a high altitudes (Zhao *et al.,* 2009). Plants take a lot as As and gather selectively at the roots of the plants. Arsenate, an analogue of phosphate, is transported to plant cells using phosphate transporters (Meharg and MacNair 1992), and adversely affects important metabolic processes. Cadmium is one of the most toxic substances in living organisms. It goes into the diet of plants that take it very easily. Cadmium alters a large number of plant body processes (Sanita` di Toppi and Gabbrielli 1999). A great approach to protect plants is to remove semimetal toxins by combining them with metal-binding thiol-peptides, such as phytochelatin (PCs) (Cobbett 2000; Sanita` di Toppi *et al.* 2003). Phytochelatins are cysteinerich peptides chelate, using thiol groups, semimetals and various toxic metals (Liu *et al.* 2010). Tobacco (*Nicotiana tabacum*) is one of the most important agricultural products throughout the world with social and economic importance. Tobacco can easily accumulate certain heavy metals and especially cadmium (Cd) in the leaves (Clarke and Brennan 1983; Wagner, Sutton, and Yeargan 1988; Tso 1990; Ruso et al. 2001). In the present study, to transfer the potent Phytochelatin synthases gene into commercial cultivar of Tobacco and to observe the effect of plant growth under different concentrations of cadmium and arsenic.

MATERIALS AND METHODS

Arabidopsis thaliana phytochelatin synthase1 isolation and cloning

Total RNA, from 15-day-old *Arabidopsis* seedlings (ecotype Columbia) was isolated using the RNeasy plant mini kit (Qiagen) following the manufacturer's instructions. Two µg of total RNA were reverse transcribed using the SuperScript™ III First-Strand Synthesis System (Thermo Fisher Scientific). Two µl of cDNA was used to perform PCR using pfu polymerase employing forward and reverse primers, viz., 5′-GGATCCATGGCTATGGCGAGTTTA-3′ and 5′-GAGCTCCTAATAGGCAGGAGCAGCGAG-3′, specific to *Arabidopsis thaliana phytochelatin synthase1* coding sequence. The underlined regions in the primers depict BamHI and SacI restriction sites introduced for subsequent cloning purpose. Amplified fragments of 1470 bp was cloned at SmaI site of pBluescript KS (+) (Stratagene, USA), then transformed into E. coli Top10 cells and was sequenced using automated DNA sequencer.

Construction of binary vector containing *Arabidopsis thaliana phytochelatin synthase* 1 and GFP expression cassettes

Arabidopsis *phytochelatin synthase1* coding sequence was excised from BamHI and SacI sites of recombinant pBSSK (+) – *Arabidopsis thaliana phytochelatin synthase* 1 vector and cloned between CaMV35S promoter and polyA terminator of *pCambia*1302 hygromycin binary vector constructed earlier in our laboratory. The *pCambia* 1302 binary vector contains expression unit of hyg (CaMV35S-hyg-polyA) which was used as a plant selection marker (www. cambia.org). The recombinant vectors, *viz., pCambia* 1302-hyg- *Arabidopsis thaliana phytochelatin synthase* 1 were maintained in HB101 cells and was mobilized into A. tumefaciens strain LBA4404 by triparental mating (Lichtenstein and Draper, 1985) using the helper vector pRK2013 and the resulting binary vector was designated as *pCambia* 1302-hyg *Arabidopsis thaliana phytochelatin synthase* 1.

Expression of *Arabidopsis thaliana phytochelatin synthase* 1gene in Tobacco plants for their functional validation.

Preparation of tobacco explants:

Seeds of tobacco were treated with 0.1% tween 20 for 5 min and then washed with sterile water later, these seeds were surface sterilized with 0.1% Mercuric chloride and 2% sodium hypochlorite for 5min each followed by sterile distilled water thrice. The seeds were kept for germination in MS basal media at 25° C. After 10-15 days leaf disc of 0.5cm were dissected from mature leaves and inoculated on to Petri dishes containing MS basal media and incubated at 24° C in dark condition for two days before infecting with *Agrobacterium.*

Agrobacterium-mediated transformation and regeneration of tobacco transgenic plants

Single colonies from sub-culture plants *Agrobacterium tumefacians* with *Arabidopsis thaliana phytochelatin synthase* 1gene constructs were inoculated into 50ml of YEP broth and grown for 24hr at 28° C in an orbital sheker set 200rpm. The cells were pelleted at OD_{600} 1.0 and resuspended in MS basal media. Leaf disc collected aseptically from invitro grown tobacco plant were placed in *Agrobacterium* suspension and shaken gently for 15min in orbital shekar set at 200rpm at 28° C. Later, the explants were placed on sterile Whatmann filter paper to blot

excess bacteria from the explants and to reduce the overgrowth. The explants were then co-cultivated for two days on co-cultivation medium. After 48hr the explants were washed thrice with a solution of Cephotaxime 250mg/lit for one hr. Later, the explants were transfer to selection media containing hygromycin 50mg/lit.

Analysis of transgenic lines under cadmium and arsenic

Homozygous transgenic tobacco line expressing *Arabidopsis thaliana phytochelatin synthase* 1 of T3 generation along with wild type controls were sown in pots. Plants were grown for 10 days on MS medium and then transferred in fresh MS media containing various concentrations *viz.,* 50 µM, 100µM and 150 µM of CdCl2 and Na2AsO4. After 10 days of treatment length of roots (cm) and fresh mean weight (mg) were recorded.

RESULTS AND DISCUSSION

The mean fresh weight (mg) of transgenic line under 50µM stress of Cadmium and Arsenic were observed as 380mg and 450mg respectively. While the wild type was showed 320mg and 420mg under 50µM stress of Cadmium and Arsenics respectively. Under under 100µM stress of Cadmium and Arsenic were observed as 240mg and 270mg respectively. While the wild type was showed 180 mg and 225mg respectively. Under under 150µM stress of Cadmium and Arsenic were observed as 185mg and 195mg respectively. While the wild type was showed 120mg and 140 mg respectively.

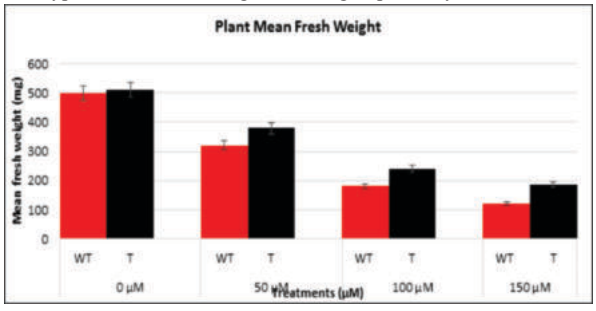

Fig. 1. Effect of Cd on fresh mean weight (g) root length of AtPCS1 expressing transgenic lines and wild type controls

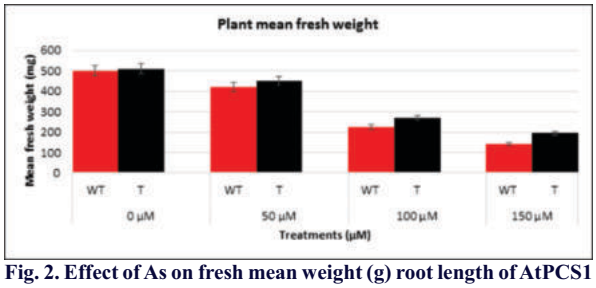

Fig. 2. Effect of As on fresh mean weight (g) root length of AtPCS1 expressing transgenic lines and wild type controls

Tolerance to Cadmium and Arsenic were evaluated in homozygous *Arabidopsis thaliana phytochelatin synthase1* transgenic tobacco lines as well as wild type control plants. Initially, no significant differences were observed in root length between wild type control and transgenic rice lines in the absence of Cd/As. The effect was more prominent in root compared to shoot because these are the primary site of defense. The root growth in terms of length was found to be more in transgenic lines when compared to untransformed control plants under 150µM $CdCl_2$.

Arabidopsis thaliana phytochelatin synthase1 transformants exhibited a mean root length of The mean root length of *AtPCS1* transformants exhibits 1.75cm and wild type tobacco line exhibits 1.80cm under normal condition. Similarly, under 50µM Cd and As stress *Arabidopsis thaliana phytochelatin synthase1* transformants exhibited a mean root length of 1.7cm and 1.69 cm respectively. Whereas, wild type tobacco plants exhibits a length of 1.65 cm and 1.60cm respectively. Under 100 µM Cd and As stress *Arabidopsis thaliana phytochelatin synthase1* transformants exhibited a mean root length of 1.68cm and 1.55cm respectively. Whereas, wild type tobacco plants exhibits a length of 1.5cm and 1.35cm respectively. The primary root length was observed under 150 µM Cd and As stress *Arabidopsis thaliana phytochelatin synthase1* transformants recorded 1.5cm and 1.35cm respectively. Whereas Whereas, wild type tobacco plants exhibits a length of 1.2cm and 1.12cm respectively.

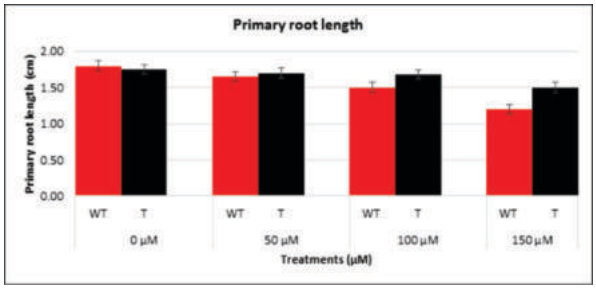

Fig. 3. Effect of Cd on root length of AtPCS1 expressing lines and untransformed controls

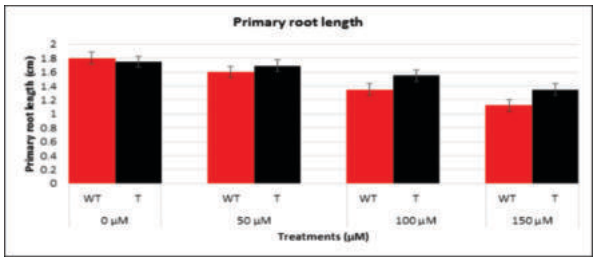

Fig.4. Effect of As on root length of AtPCS1 expressing lines and untransformed controls

Similarly it was reported earlier by Pomponi *et al.,* 2006 and Sylwia Wojas *et al.,*2008 in tobacco. Transgenic *Arabidopsis* plants over-expressing a native *Arabidopsis thaliana phytochelatin synthase1* exhibited Cd hypersensitivity (Lee *et al.,* 2003; Li *et al.,* 2004), while transgenic tobacco plants expressing a wheat *TaPCS1* showed slight increases in Cd tolerance (Gisbert *et al.,* 2003). The observed discrepancies were attributed to possible functional differences between *Arabidopsis thaliana phytochelatin synthase1* and *TaPCS1* enzymes, and more likely attributed to differences in downstream processing of Cd-PC complexes between tobacco and *Arabidopsis* (Li *et al.*, 2004) as well as to the toxicity of phytochelatins at supra-optimal levels (Lee *et al.,* 2003). It was also reported that heterologous expression of *CdPCS1* in tobacco and *Arabidopsis* led to significantly enhanced Cd or As accumulation in roots accompanied by increased PCs content (Shukla *et al.,* 2012 and 2013). Furthermore, ectopic expression of *CdPCS1* complements cad1-3 (PC-deficient) mutant of *Arabidopsis* to the similar level of synthetic PC (Shukla *et al.,* 2013). Earlier, it was reported that expression of *NnPCS1* in *Arabidopsis*, *PtPCS1* and *TaPCS1* in poplar and *TcPCS1* in tobacco disclosed accumulation of various metalloids and heavy metals, viz., Cd, As, Pb and Zn (Liu *et al.,* 2012; Couselo *et al.,* 2010; Adams *et al.,* 2011; Liu *et al.,* 2011).

CONCLUSION

The cloning and expression of *Arabidopsis phytochelatin synthase1* gene in transgenic tobacco plants. Effect of over expression of the *Arabidopsis thaliana phytochelatin synthase1* gene on plant growth tolerance to cadmium and arsenic. It was concluded that the transgenic tobacco lines showed marked enhancement in tolerance to cadmium and arsenic and also revealed increased accumulation of these heavy metals both in roots and shoots.

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