ABSTRACT

Three species of saltcedar (*Tamarix* L.; Tamaricaceae) occur in South Africa, although only *T. usneoides* is indigenous and is effectively used in southern Africa mines for phytoremediation. *Tamarix ramosissima* and *T. chinensis* were reportedly introduced into South Africa from Eurasia also for phytoremediation, but have since invaded riparian zones. *Tamarix* species are morphologically similar and hybridization adds to their taxonomic confusion. Biological invasions of *Tamarix* species have serious negative impacts in most riparian zones from changing the ecosystem function to altering the river flow system, to depositing salt crystals on the top soil and displacing native biodiversity. Mechanical and chemical control of the invasive *Tamarix* has been reported ineffective, leaving biocontrol as an alternative option to suppress *Tamarix* infestations thereby reducing its negative impacts. However, for the biocontrol programme of *Tamarix* in South Africa, hybridization and introgression events, and the presence of the indigenous species poses a challenge of host shifts for non-target effects to the indigenous species. Assessing the levels of genetic diversity and differentiation in invasive species and their closely related indigenous species is essential to better understand the invasion process and population genetic relations for an effective biocontrol programme. Molecular techniques are widely used during the initial phase of biocontrol programmes before the release of agents to accurately identify the invading species, assess the genetic diversity and compare the genetic structure between and within species.

In South Africa, the indigenous *Tamarix usneoides* is used for phytoremediation in rehabilitation efforts of mine lands contaminated with acid mine drainage. Although the plant has been planted on tailing storage facilities, its ability to tolerate the highly acidic conditions and heavy metals has not been fully investigated. Plants suitable for phytoremediation should have the ability to tolerate high concentrations of heavy metals and accumulate them in their above ground biomass. Therefore, this study investigated the physiological responses of *T. usneoides* to metal toxicity by assessing growth parameters. Profiles of gene expression patterns of *T. usneoides* for heavy metal tolerance were also investigated to assess the ability of the plant in activating its genome for tolerance against environmental stress conditions.

Nine microsatellite loci were used to assess the genetic diversity and differentiation of 150 *Tamarix* samples (117 individuals from South Africa and 33 from the U.S.). In South Africa,

samples were collected from four *Tamarix* taxa viz. *T. usneoides*, *T. chinensis*, *T. ramosissima* and *Tamarix* hybrids with 30 samples per taxon; while from the U.S. we collected *Tamarix* hybrids to compare against those from South Africa. This study showed that the indigenous *T. usneoides* is genetically more diverse than the invasive *T. chinensis* but is less diverse than *T. ramosissima*, another invasive species. There was great genetic differentiation between the indigenous and the invasive *Tamarix* species in South Africa. In addition, private alleles unique to *T. usneoides* were obtained in some remote places in the north-western part of the Northern Cape Province suggesting unpolluted populations of the indigenous *T. usneoides* germplasm. The low genetic diversity in *T. chinensis* seems to be as a result of its autogamous nature other than caused by founder effects. Higher genetic diversity was observed in the South Africa *Tamarix* hybrids compared to their U.S. counterparts and there is substantial genetic differentiation between the two hybridization incidences. The high genetic differentiation suggests that there might be minimal or no non-target effects on the indigenous species from biocontrol control agents against the invasive genotypes. This study also suggests that exploration of biocontrol agents should be done in the place of origin of the invasive plants other than in the U.S. where species of the *Diorhabda* beetles are effectively controlling *Tamarix* infestations.

Three different concentrations of cadmium (Cd) – 6 ppm, 12 ppm, 18 ppm, were applied to 240 individuals of indigenous *T. usneoides* propagated from 20 trees from different localities. The plants were exposed to Cd for eight weeks where plant height, shoot growth and chlorophyll content were measured weekly to assess the physiological responses against Cd toxicity. Of the 240 individuals, 80 samples were randomly selected for gene expression profiling using cDNA-AFLP analysis. Height, shoot growth and chlorophyll content of *T. usneoides* were all found to decrease with an increase in Cd concentrations over time as there were significant differences between the Cd treated groups and the controls. Chlorophyll content was found to be the most sensitive to Cd toxicity followed by shoot growth with plant height the least affected parameter. Although Cd toxicity affected the physiology and growth of *T. usneoides*, the plants were observed to be tolerant to the low (6 ppm) and medium (12 ppm) Cd concentrations as the measured parameters were not significantly reduced compared to the controls. Additionally, no visible signs of metal toxicity were observed at low and high concentrations. However, phenotypic signs such as leaf chlorosis, drying of branch bottoms, even the death of some plants

were observed at the high (18 ppm) Cd concentration. The high Cd concentration level resulted in a significant reduction in the growth of *T. usneoides*. cDNA-AFLP analysis of *T. usneoides* showed different profiles in expression patterns where high numbers of AFLP bands were obtained with an increase in Cd concentrations over time. The medium concentration was observed to generate the highest transcript derived fragments (TDFs) associated with heavy metal tolerance. The ability of *T. usneoides* to tolerate heavy metal concentrations < 12 ppm and the wide regions of the genome being expressed with an increase in metal concentration suggest that *T. usneoides* is a good candidate for phytoremediation.

To sum up, the indigenous *Tamarix usneoides* is moderately diverse genetically and is greatly differentiated from the exotic and invasive *T. chinensis*, *T. ramosissima* and their hybrids. The invasive *Tamarix* genotypes can be considered for biocontrol with suggested minimum non-target effects on the native species which is considered for conservation and presents a good candidate for use in phytoremediation because it can tolerate more than usual concentrations of heavy metals in contaminated soils.

Key words: Biocontrol agents, Biotic and abiotic pressure, Genetic variation, Gene expression, Habitat fragmentation, Metal tolerance, Reproductive systems, Tamaricaceae.