The genetic diversity of a clonal sedge (Terraria capillaris) was assessed using isozyme analysis of 11 loci. Of 29 enzyme systems tested, eight were selected which gave interpretable bands with consistently good resolution. Though seedlings of the species are rarely observed in nature, isozyme analysis showed that for the study transects containing 100 sample plants, the majority of plants at the site were sexually rather than clonally derived. Young plants generated from embryos grown in vitro from excised seeds showed a high level of genetic diversity which could account for the genetic diversity measured in the parent population. In terms of restoration of the species, 85% of the assessed genetic diversity at the study site could be retained if 25 T. capillaris plants were taken at random. The study illustrates how genetic assessment coupled with tissue culture methods provides a feasible model for the recovery of most of the assessed local genetic diversity of a clonal species.

Keywords: sedge; Cyperaceae; clone; zymogram; genetic diversity; recovery

Introduction

In addition to replacing plant density and biomass, preservation of floristic biodiversity, both in terms of species richness and genetic diversity of individual species, is now recognized as being important in mining rehabilitation and conservation programs (Common and Norton, 1992). In this study, assessment of genetic diversity was used as a tool for devising prescriptions for the restoration of a clonal species. In the past two decades, the number of studies conducted on clonal plant species has increased. However, no previous study of genetic diversity related to the replacement of a clonal plant species affected by mining appears to have been carried out.

Isozyme analysis is a biochemical tool which has been embraced by researchers for assessing genetic variation in plant and animal sciences. Isozyme analysis can detect variability between individuals at single loci and has been widely used to determine genetic variation existing within and between populations (Hamrick, 1989), for characterization of genetic resources and plant conservation (e.g. Coates and Sokolowski, 1989) and for the analysis of plant breeding systems (e.g. Innes and Hermanutz, 1988). More importantly for this study, isozyme analysis has been used extensively for the study of clonal plant species in terms of clone number (e.g. Silander, 1979) and genetic diversity (e.g. Horak et al., 1987).

To whom correspondence should be addressed.
Tetraria capillaris (F. Muell.) J. Black (hair sedge) is a widespread and common monocotyledonous perennial herb from south western Australia. It reproduces mainly by growth and extension of underground rhizomes which give rise to daughter ramets and clusters of plants. Since seed viability is extremely low and no seedlings of T. capillaris have been recorded in the wild (Meney et al., 1990) it has been postulated that a local population of plants might consist of a limited number of highly invasive clones. Propagation for rehabilitation of the species into disturbed sites has only been successful using in vitro plantlets generated from excised embryos of seed, a technique which has been developed and successfully applied for the propagation of recalcitrant plant species (Meney and Dixon, 1988; Meney et al., 1990; Rossetto et al., 1992).

The aim of this study was to assess the genetic diversity of wild clones of T. capillaris and to investigate excised embryo culture from seed as a means for recovering the species after mining in terms of genetic diversity. The protocol adopted for T. capillaris in this study may provide a basis for devising prescriptions for rehabilitation and recovery of other clonal plant species.

Materials and methods
Groups of 20 T. capillaris plant samples were sampled randomly at 10 plots in Jarrah (Eucalyptus marginata) forests, at Jarrahdale, Western Australia (Fig. 1). Five plots were located along each of two transects, one of 150 m and the other of 200 m. Transects lay on gently undulating land and ran parallel to and on either side of a creek (Fig. 2). The location of plots along each transect was determined to give approximately equal spacing between plots and to provide reasonable T. capillaris plant density.

T. capillaris plants with 1–2 growing shoots were collected within each plot. For each plot, a random sample of ten plants (referred to as the population samples) was taken from each collection of 20 plants and used for isozyme analysis. These will be referred to as ‘the population samples’.

Investigation of somatic variation
To investigate the level of somatic variation occurring within clones of T. capillaris plants at Jarrahdale, five clumps were sampled from different locations within the same vegetation zone. These clumps were washed and gently teased apart to reveal the rhizome connections between ramets of a genet. From the five clumps, a total of 12 segments, each made up of several visibly connected ramets, were removed (Fig. 3). The interconnected ramets represented approximately 2–3 years of growth. Isozyme analysis of tissue from each interconnected ramet was carried out.

Growth of T. capillaris plantlets by embryo culture
Seed was collected from 3 distinct T. capillaris clumps in the Jarrahdale area. Individual seeds that were solid under finger pressure were sterilized in 3 ml of 1% sodium hypochlorite solution with a drop of Tween 20 under vacuum for two 5 min periods with a 5 min rest period. They were then left to imbibe overnight. Seeds were handled aseptically and dissected to remove the white, disc-like embryo (fit broad-basal type, Radford et al., 1974). Embryos were placed directly onto filter paper bridges in sterile 30 ml tubes containing 10 ml of ½ strength Murashige and Skoog medium (Murashige and Skoog, 1962) containing 7.5 μM zeatin (Meney and Dixon, 1988).