ASSESSMENT OF THE ORIGINS OF *TRIBULUS TERRESTRIS* IN AUSTRALIA

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Summary. *Tribulus terrestris* is an introduced weed of unknown origin in Australia. It is a problem to the Australian dried fruit industry and is toxic to livestock. Correct identification of the weed is important because several native forms of *Tribulus* are confused with *T. terrestris*. Isozyme analysis separated seven Australian and six overseas populations of *T. terrestris* into four groups. The southern Australian, Broome (northern Western Australia), South African and U.S.A. populations formed a close group. Goomalling (southern Western Australia), Middle East and Crete populations formed a second group. The Indian population was separate from other groups and the Queensland and Northern Territory populations were closely related to each other. This preliminary analysis points to a foreign origin for weedy populations of *T. terrestris* in southern Australia.

INTRODUCTION

Tribulus terrestris (Zygophyllaceae) (caltrop) occurs in semi-arid and Mediterranean-type climates world-wide. It is a prostrate, summer growing annual. The seeds germinate after late spring and summer rains in warm conditions (24 - 27°C). The fruit consists of a woody burr comprised of five wedge-shaped cocci each with one or two pairs of large spines (8). There are 2 to 4 seeds per coccus (16). Seeds can remain viable for many years if buried in the soil, thus after successive generations a large reservoir of seed can accumulate (16).

Weedy populations of *T. terrestris* are found in many locations in Australia (10, 16). It is thought to have been accidentally introduced to New South Wales before 1895 (16). *Tribulus terrestris* is a problem to the Australian dried fruit industry because the spiny burrs may contaminate harvested dried fruit (7). Additionally the plant can be toxic to livestock, especially sheep. (2, 3). The taxonomic status of *T. terrestris* is unclear (2, 10, 12 19) and forms of the plant are variously considered to be native or introduced. Before considering biological control of this weed, it is essential to identify the introduced weedy form of *T. terrestris* and to determine its overseas origin (12). Here we report on a preliminary analysis, using isozyme techniques, to aid the identification of forms of *T. terrestris* as an indication of their geographic origin.

MATERIALS AND METHODS

Tribulus terrestris accessions were obtained by written requests to potential collectors. Over 80 samples were received from mainland Australia, and 13 from overseas locations with Mediterranean-type climates. The analysis presented here used seven populations from Australia and six from overseas. Dry seeds were stored at room temperature until needed. Seeds were excised from the woody burr and dipped in 1.25% sodium hypochlorite for 5 minutes, then washed in distilled water. The seeds were germinated on moist filter paper in a sealed petri dish incubated at a 16 hour 20°C, 8 hr 35°C cycle. Seedlings were used for isozyme work when they were 2 to 4 days old.

Cotyledon and root tip tissue of each seedling were homogenised together in 7 µL of 0.16M phosphate buffer (pH 7.0), containing 2.5% sucrose, 0.12% bromophenol blue and 0.75 mg mL^{-1}

dithiothreitol, in a plastic microwell tray on an ice bath (9). Three 2 x 4 mm filter paper wicks were placed in each sample. Five to ten seedlings from each of three plants was assayed for each population, except for Crete where seeds from only one plant was available.

Horizontal starch gels were made from 9.6% hydrolysed starch (Connaught) in each of three different buffer systems. Three enzymes were assayed on each of three buffer systems as follows: histidine gels at pH 8.0 (4), phosphoglucomutase (PGM) EC 2.7.5.1, 6-phosphogluconate dehydrogenase (6PGD) EC 1.1.1.44, phosphoglucoisomerase (PGI) EC 5.3.1.9; tris-citrate gels at pH 7.0 (11), aconitase (AC) EC 4.2.1.3, isocitrate dehydrogenase (IDH) EC 1.1.1.42, malate dehydrogenase (MDH) EC 1.1.1.37; lithium borate gels at pH 8.0 (9), esterase (EST) EC 3.1.1.1, leucine aminopeptidase (LAP) EC 3.4.11 or 13, glutamate-oxaloacetate transaminase (GOT) EC 2.6.1.1.

A wick from each sample was inserted into a slot cut in the cathodal side of each gel. To prevent the gels overheating, electrophoresis was carried out at 4°C in a refrigerator with an ice bath placed over each gel and a maximum current of 50 mA. Electrophoresis was terminated when the bromophenol blue front had moved about 10 cm. Each gel was sliced horizontally into 3 sections and each slice stained for an individual enzyme.

The assay methods used for EST, IDH and PGM and LAP were those described by Shaw and Prasad (13), except that an agar overlay was used for PGM. PGI, AC and MDH assays followed the methods of Richardson *et al.* (11), and 6PGD and GOT the method of Brown *et al.* (5). Stained gels were fixed with acetic acid, then rinsed in water, wrapped in plastic and photocopied for a permanent record. Isozyme banding patterns were scored immediately after staining.

Cluster analysis was performed using the UPGMA method (14) with modified Rogers distance (20) on the Biosys-1 computer program (17).

RESULTS AND DISCUSSION

Six of the enzymes with a total of eleven polymorphic loci were used in the analysis. In GOT and LAP systems the resolution proved too poor to score confidently, so they were excluded. PGI loci were clearly polymorphic, but were also excluded because they expressed complex zymograms with multiple overlapping bands. This indicates that there are more than the expected two loci for PGI (18), possibly with heteropolymers between the loci. It is therefore likely that the populations examined were polyploid. Additionally, fixed heterozygosity at the 6PGD - 2 locus in all populations examined, plus preliminary chromosome counts (4n = 24) support this view. Only those enzymes that demonstrated a diploid-like expression for the polymorphic loci examined were used in the analysis.

The dendrogram (Fig. 1) shows that some of the populations are very similar to each other. The southern Australian, Broome (northern Western Australia), South African and U.S.A. populations formed a close group. Goomalling (southern Western Australia), Middle East and Crete populations formed a second group. The Indian population was separate from any other groups and the Queensland and Northern Territory populations are closely related to each other, but were the greatest distance from the other populations. The percentage standard deviation of the best fitting dendrogram was 15.3% (6). Other methods used to derive genetic distances (17) produced essentially the same overall dendrogram.

This preliminary analysis supports the view that there are two forms of *T. terrestris* in Australia, an introduced form and a native form. The form found mainly in southern Australia is very similar to the plants from some of the overseas collections. The Northern Territory and Queensland forms of *T. terrestris* had the greatest genetic distance from overseas populations indicating that they are native to Australia. Possible origins of the introduced form of *T. terrestris* are the Mediterranean, Middle East and South Africa. The inclusion of further samples and cytogenetic data in the analysis will help to clarify the likely origins of the introduced *T. terrestris*.

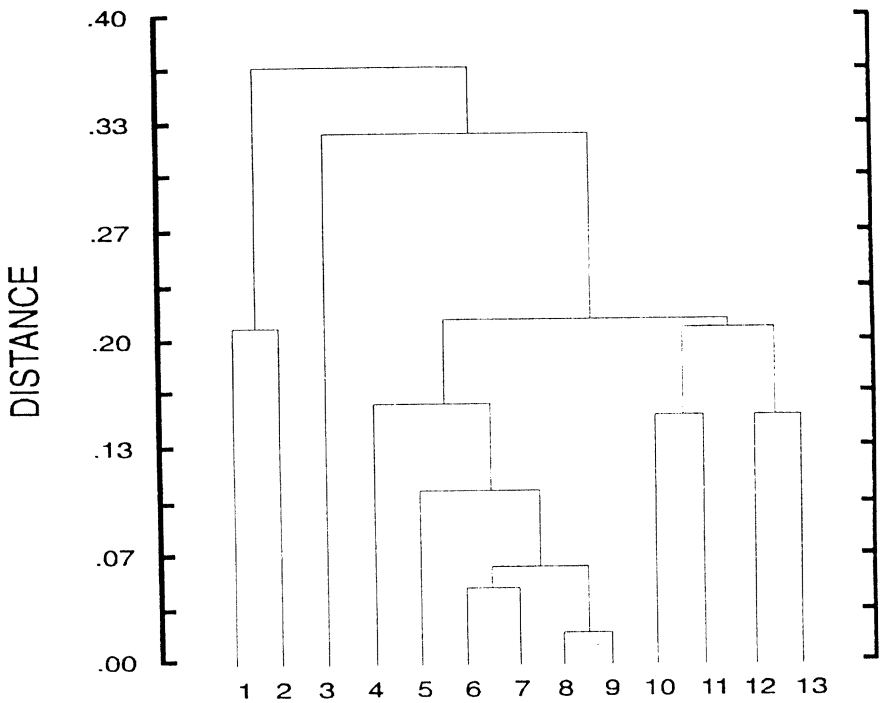

Figure 1. Dendrogram showing relationships among *Tribulus terrestris* populations, based on isozyme dissimilarity. Populations are as follows: (1) Darwin, Northern Territory, (2) Charters Towers, Queensland, (3) Kashmir, India, (4) Rondebosch, South Africa, (5) Loxton, South Australia, (6) Mudgee, New South Wales, (7) Mildura, Victoria, (8) Prosser, WA, USA, (9) Broome, Western Australia, (10) Crete (11) Kuwait City, Kuwait, (12) Tehran, Iran, and (13) Goomalling, Western Australia.

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