Assessing population genetic structure of sorghum landraces from North-western Morocco using allozyme and microsatellite markers

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Abstract The level of genetic diversity and the population genetic structure of sorghum landraces from North-western Morocco have been investigated based on direct field-sampling using both allozyme and microsatellite markers. As expected, microsatellite markers showed a much higher degree of polymorphism than allozymes, but relative measures of genetic structure such as Wright’s inbreeding coefficient $F_{IS}$ and Nei’s coefficient of genetic differentiation $G_{ST}$ were similar for the two sets of markers. Substantial inbreeding was found to occur within fields, which confirms that sorghum is predominantly selfing under cultivation. Most of the genetic diversity in Moroccan landraces occurs within fields (more than 85%), as opposed to among fields or among regions, a result which contrasts to those of studies based on accessions from germplasm collections. It is suggested that individual fields of sorghum constitute valuable units of conservation in the context of in situ conservation practices.

Key words Allozymes · Genetic diversity · Genetic resources · Microsatellites · Sorghum

Introduction

Molecular markers are recognised as significant tools to orient plant genetic resource conservation management, providing a means to accurately estimate the genetic diversity and genetic structure for species of interest (Hamrick and Godt 1997). They have been used, in the context of the ex situ conservation of domesticated species, to assess the pattern of genetic diversity in large germplasm collections (e.g. Lubbers et al. 1991; Zhang et al. 1992), to suggest priorities in future sampling missions, or to optimise the assembly of core collections (Schoen and Brown 1995). In the context of the in situ conservation of landraces, molecular markers could be useful to facilitate the selection of optimum sites, as well as to monitor ongoing changes in the pattern of diversity in the course of conservation practices (Newbury and Ford-Lloyd 1997). Currently, the pattern of genetic diversity within large germplasm collections is well characterised for most crops (e.g. Morden et al. 1989), but this does not necessarily reflect the extant genetic structure of landraces under cultivation conditions. Several factors could account for discrepancies between genetic-diversity estimates based on direct field samples and on accessions taken from germplasm collections: (1) most studies involve extremely low sample sizes at the accession level (less than ten seeds per accession in most cases: Doebley et al. 1985; Morden et al. 1989); (2) the plant material in germplasm collections is likely to have passed through genetic bottlenecks because of sampling and regeneration procedures; (3) the geographic scale of interest differs between genebank (world-wide scale) and in situ studies (regional and narrow scale). For these reasons, investigations on the pattern of genetic diversity of landraces in situ are urgently needed in order to orient in situ conservation programmes.

Sorghum (*Sorghum bicolor* L.) is one of the major food grains in the world, cultivated mainly in North...
A hierarchical sampling design was used with five representative regions from North-western Morocco prospected in August 1996 and four fields chosen within each region (Fig. 1). The prospected areas were situated in the vicinity of Tanger and Tetouan and ranged from low (running along the Mediterranean sea) to moderately high altitude (800 m). In each field, 15 inflorescences from different individuals were harvested at random. One seed per inflorescence was allowed to germinate in a Petri dish in a dark room at 22°C and seedlings were then transferred to the greenhouse and grown for about 1 month.

Isozyme procedures

Leaves from 4–6 week-old seedlings were harvested and ground on ice in the extraction buffer [Tris HCl 78.68 mM, polyethylene glycol (PEG) 6000: 1%, polyvinilpyrrolidone (PVP-40): 2%, dithiothreitol (DTT) 2.54 mM, sucrose 146 mM, ascorbic acid 2.02 mM, sodium metabisulfitte 20 mM, 2-mercaptoethanol 0.1%]. Extracts were centrifuged for 20 min at 13000 g and then the supernatant stored at −75°C. The homogenates were used in vertical 7.5% polyacrylamide gels in a Tris-glycine buffer (pH 8.6) according to Hames and Rickwood (1990). Running conditions were 75 mA for the upper gel (45 min) and 150 mA or 300 V for the lower gel (2 h 30 min) with a Protean II xi Slab Cell. In addition, part of the supernatant was adsorbed on Whatman 3M paper just after centrifugation and conserved at −75°C for starch-gel electrophoreses. The latter were run on 12% starch (Sigma ® S-8501) gels (180 V for 5 h) in a continuous Tris-borate-EDTA pH 8.6 buffer (Wendel and Weeden 1990). All electrophoreses were carried out in a cold chamber at 4°C. Nine enzymes giving clear patterns were employed in routine procedures [AAT (EC 2.6.1.1), ADH (EC 1.1.1.1), DIA (EC 1.8.1.4), EST (EC 3.1.1.-), GPI (EC 5.3.1.9), PER (EC.1.11.1.7), PGDH (EC 1.1.1.44), PGM (EC 5.4.2.2) and SOD (EC 1.15.1.1)]. Visualisation recipes are those given by Wendel and Weeden (1990). Genetic control of the analysed enzymes is discussed by Ollitrault et al. (1989a) and Wendel and Weeden (1990).

Materials and methods

Plant material

A hierarchical sampling design was used with five representative regions from North-western Morocco prospected in August 1996 and five basic races of cultivated sorghum are recognised, with a number of intermediates registered in ten categories. Sorghum is a wind-pollinated annual crop with outcrossing rates of about 0.10–0.15 (Doggett 1988), and hybridisation with spontaneous species seems to occur frequently. Allozyme studies in cultivated sorghum showed that it is strikingly less variable than other cereals like maize or barley, and that the considerable differentiation among accessions follows differences in geographic origin rather than racial classification (e.g. Morden et al. 1989; Ollitrault et al. 1989 b). Most of these conclusions were confirmed from investigations on molecular markers such as RAPDs and nuclear RFLPs (e.g. Tao et al. 1993; Deu et al. 1994; Cui et al. 1995), mitochondrial DNA (Deu et al. 1995) and chloroplast DNA markers (Aldrich and Doebley 1992). However, only genebank accessions from world-wide origins were used in these studies, and the in situ pattern of genetic diversity at a regional scale remains unknown.

In this paper, we investigate the population genetic structure of sorghum landraces from North-western Morocco, based on direct sampling in farmers’ fields. In this region, sorghum is mostly cultivated under traditional practices, and used for feeding livestock and poultry, as well as a backup in human nutrition (Kadiri and Ater 1997). A high degree of morphological variation has been observed within Moroccan landraces, which were assigned to the races durra, bicolor, and their intermediates (Kadiri and Ater 1997). In a previous study, we analysed the patterns of morphological and allozyme variation in Moroccan sorghum based on samples from six fields (Djé et al. 1998). We showed that the differentiation among fields was considerably larger for morphological characters than for allozyme markers (respectively 63% and 20% of the total variation expressed among fields), but this sampling scheme did not allow a detailed inference on the pattern of genetic variation. In the present paper, we used a hierarchical sampling design, with 20 fields of sorghum from North-western Morocco sampled within five separate areas, and investigated both allozyme and microsatellite markers. We addressed the two following issues: (1) the partition of genetic diversity at three different levels (individuals, fields, regions); (2) the comparison of population genetic statistics obtained with microsatellites versus allozymes.