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Plant Genetic Resources Newsletter

Bulletin de Ressources Phytogénétiques

Noticiario de Recursos Fitogenéticos

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Cover:
Collection of seed of a pea landrace from harvested bundles in a farmer's field in Gonghe county, Qinghai province, China (discussed by Redden et al. on pp. 1–10).
Photo: Dirk Enneking.

Couverture :
Graines d'une variété locale de pois récoltées dans un champ du comté de Ganghe, province de Qinghai, Chine (article de Redden et. al., pp. 1-10).
Photo: Dirk Enneking.

Portada:
Colección de semillas de una variedad local de guisante obtenidas de manojos cosechados en un campo agrícola del condado de Gonghe, provincia de Qinghai, China (ver el artículo de Redden et al. en las pp. 1-10).
Fotografía: Dirk Enneking

The Plant Genetic Resources Newsletter Web portal, with contents and summaries of all articles from issue 104 and full text from issue 121, can be accessed at http://www.bioversityinternational.org/publications/pgrnewsletter/archive.asp

Plant Genetic Resources Newsletter

Aims and scope
The Plant Genetic Resources Newsletter publishes papers in English, French or Spanish, dealing with the genetic resources of useful plants, resulting from new work, historical study, review and criticism in genetic diversity, ethnobotanical and ecogeographical surveying, herbarium studies, collecting, characterization and evaluation, documentation, conservation, and genebank practice.

Management
The Plant Genetic Resources Newsletter is published under the joint auspices of Bioversity International and the Plant Production and Protection Division of the Food and Agriculture Organization of the United Nations (FAO).

Availability
The Plant Genetic Resources Newsletter appears as one volume per year, made up of four issues, published in March, June, September and December. Plant Genetic Resources Newsletter is available free of charge to interested libraries of genebanks, university and government departments, research institutions, etc. The periodical may also be made available to individuals who can show that they have a need for a personal copy of the publication.

Types of paper
Articles
An article will publish the results of new and original work that makes a significant contribution to the knowledge of the subject area that the article deals with. Articles, which should be of a reasonable length, will be considered by the Editorial Committee for scope and suitability, then assessed by an expert referee for scientific content and validity.

Short communications
A short communication will report results, in an abbreviated form, of work of interest to the plant genetic resources community. Short communications in particular will contain accounts of germplasm acquisition missions. The papers will be assessed by an expert referee for scientific content and validity.

Other papers
The Plant Genetic Resources Newsletter will publish other forms of reports such as discussion papers, critical reviews, and papers discussing current issues within plant genetic resources. Book reviews will be printed, as well as a News and Notes section. Suggestions for books to review are invited, as are contributions to News and Notes.

Submission
Papers should be submitted online at http://pgrn.bioversity.cgiar.org. Correspondence on Editorial matters should be addressed to: PGRNManuscripts@cgiar.org.

Bulletin des ressources phytogénétiques

Domaine d'intérêt
Le Bulletin des ressources phytogénétiques publie des articles en anglais, en espagnol et en français, sur les ressources génétiques de plantes utiles, fruit de nouvelles recherches, d'études historiques, d'examens et de critiques concernant la diversité génétique, d'études ethnobotaniques et écogéographiques, d'études d'herbiers, d'activités de collecte, de caractérisation et d'évaluation, de documentation, de conservation et les pratiques des banques de gènes.

Parrainage
Le Bulletin des ressources phytogénétiques est publié sous les auspices de Bioversity International et de la Division de la production végétale et de la protection des plantes de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO)

Distribution
Le Bulletin des ressources phytogénétiques paraît une fois par an en un volume regroupant quatre numéros publiés en mars, juin, septembre et décembre. Il est distribué gratuitement aux bibliothèques des banques de gènes, universités, services gouvernementaux, instituts de recherche, etc. s'intéressant aux ressources phytogénétiques. Il est aussi envoyé sur demande à tous ceux pouvant démontrer qu'ils ont besoin d'un exemplaire personnel de cette publication.

Types de documents publiés
Articles
Un article contient les résultats de travaux nouveaux et originaux qui apportent une contribution importante à la connaissance du sujet dont traite l'article. Les articles, qui doivent être d'une longueur raisonnable, sont d'abord examinés par le Comité de rédaction qui en évalue la portée et la validité, puis par un expert qui en examine le contenu et l'intérêt scientifiques.

Brèves communications
On entend par brève communication un texte contenant, sous une forme abrégée, les résultats de travaux présentant un intérêt pour tous ceux qui s'occupent de ressources phytogénétiques. Elle contient en particulier des comptes rendus des missions d'acquisition de matériel génétique.

Autres documents
Le Bulletin des ressources phytogénétiques publie d'autres types de rapport tels que des documents de synthèse, des études critiques et des articles commentant des problèmes actuels concernant les ressources phytogénétiques. Le Bulletin publie une revue de livres ainsi qu'une section intitulée Nouvelles et Notes. Les auteurs sont invités à envoyer leurs suggestions pour les livres à passer en revue ainsi que des contributions aux Nouvelles et Notes.

Présentation
Les articles devraient être soumis en ligne à http://pgrn.bioversity.cgiar.org. Toutes correspondances sur les aspects éditoriaux devraient être adressées à : PGRNManuscripts@cgiar.org.

Boletín de Recursos Fitogenéticos

Objetivos y temas
El Noticiario de Recursos Fitogenéticos publica documentos en inglés, francés y español que tratan de los recursos genéticos de plantas útiles, fruto de nuevos trabajos, estudios históricos, revisiones y análisis críticos relacionados con la diversidad genética, investigaciones etnobotánicas y ecogeográficas, estudios de herbarios, actividades de colección, caracterización y evaluación, documentación, conservación, y prácticas en bancos de germoplasma.

Dirección
El Noticiario de Recursos Fitogenéticos se publica bajo los auspicios conjuntos del Bioversity International y la Dirección de Producción y Protección Vegetal de la Organización de las Naciones Unidas para la Agricultura y la Alimentación.

Distribución
El Noticiario de Recursos Fitogenéticos aparece como un volumen anual compuesto por cuatro números, que se publican en marzo, junio, septiembre y diciembre. Se distribuye gratuitamente a las bibliotecas de bancos de germoplasma, facultades universitarias y servicios gubernamentales, centros de investigación, etc. que se interesan en los recursos fitogenéticos. También pueden obtener este noticiario las personas que demuestren necesitar una copia personal.

Tipos de documentos
Artículos
Los artículos divulgarán los resultados de trabajos nuevos y originales que contribuyan de modo importante al conocimiento del tema tratado. Dichos artículos, que deberán tener una longitud razonable, serán examinados por el Comité de Redacción en cuanto a su pertinencia e idoneidad y posteriormente un experto juzgará su contenido y validez científicos.

Comunicaciones breves
Las comunicaciones breves informarán de modo conciso sobre los resultados de trabajos de interés para las personas que se ocupan de los recursos fitogenéticos. Las comunicaciones breves incluirán, en particular, resúmenes sobre las misiones de adquisición de germoplasma.

Otros documentos
El Noticiario de Recursos Fitogenéticos publicará otros tipos de informes, como documentos de trabajo, análisis críticos, y documentos que examinen cuestiones de actualidad relacionadas con los recursos fitogenéticos. El Noticiario publicará una reseña de libros así como una sección de Noticias y Notas. Las propuestas de libros para reseñar y las contribuciones a la sección de Noticias y Notas serán bien acogidas.

Presentación
Para enviar sus artículos, favor dirigirse a la dirección electrónica http://pgrn.bioversity.cgiar.org. Para información adicional sobre asuntos editoriales, dirigirse a: PGRNManuscripts@cgiar.org.

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Collecting and surveying landraces of pea (*Pisum sativum*) and faba bean (*Vicia faba*) in Qinghai province of China

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Summary

Collecting and surveying landraces of pea (*Pisum sativum*) and faba bean (*Vicia faba*) in Qinghai province of China

Twenty-eight pea (*Pisum sativum*) landraces and 26 faba bean (*Vicia sativa*) landraces were collected from farmers across 10 counties in Qinghai province of north-west China during August and September 2004 by staff from Chinese and Australian institutes. Passport data of site descriptors and GPS observations were recorded for individual accessions, plus associated historical and sociological data on the source of the landrace, crop management, gender roles and seed use. The landrace histories revealed that some were introductions from other areas, and there was historical evidence for genetic erosion. Specific gender roles were reported for some crop management operations, but not for choice of variety. No ethnic influence was detected on retention of landrace, cropping system or end use amongst the five ethnic groups interviewed.

Key words: landrace, pea, *Pisum sativum*, faba bean, *Vicia faba*, collecting mission, genetic erosion, Qinghai, China.

Résumé

Collecte et prospection des variétés locales de pois (*Pisum sativum*) et de fèves (*Vicia faba*) dans la province de Qinghai en Chine

Vingt-huit variétés locales de pois (*Pisum sativum*) et 26 de fèves (*Vicia faba*) ont été collectées auprès d'agriculteurs dans 10 comtés de la province de Qinghai dans le nord-ouest de la Chine en août et septembre 2004 par une équipe issue d'instituts chinois et australiens. Des données passeport de descripteurs de site et des observations GPS ont été consignées pour différentes accessions, ainsi que des données historiques et sociologiques sur la source des variétés locales, la gestion des ressources agricoles, les rôles masculins/féminins et l'utilisation des semences. L'historique des variétés locales révèle que certaines ont été introduites à partir d'autres régions et il existe des preuves historiques de l'érosion génétique. Il existe des rôles spécifiquement masculins/féminins pour certaines opérations de gestion des ressources agricoles, mais pas pour le choix des variétés. Aucune influence ethnique n'a été détectée pour la conservation des variétés locales, le système agraire ou l'arrêt de l'utilisation parmi les cinq groupes ethniques interrogés.

Resumen

Recolección y examen de variedades locales de guisantes (*Pisum sativum*) y habas (*Vicia faba*) en la provincia china de Qinghaititle

En agosto y septiembre de 2004 personal de instituciones de China y Australia recogieron veintiocho variedades locales de guisantes (*Pisum sativum*) y veintiséis de habas (*Vicia faba*) entre agricultores de 10 condados de la provincia de Qinghai en el noroeste de China. Para cada accesión se registraron datos de pasaporte de descriptores de sitios y observaciones de GPS, además de datos históricos y sociológicos sobre la fuente de las variedades locales, ordenamiento de cultivos, roles de cada sexo y utilización de las semillas. La historia de las variedades locales reveló que algunas fueron introducidas desde otras zonas, y había evidencia histórica de erosión genética. Para algunas operaciones de administración se informó que había funciones específicas según el sexo, no así respecto de la elección de variedades. No se detectó influencia étnica respecto de la conservación de variedades locales, sistema de cultivo o uso final entre los cinco grupos étnicos entrevistados.

Introduction

Qinghai is a western province of China, bordered by Xinjiang in the north-west, Tibet to the south-west, Gansu to the north-east and Sichuan to the south (Figure 1).

This region is part of the Qinghai–Tibetan plateau. It lies at high elevation, and its agriculture is dominated by pasture and livestock production. Crop production is mainly confined to river valleys and areas of lower elevation bordering Gansu and Sichuan. The Huang He (Yellow) river is a dominant geographic feature in the east of Qinghai province and dissects the plateau. Sixty percent of the cultivated area is rain-fed and 40% is irrigated. Cold winters and frosts during spring and autumn restrict the crop growing season to late spring–summer.

In 2003, total sown area for all crops was 466 000 ha, with canola (149 000 ha) and wheat (107 000 ha) being the major crops. Total human population was 5.34 million (ERS-USDA 2005). In 1990 ethnic minorities made up 40% of the total population of the province and 60% of its rural population (Longworth and Williamson 1993); six ethnic minorities were represented in the province: Han, Hui, Mongolian, Sala, Tu and Tibetan. Major crops are spring wheat, canola, potato and barley. Faba beans (*Vicia faba*) and peas (*Pisum sativum*) are important legume crops for food and feed, with large-seeded faba bean also exported. Both crops are consumed as vegetables and as grain. Faba beans are cultivated on 30 000

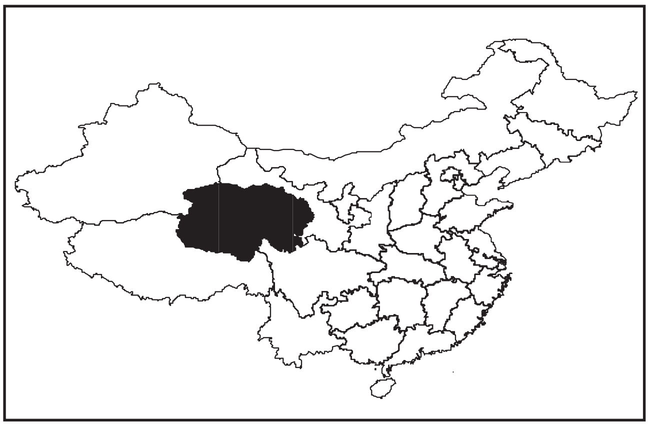

Figure 1. Quinghai province, China.

ha, most of which is irrigated, while peas are grown on some 24 000 ha of rain-fed upland fields. Most commercial pea production is for food processing to make noodles, but some is used for livestock feed.

Throughout China, collections of landrace germplasm were carried out during the 1950s and 1980s, organized by the Institute of Crop Germplasm Resources (ICGR), Beijing, with assistance from local county, district and regional administrations. This work is documented by an *Atlas for Genetic Resources in China* (Cao Yongsheng et al. 1995) and a series of germplasm catalogues (ICGR 1987; 1990; 1998). These collections were complemented by further landrace collections in 2002 by staff of the Qinghai Academy of Agricultural and Forestry Science (QAAFS) in areas where legume production was still subsistence oriented rather than commercially oriented. In the major production areas most produce was marketed and the varieties were from plant breeding programmes.

In 2004, Chinese staff in collaboration with Australian scientists, as part of project CS1/2000/035, funded by the Australian Centre for International Agricultural Research (ACIAR), re-sampled these *in situ* pea and faba bean landraces. Additional information was collected on physical and climatic characteristics of the sites, landrace history and cultivation, and potentially significant traits. Pea and faba bean were minor crops in the areas where landraces were collected, grown at least partly for subsistence food and feed. Thus Xining and Haidou districts, which account for 70% of crop cultivation in Qinghai, were excluded from the collection itinerary. Since many of the collection sites were above 1500 m elevation, germplasm from these sites may have tolerance to cold/frost during pod filling.

The aims of the collecting mission were to:

1. Collect landraces of peas and faba bean before modern varieties replace them.
2. Add value to these new accessions through site descriptions and sociological data on the factors affecting the continued cultivation of landraces.
3. Identify germplasm with likely tolerance to spring radiation frosts.

Materials and methods

With the help of local administrations, the 2002 collection mission targeted counties where pea and faba bean landraces were grown. Seed samples of the landraces were taken for a germplasm collection.

The 2004 mission re-sampled the same range of altitudes and ecological conditions in areas where legume crops were produced. If faba bean and pea special landraces had previously been collected in these areas, their characteristics were checked in the three volumes of germplasm catalogues for grain legumes (ICGR 1987; 1990; 1998). Areas with prospective collection sites were visited by He Chenbang, Wu Kunlun and Yan Qingbiao during July 2004 to prepare the itinerary for seed collection and detailed surveying.

A questionnaire covering geographic, edaphic, agronomic, phenological and sociological aspects was prepared to form the basis for interviews with farmers (Appendix 1). This had two sections, one describing the edaphic and climatic conditions at each village or farm locality where collections were made, and the other investigating cultivation and food use of the landraces collected.

Collecting was conducted from 24 August to 2 September 2004. The team of breeders and genetic resources staff were from Australia—NSW Department of Primary Industries and Australian Temperate Field Crops Collection (ATFCC), Victoria—and from China—The Institute for Crop Germplasm Resources, Chinese Academy of Agricultural Sciences and the Qinghai Academy of Agricultural and Forestry Sciences (QAAFS).

The itinerary was to the north and west of Xining, then to the south and back to Xining, through Menyuan, Qilian, Gonghe, Xinghai, Xunhua, Hualong and Pingan counties (Figure 2, Table 1).

At the completion of the mission the collected seed was split into two subsamples. One set remained with QAAFS in Xining. The other was sent to the ATFCC, Australia, with samples of faba bean landraces being sent to the

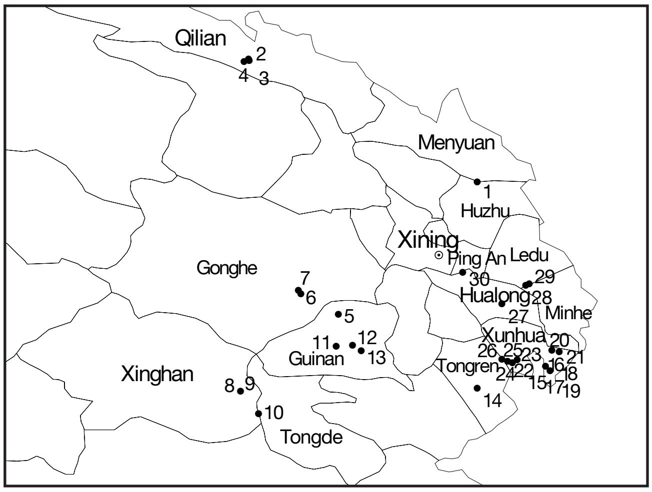

Figure 2. Qinghai counties visited during the collecting mission in 2004 showing sites where faba bean and pea landraces were collected.

Table 1. Samples of faba bean and pea collected in Qinghai province, China, August–September 2004, and site characteristics, occurrence of frosts and crop maturity as reported by farmers.

Sample	Site	County	Species[1]	Maturity[2]	Spring & summer frosts	Autumn frost	Ethnic group	pH	Longitude	Latitude	Altitude m
1	1	Menyuan	V	E	mid May	late Sept–early Oct	Han	5.5	102.05	37.20	2630
54	1	Menyuan	P						102.05	37.20	2630
2	2	Qilian	V	M	May	late Sept–early Oct	Hui	8	100.20	38.22	2658
3	2	Qilian	V	M		early Oct	Hui		100.20	38.22	2658
4	2	Qilian	P	M		early Oct	Hui		100.20	38.22	2658
5	3	Qilian	P	M	May	late Sept–early Oct	Hui	8.5	100.20	38.20	2647
6	4	Qilian	P	M	late Apr–early May	late Oct–early Nov	Hui	9	100.16	38.20	2666
7	4	Qilian	P	nil			Hui		100.16	38.20	2666
8	4	Qilian	V	E			Hui		100.16	38.20	2666
9	4	Qilian	P	nil			Hui		100.16	38.20	2666
10	5	Gonghe	P	E	late Apr–early May	late Sept	Han	9	100.92	36.13	2674
11	5	Gonghe	V	M			Han		100.92	36.13	2674
12	5	Gonghe	V				Han		100.92	36.13	2674
13	5	Gonghe	V				Han		100.92	36.13	2674
14	5	Gonghe	P	M			Han		100.92	36.13	2674
15	6	Gonghe	P	E	early–mid May	mid Sept	Hui	9	100.61	36.30	2950
16	7	Gonghe	P	L		mid Sept	Tibetan	8.5	100.60	36.33	3040
17	8	Xinghai	V	M	early May	early Sept	Hui/Han	8.5	100.15	35.51	2731
18	8	Xinghai	V	L	mid May	early Sept	Hui/Han		100.15	35.51	2731
19	9	Xinghai	V	L	early May	early Sept	Tibetan	8.5	100.14	35.51	2715
20	10	Tongde	V	L	mid May	early Sept	Tibetan	9	100.28	35.33	2846
21	11	Guinan	P	L	early Jul	early Sept	Han	8.5	100.91	35.88	2848
22	11	Guinan	P	E			Han		100.91	35.88	2848
23	11	Guinan	P	M			Han	n.d.	100.91	35.88	2848
24	12	Guinan	P	L	mid Apr	mid Sept	Tibetan	9	101.03	35.89	2868
25	13	Guinan	P	E			Tibetan	9	101.12	35.84	3029
26	13	Guinan	P	E				n.d.	101.12	35.84	3029
27	14	Tongren	V	L	mid Mar	mid Oct	Tu	9	102.05	35.56	2400
28	14	Tongren	P		mid Mar	mid Oct	Tu	n.d.	102.05	35.56	2400
29	15	Xunhua	V	M	mid Apr	mid Oct	Han	7.5	102.59	35.71	2344
30	15	Xunhua	V				Han	n.d.	102.59	35.71	2344
31	16	Xunhua	P	M	early Apr	mid Aug	Tibetan	n.d.	102.59	35.69	2900
32	17	Xunhua	V	M	mid–late Mar	mid Sept	Sala	8	102.61	35.68	2455
33	18	Xunhua	V	M	mid or late Mar	mid Sept	Sala	9	102.61	35.68	2472
34	19	Xunhua	V	M	late Mar	early Oct	Sala	9.5	102.63	35.66	2511
35	20	Xunhua	P	M	late Mar	late Sept–early Oct	Sala	8.5	102.65	35.83	1854
36	20	Xunhua	V	M			Sala	n.d.	102.65	35.83	1854
37	21	Xunhua	P	M	late Mar	late Sept	Sala	8	102.71	35.83	1845
38	21	Xunhua	V	M			Sala	n.d.	102.71	35.83	1845
39	21	Xunhua	V	M			Sala	n.d.	102.71	35.83	1845
40	22	Xunhua	V	M	early Mar	mid Sept	Tibetan	9	102.38	35.77	2265
41	23	Xunhua	V	M	early Mar	mid Sept	Tibetan	8.5	102.36	35.76	2375
42	24	Xunhua	P	M	mid Mar	mid Sept	Tibetan	9	102.32	35.74	2576
43	24	Xunhua	V	M			Tibetan	n.d.	102.32	35.74	2576
44	25	Xunhua	P	nil	late Mar	early Sept	Tibetan	n.d.	102.28	35.76	2650
45	25	Xunhua	P	M			Tibetan	n.d.	102.28	35.76	2650
46	26	Xunhua	P	M	late Apr	mid Sept	Tibetan	9	102.24	35.77	2602
47	26	Xunhua	P	M			Tibetan	n.d.	102.24	35.77	2602
48	26	Xunhua	V				Tibetan	n.d.	102.24	35.77	2602
49	27	Hualong	P	E	early May	early Sept	Hui	9	102.21	36.12	2938
50	28	Leduo	P	L	early May	mid Sept	Han	9	102.45	36.36	2572
51	28	Leduo	V	L			Han	n.d.	102.45	36.36	2572
52	29	Leduo	V	L	late Apr	late Sept	Han	9	102.46	36.37	2430
53	30	Pingan	P	L	mid May	mid Sept	Han	9	101.93	36.46	2881

1. Species: P = *Pisum sativum*, V = *Vicia faba*.
n.d. = not determined.

2. Maturity: E = early, M = medium, L = late.
blank = no record.

Table 2. History and cultivation of landraces collected in Qinghai province, China, August–September 2004.

Sample	Site	Species[1]	Grow[2]	Name	History	Source of landrace seed[3]	Reason[4]
1	1	V	5	Gadadou	>10 yr	2	1
54	1	P			>10 yr	2	
2	2	V	5	Hongdadou	>10yr from Datong county	3	1,5,6,7:yield
3	2	V	3	Baidadou	Grandfather kept, >20 yr	2	1,5,6,7:low yield
4	2	P	5	Baidou	>25 yr, from Gansu	2	1,6
5	3	P	1			1	
6	4	P	5	Xueqindou	Grandfather kept >20 yr	1	1,6
7	4	P	5	Xueqindou	Nil	2	1,5
8	4	V	5	Mayadadou	Nil	2	1,5,6,7:faba flour
9	4	P	5	nil	>20 yr introduction from Datong	2	1
10	5	P	1	Zaoshuwan dou	2 yr ago from Gansu	2	4,6
11	5	V		Wusedadau	2 yr ago from Gansu	3	1,5,6
12	5	V					
13	5	V					
14	5	P			Grandfather kept, ancestral		5,6
15	6	P	9	Heidou'er	Grandfather kept, ancestral	1	6,7
16	7	P	3	Baidou	Grandfather kept	1	1,6,7
17	8	V	1	Hongdadou	Grandfather kept since 1983 at least	1	5
18	8	V	1	Baidadou	Grandfather kept	1	1,6,7
19	9	V	1	Gadadou	Neighbour	1	5
20	10	V	5	Shanma Dadou	Grandfather kept	1	1,5
21	11	P	7	Heimadou	Ex Gonghe county 2 yrs	2	4
22	11	P	1	Baidou	Old introduction >10 yr	3	1,7
23	11	P		Dabaidou	Old introduction >10 yr	3	1
24	12	P	3	Shanma dou	>7 yr	1	1,5,6
25	13	P	3			1	
26	13	P	3			1	
27	14	V	1	Dadou	>10 yr ex Datong county	3	1
28	14	P	1	Honghuabaidou	>10 yr, introduced Huangnaihai district	3	1
29	15	V	5	Hongdadou	Grandfather kept, >30 yr	2	1,5
30	15	V		Baidadou			
31	16	P	1	Shanma dou	>15 yr introduction	2	1,5
32	17	V	1	Hongdadou	>3 yr from Linxia Gansu, Hui district where >20 yr	2	1,5
33	18	V	1	Hongdadou	Introduced from Linxia Gansu where >20 yr	2	1,5
34	19	V	5	Dadou	>10 yr introduction	2	1,5,6
35	20	P	1	Douzi	Grandfather kept, ancestral	2	1,5,6
36	20	V	5	Dadou	Ancestral	2	1,5,6
37	21	P	3	Xiaodou	Gansu province, >5 yr ago	2	1,5
38	21	V	3	Dadou	Village market seed from Dahejia Gansu	5	1,5
39	21	V	3	Dadou	Village market seed from Dahejia Gansu	5	1,5
40	22	V	5	Dadou	>10 yr ago from Linxia Gansu	2	1,5
41	23	V	3	Dadou	Ancestral	2	1,5,6
42	24	P		Shanma gadou	>10 yr introduction	2	1,5,6
43	24	V	1	Shanma	1970s	2	1,5
44	25	P	5	Shanma dou	>30 yr ancestral	6	1,5
45	25	P	5	Shanma dou	Ancestral >30 yr	6	1,5,6
46	26	P	3	Shanma dou	>25 yr	2	1,5
47	26	P	3	Shanma dou	>25 yr	2	1.5
48	26	V	3	Shanma dadou	>30 yr	2	1,5
49	27	P	3	Caoyuan 12 Caoyuan 224	>5 yr	1	1,6
50	28	P	5	Gadou'er	>10 yr	1	1,7:animal feed
51	28	V	1	Gadadou	>10 yr	2	1,7
52	29	V	1	Gadadou	>10 yr	1	6,7
53	30	P	5	Gaqindou	>10 yr	1	1,6

1. Species: P = *Pisum sativum*, V = *Vicia faba*.
2. Growing of landrace in locality: 1 = rare, 3 = occasional, 5 = frequent, 7 = abundant, 9 = dominant (blank = no record).
3. Source of landrace seed: 1 = farm, 2 = farm store, 3 = home garden, 4 = village market, 5 = town market (blank = no record).
4. Reason for growing: 1 = own seed, 2 = less disease, 3 = drought tolerant, 4 = frost tolerant, 5 = cooking/taste preference, 6 = market price, 7 = animal feed (blank = no record).

International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria.

At each collection site, the topography, soil and pH characteristics (CSIRO soil pH kit: Raupach and Tucker 1959; Manutec Pty Ltd, 4 Jeanes St., Beverley SA 5009, South Australia) were noted, and 1–4 farms with landraces were sampled according to diversity of local landraces (Table 1, Appendix 2). Modern varieties when encountered were not collected, except in one case of a mixture of two modern varieties (site 27). A total of 54 farms were sampled for seed of landraces over 30 sites or districts (Table 1). The locations varied from home gardens in urban areas to both monoculture and mixed crop farming areas near villages (Table 2).

During collecting in Aug–Sept 2004, local inhabitants provided useful guidance to locate farmers with landraces. When green crops were encountered the local farmers were generally able to provide samples of the same varieties from their seed stores, and the questionnaires for each accession were completed by both field observations and interviews with the growers.

Due to mountainous terrain with passes at altitudes exceeding 3800 m, extended travel across the high plateau (3200 m) and the need to descend into river valleys to reach cultivation areas, the number of farms visited for germplasm collection varied from one to nine per day. Latitude and longitude were recorded with a Garmin GPS 12 and altitude with an Eschenbach altimeter, calibrated by GPS (Appendix 2). In case of discrepancies between GPS and altimeter readings, a stabilized GPS value was given preference. GPS data were later converted to WGS84 datum format with the software GPS utility 4.15.17.

Results

There were 28 pea and 26 faba bean landraces collected (Table 1). The altitude of collection sites ranged from 1845 to 3040 m, with only pea landraces collected above 2850 m (Table 1). The highest altitude of observed faba bean cultivation was in Pingan county (2880 m) where a cultivar of Huangyuan county was planted. The variety did not perform well at this site and nodules were rotting. No mature seed of the variety could be collected at this site. The collecting mission covered a total of about 3000 km, traversing diverse agricultural and pastoral areas and visited farms of Han, Hui, Sala, Tu and Tibetan (but not Mongolian) ethnic groups (Table 1). The questionnaire on site characteristics and landrace cultivation was not always completed if landraces had been harvested already, or seed was only available from the farmer's seed store (Tables 1 and 2).

Genetic erosion

The prevalence of landraces was unrelated to either the frequency of pea or faba bean cultivation on local farms (Table 2) or the presence of improved cultivars. However, a dynamic picture of transition was found for landraces. Only 18 of the 54 samples collected (9 pea landraces and 9 faba bean landraces) were regarded as ancient with continual cultivation on the farm, i.e. older than 25 years or grown by grandparents (Table 2). Half of the landraces had been introduced in the past 25 years, from other districts or from Gansu province. The majority of farmers with landraces also grew modern pea or faba bean varieties; only two preferred only landraces. At least one landrace was admixed with modern improved varieties, and two would no longer be cultivated due to relatively low yields. Possibly the relatively small number of landraces found may reflect continuous long-term selection over centuries for adaptation in a single locality. Cultivation of landraces ranged from rare to frequent; landraces were abundant or dominant at only two locations (Table 2). In two cases the landraces were confined to the bunds at the edges of fields (sample 17, site 8, and sample 19, site 9).

Compared with the numbers of samples collected on previous expeditions (Table 3) there has been a dramatic reduction of pea landrace samples in the same counties from 47 to 28 over a 50-year period. A further 155 landraces had previously been found in other counties that were not visited in 2004 because farmers there were no longer cultivating landraces. However, the reverse was found with faba bean samples, with 26 samples found on this mission compared with only seven in the same counties in previous missions. While the number of samples collected is an indication, such figures can be misleading and do not necessarily reflect genetic diversity. Some of the 2004 samples were from counties not represented in previous collections.

One pea landrace had been grown for the last time since it did not yield as well as a new variety. One faba bean landrace with small red seeds (sample 17, site 8) was kept by only one family in the village because they preferred its taste. This culinary preference for a landrace was the exception. Most farmers chose a variety based on availability of their own seed, with additional considerations being food preparation characters and market price, followed by utility as animal feed (Table 2). Availability of own seed was not cited in only 7 cases.

The local names (Table 2) for the landraces described seed colour or size in 22 cases (gadadou = small-seeded faba; baidou = white-seeded pea; Xueqindou = purple-seeded faba; hong dadou = red-seeded faba; heidou'er = black pea; wusedadou = coloured faba) and were non-descriptive in 17 cases (dadou = faba bean, shanma dou/dadou = pea/faba bean); in 8 cases there was no local name given. Only a few local names were more specific, e.g. mayadadou = horse tooth bean; zaoshuwandou = early pea; hongghuabaidou = red-flowered white-seeded pea. Caoyuan 12 and Caoyuan 224 (mixture in sample 49) are modern varieties bred by QAAF.

The lack of locally specific identifying names could imply an absence of a special adaptation niche in local landraces, or in many cases a pragmatic view of landraces for utilitarian food/feed use rather than for cultural/food preferences. Choice of variety, and reasons for change, were usually pragmatic and based on economics of own seed supply and productivity. Based on their seed characteristics most of the samples are different genotypes, although some samples showed similarities.

Table 3. Number of samples of pea and faba bean collected in Qinghai province counties, China[1], 1950 to 2004.

	Pea			Faba bean			
County	**1950–78**	**1979–89**	**2004**	**1950–78**	**1979–89**	**1990–99**	**2004**
Datong	15				2	2	
Dulan	5						
Gonghe	9		4		1		3
Guide	4			1			
Guinan			6		1		
Guisui					2		
Haiyan	1						
Hualong	10		1		2		
Huangyuan	8			1	3		
Huangzhong	30				6	1	
Huzhu	19				7	1	
Leduo	8		1		1		2
Menge	1						
Menyuan	4		1	1	1		1
Minghe	46				22		
Numuhong						1	
Pingan			1				
Qilian	1		5				3
Tongde	1				1		1
Tongren	6		1				1
Wulan	1						
Xinghai	5				2	1	3
Xining	15	10		1	1	7	
Xunhua	9		8		1	1	12
Grand Total	198	10	28	4	53	14	26

1. Source of data: ICGR Beijing, based on germplasm catalogues.

Climatic factors associated with landraces

To obtain information about the occurrence of radiation frosts during flowering and seed set, the questionnaire (Appendix 1) contained two questions, one on the date of the first (autumn) frosts, the other on summer frosts (Table 1). Only the records of specific frost data from an individual farmer were tabulated; where other farmers appeared to provide general agreement but did not give specific information nothing is tabulated, thus the data are under reported.

No consistent association was found between altitude and time of first frosts (Table 1). At site 11 (samples 21–23), the farmer for sample 21 specifically mentioned occurrence of frost during mid-summer, but the other two farmers were not clear about this. Site 16 (sample 31) at 2900 m experiences the earliest autumn frosts during mid-August, followed by sites 8, 9, 10, 25 and 27 during early September. This information provided by farmers points towards potential sources of germplasm with frost tolerance during the reproductive period. Interestingly, site 11 was a source of pea varieties with early, medium and late maturity, and at site 27 the pea landrace had early maturity.

Soil PH readings ranged between eight and nine across most districts except sites 1 and 15, which had acid to neutral soils and both pea and faba bean landraces (Table 1). The topography was mainly on slopes with clay–silty loam soils, and less than 2% rock (Appendix 2). The 2004 growing season was rated as usual to dry in rainfall.

Ethnic factors

The number of sites visited did not sufficiently sample ethnic groups (Table 1) to allow a statistical comparison; however, there are some trends in the data which may be followed up by later studies.

There was little difference between ethnic groups in preference for pea or faba bean, although faba bean rather than peas was grown by the Sala group, all located in Xunhua county, mainly as monoculture crops with high inputs of fertilizer and irrigation. However the same practices were followed with landraces of both species by Tibetan farmers in the same county. The comparison of ethnic group farm practices was confounded with geographic location. Han and Tibetan farmers were widely distributed, whereas the Hui were mainly in the north-west counties, where they predominantly practiced intercropping, but they were observed to mono-crop peas in Hualong County in the east.

Similarly there was a geographic association with food end-uses but no evident ethnic association. Vegetable use of peas and faba bean was found at 17 sites in Qilian, Gonghe, Xinghai, Guinan, Tongren, Xunhua and Leduo counties, across all ethnic groups except the Sala. Usually green pods were cooked in boiled water. Grain, or derived flour, was mainly boiled in the southern counties of Tongde, Guinan, Tongren and Xunhua, whereas baking was more common in northern counties of Menyuan, Qilian and Gonghe, with no apparent ethnic associations. Across all counties and ethnic groups, legumes were an important source of animal feed, especially faba bean varieties with small to medium grain size.

Geographic rather than ethnic factors also appeared to be associated with the distribution of major crops of wheat, oilseed brassicas and potato. Oats were found at only three sites, all with low temperatures, barley at five sites in Xunhua County, hemp for seed and fibre in Xunhua, Leduo, Pingan, Qilian and Xinghai counties, while linseed was noted at 11 sites in the southern counties of Xunhua, Leduo and Pingan. Thus any trends in food use or crop preference appeared to relate to ecological or geographic factors rather than ethnic factors.

Farming system

Both pea and faba bean landraces were usually intercropped in Qilian, Gonghe, Xinghai, Tongde and Guinan counties north and west of Xining. Monoculture was prevalent in Menyuan and Guinan counties, and it was the norm in the eastern counties of Tongren, Xunhua, Hualong, Leduo and Pingan (with one exception). Intercropping was noted for very small areas of pea and faba bean sown in home gardens of vegetables, herbs and sunflowers (seven sites), for faba bean sown in potato fields (five sites) and for peas sown in fields of oilseed brassicas (five sites).

Major crops observed were spring wheat in most counties, except in Ledou and Pingan, followed by oilseed brassicas (canola and rape), potato, linseed, maize, faba bean and pea. Oilseeds made up about 35% of Qinghai cropping area, spring wheat 30%, highland barley 10%, potato 10%, faba bean 6% and pea 4%, while Jerusalem artichoke (*Helianthus tuberosus*) was about 0.3% and other crops (poppy, hemp seed/fibre, poplar, saffron thistle, fruit trees) were of very low frequency. In general peas and faba beans were noted as minor crops, consistent with provincial records.

Agricultural practices were commercially orientated, with 36 farms using fertilizer, 26 applying at least one irrigation and 22 using both irrigation and fertilizer, particularly in Tongren, Xunhua, Hualong, Leduo and Pingan counties. Only five of the farms used no inputs, not even manure, on pea and faba bean landraces, although manure was widely applied on 36 farms. Sites 3–7 with the least or zero crop inputs of fertilizer/manure were in Qilian County, plus one site each in Menyuan and Gonghe counties.

Market prices were provided for 23 of 54 samples. In other cases the absence of marketing indicated that crops were grown only for home consumption. This is consistent with some legume production being mainly for subsistence, or only partly commercially focused in the particular districts sampled. The market value of seed was broadly consistent at 0.7–1.0 yuan/jin (0.30–0.43 Aus$/kg. 1 jin=500 g).

Farm sizes were small, from 0.6–1.0 mu/person in irrigated areas for three to four person families, and up to 3 mu/person at non-irrigated sites (1 mu = 1/15 ha).

Gender issues

Many farm operations almost always had gender-based roles (data not shown). Men were almost wholly responsible for tillage, although this activity was shared with women in two cases, whereas women were wholly responsible for weeding and cooking. Sowing was mainly done by women (21 cases reported), entirely so in Qilian, Gonghe and Ledou counties, though this was a shared role in 16 other cases. There was no association between gender role and ethnic group. Men mainly applied fertilizer, though in seven cases this was shared with women. Both sexes shared harvest operations; however, in two cases women were responsible. Both sexes were usually involved in marketing of produce, although this was a female role in five cases, all from Longyang in Gonghe and all of the Han ethnic group; in seven other cases, from different counties and ethnic groups, marketing was a male role. There was no indication that gender influenced a decision on whether to retain a landrace or to phase in a modern variety.

Discussion

A considerable diversity of germplasm was collected during the 2004 survey. Spot checks of adjacent fields and threshing floors at times revealed wide variation, as judged by seed coat patterns of peas. Villages in the vicinity of initial collecting sites were found to have different varieties of faba bean. With varieties changing from village to village the possibility exists that uncollected landraces still exist in remote areas not visited by this and previous missions.

Genetic erosion of landraces was very advanced in Qinghai. Even where legumes were minor crops the agriculture was market oriented rather than subsistence oriented. In this situation there were few reasons for farmers to retain landraces in cultivation. Few of the landraces appeared to be ancient, locally adapted materials. This was indicated by a lack of locally specific names—none had locality names. In many cases landraces were actually introductions made over the last 20 years. Landraces are under threat from contamination with modern varieties which are threshed in the same place, and there is wide opportunity for cross-pollination of faba beans with modern introductions, especially where the cultivation of the landraces was confined to borders of fields.

This collecting mission may have been one of the last opportunities for collection of pea and faba bean landraces in Qinghai. The widespread movement of landraces between districts and neighbouring provinces reduces the probability that these landraces are the outcome of centuries of selection for adaptation to local climatic, edaphic and cultural selection forces and contain unique gene complexes reflecting local agro-climatic evolution (Frankel and Bennett 1970). Few of the newly collected samples appear to be ancient landraces as described by Frankel and Bennett. Such landraces may have been more prevalent in the less commercial and more subsistence-oriented agriculture of over 50 years ago, when systematic germplasm collecting began in China. This suggests that the remaining landraces of pea and faba bean in Qinghai are more regional rather than local entities. Molecular diversity analyses may resolve this question. This collecting mission provided evidence on the history and adaptation of Qinghai landraces based on a relatively small sample, and illustrated the importance of enquiring about the socio-economic context in conjunction with germplasm collecting (Vavilov 1951).

Ancestral varieties were readily phased out with the advent of a better one. We encountered only one occasion where taste played an important role in retention of a landrace. A constant flux of varieties may be an important local coping strategy to obtain the best available varieties for sustainable production.

At site 11 farmers have observed frosts during July. Interestingly, peas collected from this location were described as having early, medium and late maturities, which suggests that planting varieties with different phenologies may be a strategy to manage risk from frost damage during seed and pod setting.

The occurrence of autumn frosts in mid-August and early September perhaps may be avoided in certain local environments by either very early maturity or medium to late maturity. Landraces may also have been selected for enhanced frost resistance during reproductive development. The accessions collected from these locations and those from high altitudes should be fast tracked for evaluation of frost tolerance during the reproductive growth phase.

The use of nitrogenous fertilizer at half of the collection sites indicated that utilization of nitrogen fixation instead may not be well understood, or that there are local impediments to biological nitrogen fixation.

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References

Cao Yongsheng, Zhang Xianzhen, Gong Gaofa, Li Li, Chief editors. 1995. Atlas of main crop germplasm resources in China. China Agriculture Press, Beijing. 450 pp.

ERS-USDA 2005. China Agricultural and Economic Data: Provincial Data [online]. Available from: www.ers.usda.gov/Data/China/ProvincialForm.aspx. Date accessed: 17 July 2009.

Frankel OH, Bennett E. 1970. Genetic resources. In: Frankel OH, Bennett E, editors. Genetic Resources in Plants – their Exploration and Conservation. IBP Handbook No. 11, Blackwell Scientific Publications, Oxford and Edinburgh, UK. pp. 7–17.

ICGR. 1987. The Catalogue of Chinese Legume Germplasm. Vol. 1. Chinese Publishing House for Agriculture and Science, Beijing.

ICGR. 1990. The Catalogue of Chinese Legume Germplasm. Vol. 2. Chinese Publishing House for Agriculture and Science, Beijing.

ICGR. 1998. The Outstanding Germplasm of Chinese Legumes. Chinese Publishing House for Agriculture and Science, Beijing.

Longworth JW, Williamson GJ. 1993. China's Pastoral Region. Sheep and wool, minority nationalities, rangeland degradation and sustainable development. CAB International in association with ACIAR, Wallingford, UK.

Raupach M, Tucker BM. 1959. The field determination of soil reaction. Journal of the Australian Institute of Agricultural Science 25:129–133.

Vavilov NI. 1951. The Origin, Variation, Immunity and Breeding of Cultivated Plants. Roland Press, New York, USA.

Appendix 1. Collecting sheet used to interview farmers during survey, Qinghai province, China, August–September 2004

Site No: Collectors ________________________________

Collecting date:

Site: Province _________, county_____, location ________ km ___ direction ____ from ______

Latitude ________, Longitude _________, Elevation _____ m

Topography: 1 – plain, 2 – slope, 3 – valley, 4 – plateau, 5 – hill, 6 – mountain

Soil type: 1 – clay, 2 – clay loam, 3 – silt, 4 – silty loam, 5 – sandy loam, 6 – sand

Rock %: 1–2, 2–5, 3–15, 4–40, 5–80, 6–80+

Soil pH: _________ Weather (usual, dry or wet): _______ Date of the first frost: _____

Summer frosts: ________

Ethnic group: __________

Sample No:

Crop frequency: 1 – rare, 3 – occasional, 5 – frequent, 7 – abundant, 9 – dominant

Other varieties and crops in locality:__________________________________

Local variety frequency: 1 – rare, 3 – occasional, 5 – frequent, 7 – abundant, 9 – dominant

Variety name ________________________

English translation of the name__________________

Source of seed: farm__, farm store __, home garden __, village market __, town market __, other_________

Population in sampling: _______ plants (approximately)

Crop system: monoculture __ mixed with cereals __ mixed with crop(s): ______________

Pest or diseases (1 low–9 high) ______________________________________

Reason for growing:
own seed __, less disease risk __, drought tolerant __ frost tolerant __, cooking/taste ___, price ____ other ______

Maturity: Early ____ Medium ____ Late____

Use:
1. *Vegetable:* leaves __ pod __ seed __ 2. *Grain*: food ___ animal feed ___ green fodder __ dry straw
3. *Green manure* __ Other___________________________

Crop inputs:
manure __ straw cover in seedling __ fertilizer/type ___ irrigation __ chemical spray (aphids or other pests) _____________

Male (M) or female (F) roles:
tillage __ sowing __ fertilizer __ weeding__ chemicals___ harvest _____ cooking _____ marketing ____

Local variety source:
Grandfather kept (ancestral) ____ > 10 years (old introduction) ____ Neighbour____ Village market or town market ____

Seed condition: insect damage (Yes or No) _____

Main usage: 1 – grain food, 2 – vegetable, 3 – animal feed, 4 – forage

Methods of cooking vegetable: boiling ____ baking ___ fry in oil __ fermenting ___
or seed: boiling ____ baking ___ fry in oil __ fermenting ___ dehulling __

Recipes: porridge ___ cakes ___ mixed dishes___ snacks ___ fermentation __ sprouts __

Market prices (Yuan/jin): __________

Appendix 2. Geography of landrace collection sites, Qinghai province, China, 2004

Site	County	Location	Topography[1]	Soil type[2]	Rock %[3]	pH
1	Menyuan	Longlang village near Xianmi town, 60 km E of Haomen town	3	2	2	5.5
54	Menyuan	Longlang village near Xianmi town, 60 km E of Haomen town	3	2	2	5.5
2	Qilian	Huangzang village, 12 km N of Babao town	2	2	1	8
3	Qilian	Huangzang village, 12 km N of Babao town	2	2	1	8
4	Qilian	Huangzang village, 12 km N of Babao town	2	2	1	8
5	Qilian	Huangzang village, 10 km N of Babao town	2	2	4	8.5
6	Qilian	Dipanzi village, Zamashi district, 12 km NW of Babao town	3	2	2	9
7	Qilian	Dipanzi village, Zamashi district, 12 km NW of Babao town	3	2	2	9
8	Qilian	Dipanzi village, Zamashi district, 12 km NW of Babao town	3	2	2	9
9	Qilian	Dipanzi village, Zamashi district, 12 km NW of Babao town	3	2	2	9
10	Gonghe	Longyang town, market garden	7	2	2	9
11	Gonghe	Longyang town, market garden	7	2	2	9
12	Gonghe	Longyang town, market garden	7	2	2	9
13	Gonghe	Longyang town, market garden	7	2	2	9
14	Gonghe	Longyang town, market garden	7	2	2	9
15	Gonghe	Xixiangkan village, Qiabuqia district, 4 km N of Qiabuqia town	1	3	1	9
16	Gonghe	Xiala village, Qiabuqia district, 5 km N of Qiabuqia town	2	2	1	8.5
17	Xinghai	Tangnaihei, 18 km SE of Ziketan town	1	4	1	8.5
18	Xinghai	Tangnaihei, 18 km SE of Ziketan town	1	4	1	8.5
19	Xinghai	Xia village, 14 km SE Ziketan town	1	4	1	8.5
20	Tongde	Banduo village, near Bagou town, 38 km NE from Tongde county	2	2	2	9
21	Guinan	Chana village, near Shagou town, 26 km NW from Guonayin town	2	2	1	8.5
22	Guinan	Chana village, near Shagou town, 26 km NW from Guonayin town	2	2	1	8.5
23	Guinan	Chana village, near Shagou town, 26 km NW from Guonayin town	2	2	1	8.5
24	Guinan	Luohecai village, near Shayou town, 17 km NW from Guomayin town	2	5	1	9
25	Guinan	5 km NW Guomayin town	2	4	2	9
26	Guinan	5 km NW Guomayin town	2	4	2	9
27	Tongren	Guomari village, Nianduhu district, 5 km N from Tongren county	2	4	1	9
28	Tongren	Guomari village, Nianduhu district, 5 km N from Tongren county	2	4	1	9
29	Xunhua	Tangluoga village, Baizhuary district, 21 km E from Xunhua county	2	1	1	7.5
30	Xunhua	Tangluoga village, Baizhuary district, 21 km E from Xunhua county	2	1	1	7.5
31	Xunhua	Danma village, Daowei district, 24 km E from Xunhua county				
32	Xunhua	Lihong village, Daowei district, 26 km E from Xunhua county	2	1	1	8
33	Xunhua	Lihong village, Daowei district, 26 km E from Xunhua county	2	2	2	9
34	Xunhua	Erjia village, Daowei district, 30 km E Xunhua county	2	2	1	9.5
35	Xunhua	Dazhuang village, Mengda district, 20 km E from Xunhua	3	2	1	8.5
36	Xunhua	Dazhuang village, Mengda district, 20 km E from Xunhua	3	2	1	8.5
37	Xunhua	Muchang village, Mengda district, 22 km E from Xunhua	3	4	1	8
38	Xunhua	Muchang village, Mengda district, 22 km E from Xunhua	3	4	1	8
39	Xunhua	Muchang village, Mengda district, 22 km E from Xunhua	3	4	1	8
40	Xunhua	Mari village, Wendu district, 20 km S from Xunhua	2	2	1	9
41	Xunhua	Maoyu village, Wendu district, 21 km S from Xunhua	2	2	1	8.5
42	Xunhua	Wangangina village, Wendu district, E from Xunhua	7	2	1	9
43	Xunhua	Wangangina village, Wendu district, E from Xunhua	7	2	1	9
44	Xunhua	Yaga village, Galen district, 33 km SW from Xunhua				
45	Xunhua	Yaga village, Galen district, 33 km SW from Xunhua				
46	Xunhua	Bitang village, Galen district, 44 km W from Xunhua	5	2	1	9
47	Xunhua	Bitang village, Galen district, 44 km W from Xunhua	5	2	1	9
48	Xunhua	Bitang village, Galen district, 44 km W from Xunhua	5	2	1	9
49	Hualong	Ertang village, Ertong district, 5 km S of Hualong	5	2	1	9
50	Leduo	Zhaojiapin village, Putai district, 6 km W from Putai town	7	4	1	9
51	Leduo	Zhaojiapin village, Putai district, 6 km W from Putai town	7	4	1	9
52	Leduo	Zhaojiapin village, Putai district, 2.5 km W from Putai town	7	4	1	9
53	Pingan	Hongshuiquan town, 16 km SW from Pingan town	4	4	1	9

1. Topography: 1 = plain, 2 = slope, 3 = valley, 4 = plateau, 5 = hill, 6 = mountain (blank = no record).
2. Soil type: 1 = clay, 2 = clay loam, 3 = silt, 4 = silty loam, 5 = sandy loam, 6 = sand (blank = no record).
3. Rock %: 1 = 1–2, 2 = 2–5, 3 = 3–15, 4 = 16–40, 5 = 41–80, 6 = >80 (blank = no record).

Collection of pea (*Pisum sativum*) and faba bean (*Vicia faba*) germplasm in Yunnan

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Summary

Collection of pea (*Pisum sativum*) and faba bean (*Vicia faba*) germplasm in Yunnan

Two germplasm collection missions were undertaken in Yunnan Province, China, in 2004 and 2005, as part of a joint project between China and Australia for improvement of pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) production. One objective of this project was the collection and exchange of pea and faba bean germplasm from under-represented priority regions of western China, specifically in Yunnan and Qinghai provinces. This paper reports on collection missions in eastern and in southern Yunnan. A total of 67 faba bean and 65 pea landraces were collected, with documentation of GPS site coordinates and physical characteristics, and of associated agro-economic and historical data on the cultivation of these landraces. Many but not all landraces were ancestral, possibly selected for long-term adaptation to the local environment at respective sites, but food/feed usage neither related to ethnicity nor to geographic location. Gender roles in agriculture were important, but on limited data appeared unrelated to either ethnicity or retention of landraces. There was evidence for genetic erosion, with the presence of modern varieties at many locations.

Key words: Pea, *Pisum sativum, Vicia faba,* faba bean, germplasm, collecting mission, landrace, China, Yunnan.

Résumé

Collecte de matériel génétique de pois (*Pisum sativum*) et de fève (*Vicia faba*) dans le Yunnan

Deux missions de collecte de matériel génétique ont été entreprises dans la province du Yunnan, en Chine, en 2004 et en 2005, dans le cadre d'un projet sino-australien d'amélioration de la production du pois (*Pisum sativum* L.) et de la fève (*Vicia faba* L.). Un objectif de ce projet consistait à collecter et échanger du matériel génétique de pois et de fève de régions prioritaires, sous-représentées de Chine occidentale, en particulier les provinces du Yunnan et du Qinghai. Cet article présente les missions de collecte dans l'est et le sud du Yunnan. Au total, 69 variétés locales de fève et 65 de pois ont été collectées, en précisant les coordonnées des sites par GPS et les caractéristiques physiques, ainsi que des données agro-économiques et historiques relatives à la culture de ces variétés. De nombreuses variétés locales, mais pas toutes, sont très anciennes, peut-être sélectionnées pour leur adaptation de longue date à l'environnement local dans les sites respectifs, cependant leur utilisation dans l'alimentation humaine/animale n'est ni liée aux ethnies ni à la situation géographique. La répartition des rôles entre hommes et femmes dans l'agriculture est importante, mais ne semble pas, sur des données limitées, présenter de lien avec les ethnies ou la conservation des variétés locales. L'érosion génétique a été mise en évidence, des variétés modernes ayant été observées dans de nombreux endroits.

Resumen

Recolección de germoplasma de guisantes (*Pisum sativum*) y habas (*Vicia faba*) en Yunnan

En 2004 y 2005 se emprendieron dos misiones de recolección de germoplasma en la provincia de Yunnan, China, como parte de un proyecto conjunto entre China y Australia destinado a mejorar la producción de guisantes (*Pisum sativum* L.) y habas (*Vicia faba* L.). Un objetivo de este proyecto era recoger e intercambiar germoplasma de guisantes y habas provenientes de regiones prioritarias poco representadas de China occidental, específicamente las provincias deYunnan y Qinghai. El documento informa acerca de las misiones de recolección en Yunnan oriental y meridional. Se recogieron 69 variedades locales de habas y 65 de guisantes, con documentación de las coordenadas GPS y características físicas de los sitios y de los datos agroeconómicos históricos asociados con el cultivo de estas variedades locales. Muchas aunque no todas las variedades locales eran ancestrales, probablemente seleccionadas por la adaptación a largo plazo al ambiente local de los respectivos sitios, pero su uso como alimento o forraje no se relacionaba con la etnicidad ni con la ubicación geográfica. Los roles por sexo eran importantes en las prácticas agrícolas, pero en datos limitados aparecieron sin relación con la etnicidad ni con la conservación de las variedades locales. En numerosas ubicaciones había evidencia de erosión genética, con presencia de variedades modernas.

Introduction

China is the largest producer of faba beans in the world (Cubero and Nadal 2005). The crop is grown widely in the country, with the area sown to the crop ranging from 1 350 000 hectares in 2001 to 800 000 ha in 2004. China is the third largest producer of peas, with 11% of the world production at 1.2 million tonnes. The crop is grown for grain on 800 000 ha and as a vegetable on 240 000 ha (Cubero and Nadal 2005; Redden et al. 2005).

In Yunnan Province the major cropping activities vary with altitude. At lower altitudes rice, soya bean, sugar-cane and maize dominate, with faba beans and peas grown in small areas along the edges of fields or in vegetable gardens. At higher altitudes the cropping of winter crops is more widespread and often dominated by canola, ginseng, winter cereals or faba beans. The majority of the faba beans are a medium seed-size type and are eaten in a variety of foods including soups and dry beans. Peas are also part of the diet and are consumed as pea noodles and tofu. Both crops are increasingly consumed as green vegetables in towns and cities, and use of peas and small-seeded types of faba bean for animal feed is important in rural areas (Bao Shiying et al. 1998).

Faba bean is grown on an area of 342 700 ha in Yunnan (Bao Shiying 2003). Modern varieties, most of which have been bred by the Yunnan Academy of Agricultural Sciences (YAAS), are grown on 140 000 ha, accounting for 10–17% of China's faba-bean production area. Peas are grown on 175 000 ha in Yunnan. Much of this is under traditional landraces as a pea breeding programme commenced less than five years ago.

The high penetration of faba bean varieties from the YAAS breeding programme and impending release of modern pea varieties has placed landrace varieties of both crops at a high risk of being displaced. Collection and maintenance of landraces from regions that are not currently represented in germplasm collections is therefore of a high priority.

One objective of the joint Australia–China project for improvement of pea and faba bean was the collection of pea and faba bean landrace germplasm in Yunnan Province in collaboration with YAAS. Pea and faba bean production regions of Yunnan that had not previously been the target of collecting missions were targeted for collection of landraces on two missions, in 2004 and 2005.

A questionnaire was used at each collection site to document site physical characteristics, and farmers assisted with documentation of the history, agricultural system and end uses of landraces (Appendix 1). Yunnan is home to many ethnic groups, and landraces were collected from Zhuang, Yao, Yi and Maio ethnic groups as well as Han Chinese growers. Both ethnic practices and gender roles were explored. Faba beans and peas were collected from monocropped fields, field edges, intercropped fields and vegetable growing areas.

Materials and methods

The first trip covered 1400 km, beginning west of Kunming then travelling north-east via Xundian, Dongchuan, Qiaojia, Shuifu, Xijiang, Weixing and Zhengxiong counties and was conducted between 10 and 23 May 2004 when most crops were mature (Figure 1); many had already been harvested.

The second mission was a circuit of some 2000 km to the south and south-east of Kunming (Figure 1) from 30 March to 4 April 2005, covering an area in which farmers were known not

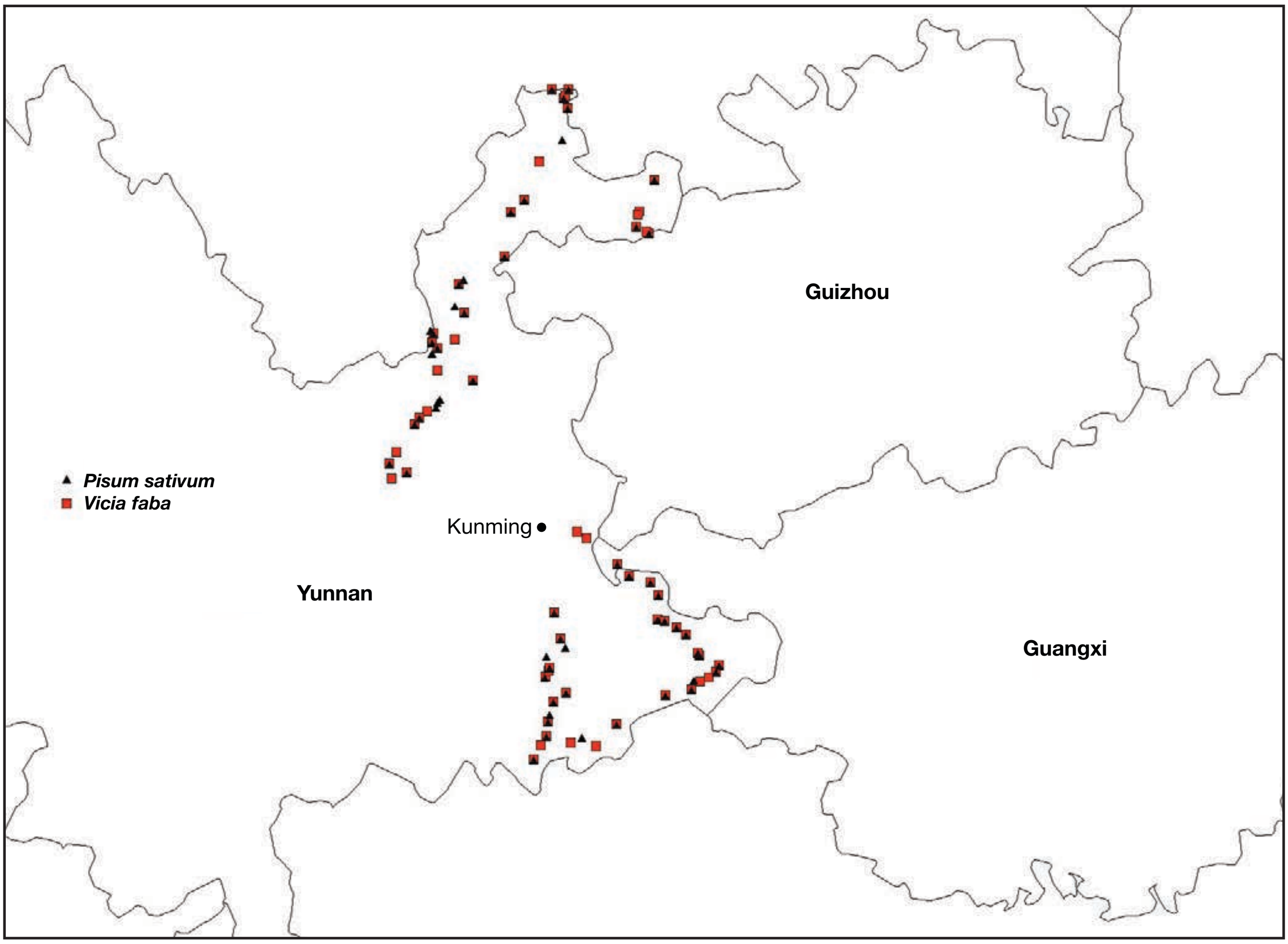

Figure 1. Sites where pea and faba bean germplasm were collected on separate expeditions north and south of Kunming in Yunnan Province, China, 2004 and 2005.

to have adopted faba bean varieties from the YAAS breeding programme. Many crops were immature at this time, which impeded the collection of landraces. The collection route briefly entered Guanxi Province and reached as far south as the Vietnamese border, the more remote fringes of the agricultural areas of the province rather than the intense faba-bean-growing areas adjacent to and north-west of Kunming.

Pea and faba bean landraces had not previously been collected from either of the areas covered by the collection missions.

Thirty-four faba bean landraces and 34 pea landraces were collected from 41 sites on the first collection mission. On the second collection mission 33 faba bean and 31 pea landraces were collected from 43 sites in Luoping, Xi Ling, Guangnan, Funing, Malipo, Ma Guan, Weishan, Yan Shan and Qiubei counties.

On both expeditions the collection sites were often in terrain that was difficult to access and were separated one from another by long distances. They were chosen after discussion with local residents to establish whether the local crops of either pea or faba bean were likely to be landraces or recently bred improved varieties. Random samples consisting of a minimum of 20 plants per field or per unthreshed heap, or 50 g from a seed store, were collected for each landrace, although samples were usually larger.

At each site data were collected describing site parameters such as location, topography, weather, soil type and ethnic group. If time permitted and the local farmers were available data were collected for each sample of either peas or faba beans on cropping system, variety name and source, reasons for and methods of growing and usage for human food, stock feed or market. In one case, it was not possible to communicate with the local Miao women whose language was different from Mandarin Chinese.

During a previous collection trip in Qinghai province in 2004, casual checking of nodulation suggested a potential problem with nitrogen fixation. To gather further data, observations of root nodulations of pea and faba bean crops were taken at every opportunity during the 2005 mission in Yunnan.

Participants in the collection trips were Bao Shiying, Zong Xuxiao, Wang Liping, He Yuhua, Tony Leonforte and Jeff Paull in 2004; and Bao Shiying, He Yuhua, Wang Liping, Zong Xuxiao, Li Lichi, Dirk Enneking and Ian Rose in 2005.

At the end of the mission, seed was left with the Chinese cooperators to be divided into two portions; one for the Chinese collection, and one to be sent to Australia via the DPI-Victoria quarantine facilities at Grains Innovation Park, Horsham.

Results

Site descriptions

Details of the 41 collection sites in 2004 are presented in Table 1. The topography varied from plains to mountains and the elevation of collection sites ranged from below 400 m (4 sites) to above 2000 m (12 sites). Soils were mainly clay but 5 sites tended to be sandy. The soil pH was acidic at many sites, although 14 sites distributed in 10 counties were alkaline.

In 2005 some site characteristics were not recorded because some samples came from markets (Table 2). However, for those sites where altitude was recorded, all were all above 600 m, with the highest location being at 1988 m in Qiubei county. Nineteen sites were on plains, four sites were in valleys, two were upland sites and three sites were on hills. Soils were clays or clay-loams, and only six sites exceeded 5% rock. The soil at most sites was neutral to alkaline; only 11 sites were acidic.

Landrace characteristics

Nearly all pea landraces collected in 2004 had white–light green primary colour, with ten having speckled secondary markings, and two had brown primary colour with speckled seed (Table 3). Three samples were mixtures of white, green (two cases) and brown primary coloured seed (Table 3). In 19 cases the samples had mixed hilum colour. Only in Wenshan county was the hilum colour uniformly yellow (sites 34 and 35), elsewhere most had black admixture or were uniformly black.

Faba bean landraces collected in 2004 were predominantly white–light green, and only one was red seeded (Table 3). Generally there was a mixture of hilum colours within a landrace but all included black, and eight landraces had uniformly black hilum.

Pea landraces collected in 2005 included two samples where secondary colour speckling was present on the seeds and two were mixed for marbling and three accessions were mixed for hilum colour (Table 4). The occurrence of a non-white colouration in pea was less frequent than in the 2004 samples collected north of Kunming.

The landraces of pea and faba bean collected in 2004 had mainly been sown with the farmer's own seed. In only two cases for each crop had seed been obtained from outside, one was from within the same village, one from a local market and two from a nearby town. The latter may not have been local landraces; however they did not differ greatly in seed traits from the local landraces. No modern improved varieties were noted in farmer’s fields, although seed of these was found in town markets.

In 2005, the seed of a landrace was sampled from a farmer’s field in only one case. The majority of samples were obtained from farmer’s stores (16 samples) or home gardens (17 samples) (Table 4). In addition, three samples were obtained from local markets and four from town markets. At two locations the seed collected was too immature to be viable and these samples are not reported. The frequency of pea or faba bean crops was noted as rare or occasional in 10 of the 14 replies obtained, and frequent or abundant in only four cases. This suggests that pea and faba bean were usually minor crops.

There was a limited dataset on reasons for growing pea or faba bean landraces, however cooking/taste was always cited (Table 4). Other reasons included price/markets and other (feed). There was no indication of selecting the landrace for agronomic reasons such as disease resistance or drought or frost tolerance. Landraces tended to be available in all areas where samples were collected but the frequency differed among the 15 sites recorded. They were rare or occasional in

Table 1. Geographic and physical characteristics of collection sites north of Kunming, Yunnan Province, China, visited in May 2004.

Site	Species[1]	County	Village	Latitude (decimal °)	Longitude (decimal °)	Elevation (m)	Topography[2]	Soil type[3]	Rock %[4]	pH
1	V	Xishangqi	Zhuangzi	25.25	102.67	1937	5	1	1	6.5
2	P & V	Xishangqi	Xiaohebian	25.38	102.65	1779	5	1	1	5.5
3	V	Fuming	Kuanzhuang	25.48	102.72	1708	3	1	1	5.5
4	P & V	Xundian	Houjiezhong	25.30	102.81	1848	3	1	1	7
5	P & V	Xundian	Luga	25.72	102.88	2127	1 & 2	1	1	5
6	P & V	Xundian	Dujia	25.78	102.92	2050	1	1	1	
7	V	Xundian	Niujie	25.84	102.99	2033			1	
8	P	Xundian	Shuifang	25.87	103.08	2075	1	1	1	
9	P	Dongchuan	Huagou	25.91	103.09	2357	5	1	1	5.5
10	P	Dongchuan	Songmaopeng	25.94	103.11	2100	3	1	1	
11	V	Dongchuan	Dengjiawan	26.19	103.09	1296	6	1	1	7.5
12	P	Dongchuan	Xiaoxing	26.34	103.04	2304	6	1	1	5
13	P	Dongchuan	Boka	26.54	103.03	2045	2	1	1	5.5
14	P & V	Dongchuan	Boka	26.43	103.04	1859	5	1	1	5.5
15	P & V	Dongchuan	Gele	26.51	103.05	851	1	5	1	8
16	Px2 & V	Dongchuan	Dahuangdi	26.38	103.09	980	1	5	4	9
17	V	Huize	Yizewu	26.46	103.25	2134	1	1	1	8
18	P & V	Huize	Daqiao	26.69	103.33	2513	1	1	1	
19	P	Qiaojia	Laoganshu	26.76	103.25	2423	1	1	1	
20	P & V	Qiaojia	Lizigou	26.94	103.28	1904	6	1	1	5.5
21	P	Qiaojia	Waluo	26.98	103.32	2079	5	1	1	4.5
22	P & V	Pudian	Shaba	26.11	103.41	1471	6	1	1	8.5
23	V	Zhongtong city	Fenghuang	27.18	103.69	1885	1	1	1	8
24	P & V	Zhongtong city	Fenghuang	27.18	103.69	1885	1	1	1	7.5
25	Px2 & V	Zhongtong city	Jing'an	27.57	103.75	1852	1	1	1	
26	P & V	Daguan	Yuwan	27.67	103.87	1289	2	1	1	6
27	V	Daguan	Jili	28.01	104.00	590	6	1	1	8
28	P	Yanjin	Jiaozi	28.20	104.21	394	6	1	1	6
29	P & Vx2	Shuifu	Huatang	28.47	104.27	383	6	1	1	6
30	P	Shuifu	Taiping	28.55	104.22	558	2	1	1	7.5
31	P & V	Shuifu	Taiping	28.56	104.23	578	2	1	1	7.5
32	V	Shuifu	Taiping	28.58	104.24	712	2	1	1	5.5
33	P & V	Xijiang	Lianyu	28.63	104.12	359	1	6	1	7.5
34	P & V	Xijiang	Qingmingwo	28.63	104.27	336	1	1	1	5.5
35	Px4 & V	Weixing	Zhaxi	27.85	105.05	1112			1	
36	V	Weixing	Ganhe	27.57	104.91	1495	2	6	1	
37	V	Zhengxiong	Banqiao	27.54	104.90	1458	2	1	1	
38	P & V	Zhengxiong	Dawan	27.44	104.88	1604	2	6	1	7.5
39	V	Zhengxiong	Wujiatun							
40	Vx2	Zhengxiong	Jianchao	27.39	104.97	1403	1	1	1	8
41	P & V	Zhengxiong	Er'longguang	27.38	104.99	1389	2	1	1	8

1. Species: P = *Pisum sativum*, V = *Vicia faba*.
2. Topography: 1&2 = plain, 3 = valley, 4 = plateau, 5 = upland, 6 = hill, 7 = mountain.
3. Soil: 1 = clay, 2 = clay loam, 3 = silt, 4 = silty loam, 5 = sandy loam, 6 = sand.
4. Rock %: 1–2 = below 5%, 3 = 6–15%, 4 = 16–40%, 5 = 41–80%, 6 = >80%.

Table 2. Geographic and physical characteristics of collection sites to the south and south-east of Kunming, Yunnan Province, China, visited in March–April 2005.

Site	Species[1]	County	Village	Latitude (decimal °)	Longititude (decimal °)	Elevation (m)	Topography[2]	Soil type[3]	Rock %[4]	pH
1	V	Luoping	Shangalai	24.79	104.35	1582	3	2	1	7.5
2	V	Luoping	Tudimiao	24.73	104.44	1324				7.5
3	P & V	Xi Ling	Guzhang market	24.50	104.71	875				
4	V	Guangnan	Zhehang	24.42	102.81	858	3	2	1	5
5	P & V	Guangnan	Tang Shang	24.40	104.82	853				
6	P & V	Guangnan	An' deng	24.35	105.01	817	3	2		8
7	P & V	Guangnan	A'ke	24.23	105.08	868	3	2	2	6
8	P & V	Guangnan	Huangpo	24.03	105.07		1	2	1	8
9	P & V	Guangnan	Kuimiao	24.01	105.14	1368	5	2		8.5
10	P	Guangnan	Yan Liujing	23.95	105.24	1386	6	2	4	5
11	P & V	Guangnan	Bajiao	23.89	105.33	667	2	2	1	8.5
12	P	Guangnan	Pingtianbao	23.72	105.45	1110	3			5
13	P & V	Guangnan	Bajia	23.73	105.44	1130	1	2	1	8
14	P	Funing	Nahong	22.16	105.03	678	1	2	1	5.5
15	P & V	Funing	Funing markets	23.62	105.63	660				
16	P & V	Funing	Podi	23.57	105.60	1068	6	2	3	8
17	V	Funing	Lida	23.52	105.54	1230	1	2	1	8
18	P	Funing	Niugun tang	23.50	105.40	1411	2	1	1	8.5
19	V	Funing	Xiazhai	23.48	105.46	1490				5
20	P	Funing	Guanshan	23.42	105.38	1522				
21	Vx2	Funing	Mujang town	23.42	105.38		5	2	3	8
22	P & V	Malipo	Donggan	23.37	105.15	1667	2	2	3	7
23	P & V	Malipo	Malipo county market	23.12	104.70	1105				
24	V	Malipo	Meng tong	23.88	104.71	1046	2	2	2	5
25	V	Ma Guan	Lu Long	22.92	104.52					
26	P	Ma Guan	Ma Guan county	23.00	104.39	1307	2	1		8
27	V	Ma Guan	Ken He	22.95	104.29	1566	2	2	3	7
28	P & V	Ma Guan	Gulinqing market	22.81	103.96	1409				
29	V	Ma Guan	Dadi	22.93	104.02	1712	6	2	4	8
30	P & V	Ma Guan	Bazhai market	23.01	104.07	1730				
31	P & V	Ma Guan	Shizhamen	23.13	104.09	1596	2	2	1	5.5
32	P	Wenshan	Sunjia	23.20	104.10	1680	2	1	1	5
33	P & V	Wenshan	Huangcaoba	23.31	104.13	1753	2	2	1	8.5
34	P & V	Wenshan	Suburb of Wenshan county	23.39	104.25	1309	2	2	1	9
35	P	Wenshan	Reshui Zhai	23.52	104.06	1334				
36	V	Wenshan	Yimude	23.53	104.06			2		8
37	V	Wenshan	Xiaopingba	23.58	104.09	1464		1		6
38	P & V	Wenshan	Xiamu	23.61	104.10	1429	1	2	1	8
39	P	Wenshan	Shanshuqi	23.71	104.07	1468	2	2	1	8.5
40	P	Yan shan	Wenmo	23.79	104.24	1476	2	2	1	5
41	P & V	Yan shan	Yide Yi	23.85	104.20	1530	1	1	1	8
42	P & V	Qiubei	Marmaochong	24.08	104.15	1447		2		8.5
43	P	Qiubei	Huangnishao	24.04	103.78	1988	1	2	1	7.5

1. Species: P = *Pisum sativum*, V = *Vicia faba*.
2. Site topography: 1&2 = plain, 3 = valley, 4 = plateau, 5 = upland, 6 = hill, 7 = mountain.
3. Soil: 1 = clay, 2 = clay loam, 3 = silt, 4 = silty loam, 5 = sandy loam, 6 = sand.
4. Rock %: 1–2 = below 5%, 3 = 6–15%, 4 = 16–40%, 5 = 41–80%, 6 = >80%.

Table 3. Characteristics of pea and faba bean landraces collected north of Kumning, Yunnan Province, China, May 2004.

ACCID[1]	Site	Species[2]	Landrace source	Primary seed colours plus secondary speckled[3]	Hilum colour[4]	100-seed weight (g)
L0356	2	P	own seed	W, Sp	B	17
L0357	4	P	own seed	W, Sp	B	18
L0359	5	P	own seed	W & G	B	17
L0360	6	P	own seed	W & G	B	20
L0361	8	P	own seed	W & G	B	16
L0362	9	P	own seed	W & G, Sp	B	17
L0363	10	P		W & G, Sp	B	18
L0364	12	P	within village	W & G	B	20
L0365	13	P		W & G	B	21
L0366	14	P		W & Br	B	21
L0367	15	P		W & G	Y & B	21
L0369	16	P	own seed	BR, Sp	Y & B	18
L0368	16	P	own seed	W&G	Y & B	23
L0370	18	P	own seed	W & G, Sp	Y & B	16
L0371	19	P	own seed	W & G	Y & B	19
L0372	20	P	own seed	W & G, Sp	Y & B	8
L0373	21	P	own seed	W & G, Sp	Y & B	14
L0374	22	P		W&G	Y & B	17
L0375	24	P	own seed	W&G	Y & B	15
L0376	25	P		W&G	Y & B	19
L0377	25	P		Br, Sp	Y & B	17
L0378	26	P		G, Sp	Br & B	13
L0379	28	P	town seed market	W & G	Y & B	10
L0380	29	P	own seed	W & G	Br & B	11
L0381	30	P		W & G	Y & B	17
L0382	31	P		W & G	Br	12
L0383	33	P	own seed		Br	11
L0384	34	P	own seed	W & B, Sp	Y & W	13
L0387	35	P		G	Y	21
L0388	35	P		G	Y	22
L0386	35	P		W & G & Br	Y & B	23
L0385	35	P		W & G & Br	Br & Y & B	17
L0389	38	P		G	Br & B	23
L0390	41	P	own seed	G	Br	14
K01367	1	V	own seed	W & G	B & G	114
K01368	2	V	own seed	W	W & B	110
K01369	3	V	own seed	W	W & B	107
K01370	4	V	own seed	W	W & B	110
K01371	5	V	own seed	W	W & B	106
K01372	6	V	own seed	W	W & B	102
K01373	7	V		R	W & B	113
K01374	11	V	town seed market	W	B	105
K01375	14	V		W	W & B	123
K01376	15	V		W	W & B	92

Table 3 (cont.). Characteristics of pea and faba bean landraces collected north of Kumning, Yunnan Province, China, May 2004.

ACCID[1]	Site	Species[2]	Landrace source	Primary Seed colours plus secondary Speckled[3]	Hilum colour[4]	100-seed weight (g)
K01377	16	V	own seed	W	W & B	138
K01378	17	V		W	W & B	104
K01379	18	V	own seed	W	W & B	138
K01380	20	V	own seed	W	B	
K01381	22	V		W	W & B	117
K01382	23	V	own seed	W	W & B	92
K01383	24	V	own seed	W	W & B	107
K01384	25	V		W	W & B	15
K01385	26	V		W	W & B	139
K01386	27	V		W	B	113
K01388	29	V	own seed	W	B	62
K01387	29	V	own seed	W	W&B	75
K01389	31	V		G	B&G	72
K01390	32	V	own seed	G	B	81
K01391	33	V	own seed	G	B	64
K01392	34	V	own seed	G	B & G	83
K01393	35	V		G	B	52
K01394	36	V		W	W & B	53
K01395	37	V		G	B & G	38
K01396	38	V		W	B	49
K01397	39	V	local market	W & G	W & B	65
K01398	40	V	own seed	W	W & B	109
K01399	40	V	own seed	W & G	B & G	79
K01400	41	V	own seed	W & G	W & B	59

1. ACCID = accession identity in YAAS collection.
2. Species: P =*Pisum sativum*, V = *Vicia faba*.
3. Primary seed colour: W = white, G = light green, Br = brown, R = red, plus secondary Sp = speckled if present.
4. Hilum colour: W = white, B = black, Y = yellow, Br = brown.

half the cases and frequent in half, but in only 11 cases was there a positive response on maintenance of the landrace. Only 13 landraces were unambiguously claimed as ancestral in the family, one case was both ancestral and town market. In another six cases seed of landraces for crop production was sourced from a market.

Ethnic influence

There were no apparent differences among ethnic groups for consumption of pea/faba beans (data not shown). Both seeds and pods were cooked by boiling in most cases; the four instances of frying were confined to Louping and Guangnan counties, with two instances in each of the Han and Zhuang groups. In 11 cases use of peas/faba bean as vegetables was cited, particularly by Han and Zhuang groups in Louping and Guangnan counties. This stated difference in use appeared to be geographically based rather than ethnic. The same counties, near large towns, also showed the highest levels of awareness of the market prices of seed and of pods. A few disadvantages of landraces were listed, mainly their poor market price but also one case of rust susceptibility and one case of sensitivity to frost, both in faba bean.

Gender

There was little data on possible gender influence on storage of landraces, with both genders responsible in three cases and males in five cases (Table 4).

The role of gender was striking in division of farm/food roles, although there were only 17 responses out of 66 interviews (data not shown). Men were largely responsible for tillage and sowing activities in Louping and Guangnan counties, while women tended to do this in Funing county (men worked in a nearby city). There was no clear gender role for fertilizer application (only a few farms) or for weeding operations. Women were mainly responsible for applying chemicals (dimethoate for controlling aphid, dichlobutiazol for rust), and all harvesting was done by men. Women appeared to be responsible for cooking and marketing across ethnic

Table 4. Characteristics of pea and faba bean landraces collected south and south-east of Kunming, Yunnan Province, China, March–April 2005.

ACCID	Sample No.	Site	Species[1]	Landrace origin[2]	Primary and secondary seed colours[3]	Hilum colour[4]	100-seed weight (g)	Source of sample[5]	Crop frequency[6]	Crop system	Reason for growing[7]	Variety maintenance[8]	Local variety frequency[9]	Gender storage of seed[10]
L1411	3	3	P									F		
L1381	6	5	P		W	W	25	4				F		
L1382	9	6	P	A	W & G	W	14	2				T		
L1383	11	7	P	A	W	W	20	3			5	T	5	M
L1384	13	8	P		W, Sp	W	18		7	Monoculture	5,6	F	5	B
L1385	14	9	P		W	W	24	2				F		
L1386	16	10	P	A	W & G	W	15	2		Intercrop with vegetables	5,6,7	T	5	M
L1387	19	11	P	A	Y & G	W	19	2	1		5,7	T	1	
L1388	21	12	P		W & G	W	18					F		
L1389	23	13	P		W, m/M	W	15					F		
L1390	24	14	P		W & G	W & B	19	2	3	Monoculture	5,6,7	F		
L1391	25	15	P		W&G	W	24					F		
L1392	28	16	P		W	W & B	16					F		
L1393	30	18	P	M	W & G	W		4		Mixed with rape	5	F		
L1394	32	20	P	TM	W	W & G	14	4			5	F		
L1395	36	22	P		W & G	W	16					F		
L1396	37	23	P		W	W	17					F		
L1397	41	26	P		W	W	17	3		Mixed with faba bean		F		
L1398	43	28	P		G, Sp	Br	18					F		
L1399	46	30	P		W & G	W	19					F		
L1400	49	31	P		W & G	W	13	3		Mixed with peas		F		
L1401	50	32	P		W	W	15	3		Monoculture		F		
L1402	51	33	P		W, m/M	W	14	3		Monoculture		F		
L1403	53	34	P		W	W	11	3		Intercrop beans persimmon		F		
L1404	55	35	P		W & G	W	21	2	5	Monoculture	5	T	5	
L1405	58	38	P		W & G	W	17	3				F		
L1406	60	39	P		W	W	24	3				F		
L1407	61	40	P		W	W	18					F		
L1408	62	41	P		W & G	W	19			Intercrop vetch manure beans		F		
L1409	64	42	P		W & G	W	18					F		
L1410	66	43	P		W & G	W	16	2				F		
K1476	1	1	V	A	Br	W & B	91	2	3	Monoculture	5	T		B
K1477	2	2	V	A	W & Br	W & B	93	2	3	Mixed with cereals	5,7	T	3	
K1508	4	3	V					5				F		
K1478	5	4	V		W & Br	W & B	120			Intercrop potato		F		
K1479	7	5	V		W & Br	W & B	110	4				F		

Table 4 (cont.). Characteristics of pea and faba bean landraces collected south and south-east of Kunming, Yunnan Province, China, March–April 2005.

ACCID	Sample No.	Site	Species[1]	Landrace origin[2]	Primary and secondary seed colours[3]	Hilum colour[4]	100-seed weight (g)	Source of sample[5]	Crop frequency[6]	Crop system	Reason for growing[7]	Variety maintenance[8]	Local variety frequency[9]	Gender storage of seed[10]
K1480	8	6	V	A				2	1	Mixed with cereals	5,7	T	3	
K1481	10	7	V	A	W & Br	W & B	119	3	3	Monoculture	5,6,7	F	5	M
K1482	12	8	V	A	W & Br	W & B	118	2	7	Monoculture	5, 6 rare,7	T	5	B
K1483	15	9	V		W & Br	W & B	80	2				F		
K1484	18	11	V	A	Br	W & B	142	2	1	Monoculture	5,7	T		
K1485	22	13	V	A	W & Br	B	89	1	3	Intercrop rape peas	5,6,7	T	3	M
K1486	26	15	V		Br	B	98					F		
K1487	27	16	V	A, TM	W & Br	W & B	78	2		Monoculture	5,6	F	3	
K1488	29	17	V		W & Br	B	89	4		Monoculture	5	F	3	
K1489	31	19	V	M	W & Br	W & B	101		3	Mixed with rape	5	F	3	M
K1490	34	21	V		W & Br	W & B	91					F		
K1491	33	21	V		Br	W & B	97	4			5	F		
K1492	35	22	V		W & Br	W & B	103					F		
K1493	38	23	V		LBr	W & B	93					F		
K1494	39	24	V		LBr	W & B	103	4	1	Monoculture	5	F	1	
K1495	40	25	V		LBr	W & B	132	3		Monoculture		F		
K1496	42	27	V		W & Br	W & B	83	3				F		
K1497	44	28	V		W & Br	W & B	87					F		
K1498	45	29	V		W & Br	W & B	95	3		Mixed with potato, rape, peas		F		
K1499	47	30	V		W & Br	W & B						F		
K1500	48	31	V		W & Br	W & B	102	3		Intercrop peas		F		
K1501	52	33	V		W & Br	W & B	126	3				F		
K1502	54	34	V		W & Br	W & B	115	3				F		
K1503	56	36	V	A	W & Br	W & B	140	2	5	Monoculture		F	5	
K1504	57	37	V	TM	W & Br	W & B	118	3		Monoculture		F		
K1505	59	38	V		Br	W & B	140	3				F		
K1506	63	41	V		Br	W & B	108					F		
K1507	65	42	V		Br	B	104			Monoculture		F		

1. Species: P = *Pisum sativum*, V = *Vicia faba*.
2. Landrace origin: A= ancestral, M=market, TM=town market, VM=village market.
3. Primary seed colour: W = white, G = green, Br = brown, LBr = light brown, plus secondary colours Sp = speckled, m/M = mixed for marbling.
4. Hilum colour: W = white, B = black.
5. Source of sample seed: 1 = farm, 2 = farm store, 3 = home garden, 4 = village market, 5 = town market.
6. Crop frequency: 1 = rare, 3 = occasional, 5 = frequent, 7 = abundant, 9 = dominant.
7. Reason for growing: 1 = own seed, 2 = less disease, 3 = drought tolerant, 4 = frost tolerant, 5 = cooking/taste, 6 = price/market, 7 = other (feed).
8. Variety maintenance: T = true, F = false
9. Local variety frequency: 1 = rare, 3 = occasional, 5 = frequent, 7 = abundant, 9 = dominant.
10. Gender who stores seed: B = both, M = male, F = female.

groups and locations. In Louping, Guangnan and Funing counties young people tended to find work in Kunming, and males in Funing also found work in Guangdong.

Farm management

There was a wide variation in farm size, from 0.3 to 20 mu (1 mu = 1/15 ha) within Guangnan county and from 1 to 20 mu in Funing, while average farm size was 2 mu in Malipo and up to 8 mu in Wen Shan county (including assorted hilly country). Pea and faba bean crops were intercropped or grown in mixtures in 15 cases, and monocropping was noted in 16 cases in 2005 (Table 4). In the 21 cases where the use of crop inputs was reported, only six farmers applied fertilizer; of these, three also supplied manure, four others supplied manure only, two used an insecticide, and two used a herbicide (Table 5).

The main winter crops in competition with peas and faba beans were canola (rape), wheat or other cereals, vegetables, cabbage, sugar-cane and other crops such as eucalypts for oil, fruits, common bean, grasses, potato, ginseng, herbs, tea, persimmon, and flowers (one to four reports each) (Table 4). Thus agriculture in Yunnan is very diverse, and winter crops are frequently rotated with summer crops of rice, maize, sugar-cane and soya bean. Pea and faba beans were clearly minor crops, stated as frequent or abundant in only four cases (Table 4).

Importance of frost

In a pre-project visit to Yunnan in January 2003 frost was

Table 5. Frost risk and farm management for pea and faba bean landraces collected south and south-east of Kunming, Yunnan Province, China, in 2005[1], by collecting sites where frost incidence was reported.

Sample no.[2]	Species[3]	Planting date[4]	Flowering date[4]	Maturity[4]	Date of first frost[4]	Frost at flowering–podding	Last frost[4]	Harvest date[4]	Crop inputs
1*	V	Oct M	Feb E	M	Dec L	Flowering	Mar E	May E	Fert PK, insecticides (aphids)
2	V	Oct E	Feb E	M	Sept E		Dec L	May E	Manure, K fertilizer
8*	V	Oct M	Jan M		Nov E	Flowering	Feb L	April L	Manure
9*	P				Nov E	Flowering	Feb L		Manure
10*	V	Oct M	Nov L	M	Oct E			Apr M	Herbicides once per year
11	P			M	Oct E				Insecticides (aphids), herbicides once one year
12*	V	Oct M	Dec E	M	Nov L	Flowering	Feb M	Apr L	None
13*	P	Oct E	Feb E	M	Nov L	Flowering	Feb M	Apr L	None
14*	P				Nov E	Flowering	Feb M		
15*	V				Nov E	Flowering	Feb M		
16*	P	Oct E	Jan M	L	Nov E		Feb E	Apr M	Manure, NPK
17	V				Nov E		Feb E		
18*	V	Oct M	Jan E	M	Feb E	Podding		Apr M	None
19*	P	Oct L	Feb L		Feb E	Podding		May E	None
22	V	Oct M	Dec L	M	Feb M			Apr L	None
23*	P	Nov E	Feb L		Feb M			May E	
24	P	Oct M	Dec E	M				May E	None
27*	V	Oct M	Dec E	L	Nov E	Flowering		Apr L	None
28*	P				Nov E	Flowering			
29*	V	Oct E	Dec E	L	Dec M	Flowering		Apr L	Manure
30	P			M					Manure, fertilizer PK
31*	V	Nov E	Jan E	L	Dec E	Flowering		Apr l	Manure
32	P				Nov E				Fertilizer PK
33	V								Fertilizer PK
39	V	Oct M	Jan L	E				Apr E	None
55	P	Nov E	Jan E					Apr L	None
56	V				Jan E				Faba bean sown next river

1. Data available for 27 landraces only.
2. * = flowering exposed to frost.
3. V = *Vicia faba*, P = *Pisum sativum*.
4. E = early, M = mid, L = late.

observed on pea crops at flowering time above 2000 m elevation. A high elevation site of 1933 m close to the YAAS research station at Kunming screened both pea and faba bean genotypes for cold tolerance in the reproductive period. Two faba bean accessions and several pea accessions were seen to have survived a snow event two weeks earlier.

Frost is widespread in Yunnan, particularly at higher elevations. During the 2005 collecting trip in 13 cases, eight for faba bean and five for pea, the landraces were exposed to frost during the flowering–podding stage (Table 5). For the remaining 48 landraces collected in 2005 the reported information was too sparse to gauge whether they may have been adapted to frost events in the flowering–podding period. In view of the significance of frost in regions of Yunnan it is possible that local landraces adapted to these conditions may be sources of genes for tolerance of frost in the reproductive period.

Biological nitrogen fixation

The presence of nodules is a prerequisite for nitrogen fixation, and if the colour of the nodule interior is pink this is an indicator of possible nitrogen fixation. Nodule colours ranged from white to intense red. The best nodulation was observed on faba beans in an area where *Vicia villosa* (purple vetch) was grown as a green manure/forage crop, followed by areas where *Vicia sativa* grew as part of the adventitious flora. In the 27 cases where crops were examined for presence of nodules, 20 were on faba bean and 7 were on pea (Table 6); 22 had pink nodules indicating possible nitrogen fixing activity, and the five cases of non-pink nodules were all on faba bean (data not shown). Possibly this indicates different strains of rhizobia interacting with either location or different faba bean landraces.

Discussion

For both pea and faba bean, the landraces in the 2004 collection in north-east Yunnan were grown from farmer-saved seed, with three exceptions grown from seed from a market source. Where source of landrace was cited in 2005, in south-east Yunnan, over one-third was from a market source while the remainder was stated to be ancestral or farmers' own seed passed down through generations. There is a possibility that the landraces obtained from markets were non-local, and in future these could be replaced by improved modern varieties as in other parts of Yunnan where modern faba bean varieties have been released. On each collection trip there was evidence of trade in seed, although this appeared to be mainly local. The landraces reported as 'ancestral' may have an ancient lineage with the farmer's family and be co-adapted to the local growing conditions. However, the collected landraces cannot be assumed to have had centuries of local selection for co-adaptation with the local environment in traditional agriculture (Vavilov 1951). In no instance was there a local name for traditional landraces as might occur if their cultivation was a long-established practice. The names given were always 'candou', which translates as faba bean, and 'wandou', which translates as pea. The attitude towards landraces appeared to be utilitarian and pragmatic, and the continued cultivation of landraces may reflect lack of choice, in comparison with districts where modern faba bean varieties are available and widely adopted (Liu et al. 1993).

None of the farms visited was so isolated that agriculture was primarily for subsistence, with local landraces isolated from outside infusion. In 2005, chemical fertilizer was applied to six landrace crops, and insecticides/herbicides were used in three cases. The cropping environment is starting to change towards commercial orientation in these areas.

The genetic value of the landraces of pea and faba bean collected awaits determination. Past collections of Chinese faba beans have found good virus resistances available to local breeding programmes in both Australia and China. The occurrence of disease-resistance traits in the current collections has yet to be assessed. The environment during the collection mission seemed ideal for chocolate spot (*Botrytis fabae*) development and there were encouraging levels of variability in plant symptoms observed.

The nodulation results suggest that there is great variability in nitrogen fixation under current farming practices in Yunnan. Further investigations are warranted to help optimize the existing potential for nitrogen fixation in Chinese farming systems with benefits to farm income and to the environment.

Table 6. Nodulation frequency scores of pea and faba bean crops during pod filling in 2005, Yunnan Province, China (1 =high; 9 = none).

Species	1	2	4	5	6	7	8	9	Total
Pisum sativum		1	1	1		2	1	1	7
Vicia faba	6	1	1	5	1	3	2	1	20

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References

Bao Shiying, Liu Zhenxi, Li Meijuan, Lai Yonghong, Zhao Zhanling, 1998. Yunnan food legume germplasm resource. In: Opportunities for high quality, healthy and added-value crops to meet European demands. Proceedings 3rd European Conference on Grain Legumes, 14–19 November, 1998, Valladolid, Spain (ed. AEP), p. 220.

Bao Shiying. 2003. Agricultural Statistical Data of Yunnan 2003. YAAS, China.

Cubero JI, Nadal S. 2005. Faba bean (*Vicia faba*). In: Singh RJ, Jauhar PP, editors. Genetic Resources, Chromosome Engineering, and Crop Improvement. Volume 1: Grain Legumes. CRC Press, Boca Raton, Florida, USA, pp. 163–186.

Liu ZS, Zhao YZ, Bao SY, Wen Guang W. 1993. An approach to the development of faba bean production and yield stability in Yunnan. In: Saxena MC, Weigand S, Li-Juan L, editors. Faba Bean Production and Research in China. ICARDA, Aleppo, Syria, pp. 75–83

Redden B, Leonforte T, Ford R, Croser J, Slattery J. 2005. Pea (*Pisum sativum* L.). In: Singh RJ, Jauhar PP, editors. Genetic Resources, Chromosome Engineering, and Crop Improvement. Volume 1: Grain Legumes. CRC Press, Boca Raton, Florida, USA, pp. 49–83.

Vavilov NI. 1951. The Origin, Variation, Immunity and Breeding of Cultivated Plants. Roland Press, New York, USA.

Appendix 1. Germplasm collecting form used on 2005 germplasm mission in Yunnan

Collecting Form, landraces________ Peas, ______ Faba bean

Sample Identification _____

Collector ________ Collecting date ______________

Site: Province ______ County ______ Location ______ km _____ direction _____from _______

GPS: Latitude _____ Longitude _______ Elevation _____ m.

Ethnic group _____

Topography: 1 – plain, 2 – plain, 3 – valley, 4 – plateau, 5 – upland, 6 – hill, 7 – mountain

Soil texture: 1 – clay, 2 – clay loam, 3 – silt, 4 – silty loam, 5 – sandy loam, 6 – sand

Rock %: 1 – 2%, 2 – 5%, 3 – 15%, 4 – 40%, 5 – 80%, 6 – 80+%

Slope: Degrees _____ Aspect (N etc) _____

Soil pH _____

Soil drainage: 3 – poor, 5 – moderate, 7 – well drained

Rainfall: _____ Frost frequency: _____

Crop frequency: 1 – rare, 3 – occasional, 5 – frequent, 7 – abundant, 9 – dominant

Other varieties and crops in locality: _______________

Landrace frequency; 1 – rare, 3 – occasional, 5 – frequent, 7 – abundant, 9 – dominant

Local name: ____________________ English translation: ____________________

Source of seed: farm __, farm store __, home garden __, local market __, town market __

Number of plants sampled, if from standing crop _____ (2 pods/plant pea to different bags CAAS & Australia)

Crop system: monoculture __, mixed with cereals __, mixed with other crops __

Plant population: 3 – low, 5 – intermediate, 7 – high

Pests and diseases (1 low, 9 high) ____________________

Reasons for choice of landrace: own seed __, less disease risk __, drought tolerant __, maturity: early __, medium __, late __, frost tolerant __, cooking/taste __, other __

Use: vegetable leaves __, pod __, seed __, grain food __, grain feed __, fodder __, straw __

Crop inputs: manure __, mulch __, fertilizer/type __ / ______, irrigation __, chemical/disease _____

Crop operations: manual ____________________ , mechanised ____________________

Male/female roles: tillage __, sowing __, fertilizing __, weeding __, chemicals __, harvest __, cooking __, marketing __

Barter: labour or cash to neighbour for inputs of seed __, fertilizer __, chemicals __

Landrace history: ancestral __,old introduction __, new introduction __: time __, source __

Seed source: own __, within village __, next village __, town market __, Government/Extension service __

Own seed supply: storage method _____, storage conditions _____, insect damage _____

Separation land race other varieties: field _____, harvest _____,

Separation landrace other varieties: own food/feed _____, market surplus _____

Separation seed types within land race: own seed supply _____, cooking _____ , market _____

Seed food frequency: 1 – daily, 2 – weekly, 3 – occasional, seasonal

Vegetable food frequency: 1 – daily, 2 – weekly, 3 – occasional, 4 – seasonal

Feed/forage frequency: 1 – daily, 2 – weekly, 3 – occasional, 4 – seasonal

Cooking vegetable or seed: none __, boiling __, baking __, roasting __, snacks __

Recipes: porridge __, cakes __, bread ingredient __, other _____, specialties _____

Taste: 1 – poor, 2 – acceptable, 3 – good

Market prices: _____

Mortality of Mexican coconut germplasm due to lethal yellowing

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Summary

Mortality of Mexican coconut germplasm due to lethal yellowing

Mortality due to lethal yellowing (LY) was recorded over 15 years for five coconut ecotypes representative of the diversity of coconut germplasm cultivated in Mexico. The trial was established in 1991 in an area of active LY outbreaks on the northern coast of Yucatan, Mexico, using a three block design with random distribution of the ecotypes within each block. The ecotypes included the susceptible Mexican Atlantic Tall (MXAT) and the resistant Malayan Yellow Dwarf (MYD), both used as references, and three Pacific Tall (PT) ecotypes, MXPT1, MXPT2 and MXPT3, to be tested. Parametric and non-parametric variance analyses of the results indicated significant differences ($p<0.05$) in mortality percentages among ecotypes and between blocks and no ecotype–environment interaction. Mortality was very high in MXAT and very low in MYD, similar to previous results in Jamaica. PT ecotypes had intermediate mortality percentages; mortality was low in MXPT1 and MXPT2 and not statistically different from that in MYD, while mortality was higher in MXPT3 and not statistically different from that in MXAT. According to these results we should expect that LY will cause lower mortality in coconut populations on the Pacific coasts, where most of the germplasm is MXPT, than the mortality rate observed in the Gulf of Mexico, where most of the germplasm is MXAT. MXPT1 and especially MXPT2 could be recommended (a) for replanting programmes; (b) as male parents for the production of dwarf × tall hybrids with the resistant MYD; and (c) for the selection of elite individuals with improved productivity for propagation purposes.

Key words: lethal yellowing, coconut, Mexican germplasm, mortality.

Résumé

Létalité des ressources génétiques de cocotier due au jaunissement mortel

La létalité due au jaunissement mortel (LY) a été consignée pendant 15 ans pour cinq écotypes de cocotier, représentatifs de la diversité du matériel génétique de cocotier, cultivés au Mexique. L'essai mis en place en 1991 dans une région où sévit le jaunissement mortel (côte nord-ouest du Yucatan, Mexique) utilise un modèle à trois blocs avec une distribution aléatoire des écotypes au sein de chaque bloc. Les écotypes comprennent le Grand atlantique du Mexique (MXAT), sensible, et le Nain jaune de Malaisie (MYD), résistant, tous deux utilisés comme témoins, ainsi que trois écotypes Grand pacifique (PT), MXPT1, MXPT2 et MXPT3 à tester. Les analyses de variance paramétriques et non paramétriques des résultats révèlent des différences significatives ($p<0,05$) de létalité parmi les écotypes et entre les blocs, mais pas d'interaction écotype-environnement. La létalité est très élevée pour MXAT et très faible pour MYD, ce qui concorde avec des résultats précédemment obtenus en Jamaïque. Les écotypes PT présentent des pourcentages de létalité intermédiaires ; la létalité est faible pour MXPT1 et MXPT2 et n'est pas statistiquement différente de celle de MYD, tandis qu'elle est plus élevée pour MXPT3 et non statistiquement différente de celle de MXAT. En fonction de ces résultats, il est prévisible que le jaunissement mortel provoque une létalité moindre dans les populations de cocotier sur les côtes pacifiques, où le matériel génétique dominant est MXPT, que dans le golfe du Mexique, où le matériel génétique principal est MXAT. MXPT1 et en particulier MXPT2 peuvent être recommandés (a) pour les programmes de replantation (b) comme parents mâles pour la production d'hybrides nain x grand avec MYD résistant, et (c) pour la sélection d'individus élites présentant une productivité améliorée pour la multiplication.

Resumen

Mortalidad de germoplasma de coco en México por amarillez letal

Durante 15 años desde1991 se registró mortalidad por amarillez letal (LY, siglas en inglés) de cinco ecotipos de coco que representan la diversidad de germoplasma de coco cultivado en México. El ensayo abarcó una zona de brotes activos de LY en la costa septentrional de Yucatán empleando un diseño de tres bloques de ecotipos distribuidos al azar que comprendían el susceptible Mexicano Atlántico Alto (MXAT) y el resistente Enano Malayo Amarillo (MYD), como referencia, para comprobar tres ecotipos Pacífico Alto (PT): MXPT1, MXPT2 y MXPT3. Los análisis de varianza paramétricos y no paramétricos indicaron diferencias significativas ($p<0,05$) de porcentajes de mortalidad entre ecotipos y entre bloques, sin interacción ecotipo-medio ambiente, y mortalidad muy elevada en MXAT y muy baja en MYD, similar a resultados previos en Jamaica. La mortalidad de ecotipos PT era intermedia; en MXPT1 y MXPT2 era baja y estadísticamente no diferente de la de MYD; en MXPT3 era mayor y estadísticamente no diferente de MXAT. Por esto puede esperarse que LY provoque menos mortalidad en las costas del Pacífico donde la mayor parte del germoplasma es MXPT, que en el Golfo de México donde el grueso es MXAT. MXPT1 y especialmente MXPT2 pueden recomendarse (a) para programas de replantación, (b) como progenitores masculinos para producir híbridos enanos x altos con el resistente MYD y (c) para seleccionar individuos de élite con productividad mejorada para la propagación.

Introduction

Lethal yellowing (LY) is a devastating disease caused by phytoplasmas (Plavsic-Banjac et al. 1972; McCoy et al. 1983) that affects nearly 40 palm species (Harrison et al. 1999). There is evidence that it can be transmitted by *Myndus crudus* Van Duzee (Homoptera: Cixiidae) (Howard et al. 1984a). In the past 50 years it has killed millions of coconut (*Cocos nucifera* L.) palms in several countries in Latin America and the Caribbean (LAC), drastically affecting both coconut production and the scenery in tourist resort areas (Oropeza et al. 2005). LY has become the main threat to coconut cultivation in LAC (Ashburner and Been 1997). Similar diseases known as lethal yellowing-type diseases (LYD) have also been described in Cameroon, Ghana, Kenya, Mozambique, Nigeria, Tanzania and Togo in Africa (Nienhaus et al. 1982; Eden-Green 1997).

Unfortunately, there are no effective measures to eliminate phytoplasmas from diseased palms. Insecticides were found to reduce dispersal rates but not enough to be useful (Reinert 1977; Howard and McCoy 1980; McCoy et al. 1983). Tetracycline antibiotics were found to reduce the symptoms of the disease (McCoy et al. 1983) but their use is very limited. They are expensive to use because they require permanent periodic applications and in some countries their use is not allowed because of health concerns. So far, the only effective way known to deal with this disease is the removal of diseased trees combined with sanitation and replanting with resistant germplasm (Been 1981, 1995). Therefore, the search for resistant coconut varieties has become a priority activity in LAC (Ashburner and Been 1997).

Evaluation for resistance is difficult since the disease cannot be transmitted in a controlled manner (Oropeza et al. 2005). As a result, testing depends on natural transmission and since LY establishes mainly in bearing palms (McCoy et al. 1983), evaluation of LY resistance in coconut can take 10 years or more. In addition, these efforts are often disrupted by hurricanes (Johnston et al. 1994), which alter test conditions, such as plant development and vector population dynamics, or may bring an abrupt end to the tests.

Field testing for LY resistance of coconut germplasm was carried out in Jamaica from the early 1960s to the early 1980s (Been 1981). Results showed differences in mortality between genotypes: the Jamaican Tall (known in other countries as Atlantic Tall, AT) was the most susceptible, with 90% mortality, and the Malayan Dwarf varieties were the most resistant ones, particularly the Malayan Yellow Dwarf (MYD) with 4% mortality. The Panama Tall, with an intermediate mortality of 44%, was used as pollen donor in a programme to produce hybrid palms (Harries and Romney 1974; Been 1995). The MYD × Panama Tall hybrid (Maypan) and the MYD have been the most extensively planted coconuts in Jamaica, Mexico, Belize and Honduras. A review of the data from the various trial sites in Jamaica indicated a genotype–environment interaction which is believed to be related to differences in the compositions and densities of the vector insects and host-plant populations (Ashburner and Been 1997). Similar observations have been reported in trials in Africa (Sangare et al. 1992; Shuiling et al. 1992).

Further screening for LY resistance in coconut continues as a priority in LAC. Interest has increased after observations in sites in Florida (Howard et al. 1987) and Jamaica (Broschat et al. 2002) showed higher than expected mortalities in MYD and Maypan. In Mexico, coconut germplasm for screening can be sourced from the coasts of the Gulf of Mexico and the Yucatan Peninsula, where coconut palms from the West Coast of Africa were introduced during the 16th century, and from the Mexican Pacific coast, where germplasm from Melanesia, Polynesia, Malaysia and the Pacific Coast of Panama is grown (Zizumbo-Villarreal et al. 1993; Zizumbo-Villarreal 1996). Coconut populations that represent the coconut genetic diversity in Mexico have been collected from all producing areas and morphological, phenological, biochemical and molecular characterizations have been carried out. Analyses of the data led to the identification of five ecotypes, consisting of one dwarf and four tall ecotypes (Zizumbo-Villarreal and Colunga-GarcíaMarín 2001; Zizumbo-Villarreal et al. 2005, 2006).

Nuts from these characterized populations were collected in 1990 to establish a trial for the evaluation of LY resistance in the Yucatan Peninsula in a site that was affected by the disease (Zizumbo-Villarreal et al. 1999). Based on the knowledge previously gained in Jamaica, we decided: (a) to establish the trial on one site with a soil humidity gradient with the gradient reflected in the experimental blocks; (b) to carry out cultural practices that promote the occurrence of vector insects; and (c) to include two genotypes that can be indicators of the level of disease incidence—the susceptible MXAT and the resistant MYD; MXAT would also promote the occurrence of phytoplasmas. In this paper, we report the analysis of the response to LY of the germplasm tested in this trial.

Materials and methods

Collection of germplasm

Seventeen tall populations and one dwarf population representing the genetic diversity in Mexico were selected (Table 1, Figure 1) from 41 populations previously identified according to the pattern of morphological variation of the nut (Zizumbo-Villarreal et al. 1993). After further characterization, the 18 populations were grouped into five ecotypes: Malayan Yellow Dwarf (MYD), Mexican Atlantic Tall (MXAT), Mexican Pacific Tall 1 (MXPT1), Mexican Pacific Tall 2 (MXPT2) and Mexican Pacific Tall 3 (MXPT3) (Zizumbo-Villarreal and Colunga-GarcíaMarín 2001; Zizumbo-Villarreal et al. 2006). Nuts of these selected populations were collected and taken to Yucatan, where they were established in the field and exposed to LY for 15 years.

Establishment of the trial

The trial was established in 1991 in an area on the northern coast of Yucatan where LY was active. The site was located in a sand-dune area that separated the sea from a freshwater lagoon system. The site has a dry, warm climate with an average annual precipitation of 680 mm from summer rains and average annual temperature of 26 °C (García 1988). The soil type is Quartzipsamment (Soil Survey Staff 1999) with a slight slope north–south associated with the depth of the freatic level (between 50 cm and 100 cm). After germination, the plants were grown in a nursery for 1.5 years before they were transplanted to the trial site. The palms were separated by 9 m from each other in an equidistant triangular arrangement (Figure 2).

Table 1. Data on ecotypes and source location of coconut populations tested in San Crisanto, Yucatan, Mexico.

Code	Population	Ecotype	Location			
			Municipality	State	Latitude	Longitude
K1	Champotón	MXAT	Champotón	Campeche	19° 20'	90° 43'
K3	Sabancuy	MXAT	Escárcega	Campeche	18° 00'	91° 00'
G1	Marquelia	MXPT3	Ayozu	Guerrero	16° 45'	98° 35'
G2	El Carrizo	MXPT3	Copala	Guerrero	16° 50'	98° 35'
G4	Tecpan	MXPT1	Tecpan	Guerrero	17° 31'	101° 13'
M1	El Caimán	MXPT1	L. Cárdenas	Michoacán	18° 15'	101° 55'
M2	El Manglar	MXPT1	L. Cárdenas	Michoacán	18° 15'	101° 55'
M3	Coahuayana	MXPT1	Coahuayana	Michoacán	18° 19'	103° 30'
C1	Callejones	MXPT2	C. de Ortega	Colima	18° 56'	103° 58'
C2	C. de Ortega	MXPT2	C. de Ortega	Colima	19° 12'	103° 48'
C3	Tecomán	MXPT2	Tecomán	Colima	18° 54'	103° 52'
C4	Cuyutlán	MXPT1	Cuyutlán	Colima	18° 55'	104° 05'
C6	Centinela	MXPT1	Manzanillo	Colima	19° 10'	104° 30'
J1	Cihuatlán	MXPT1	Cihuatlán	Jalisco	19° 15'	104° 35'
J2	B. Navidad	MXPT1	Cihuatlán	Jalisco	19° 15'	104° 45'
N1	San Blas	MXPT2	San Blas	Nayarit	21° 33'	105° 17'
N2	Matanchén	MXPT2	San Blas	Nayarit	21° 33'	105° 17'
C5	Tecomán	MYD	Tecomán	Colima	18° 54'	103° 52'

Source: Zizumbo-Villarreal and Colunga-GarcíaMarín 2001, Zizumbo-Villarreal et al. 1993, 1999, 2006.

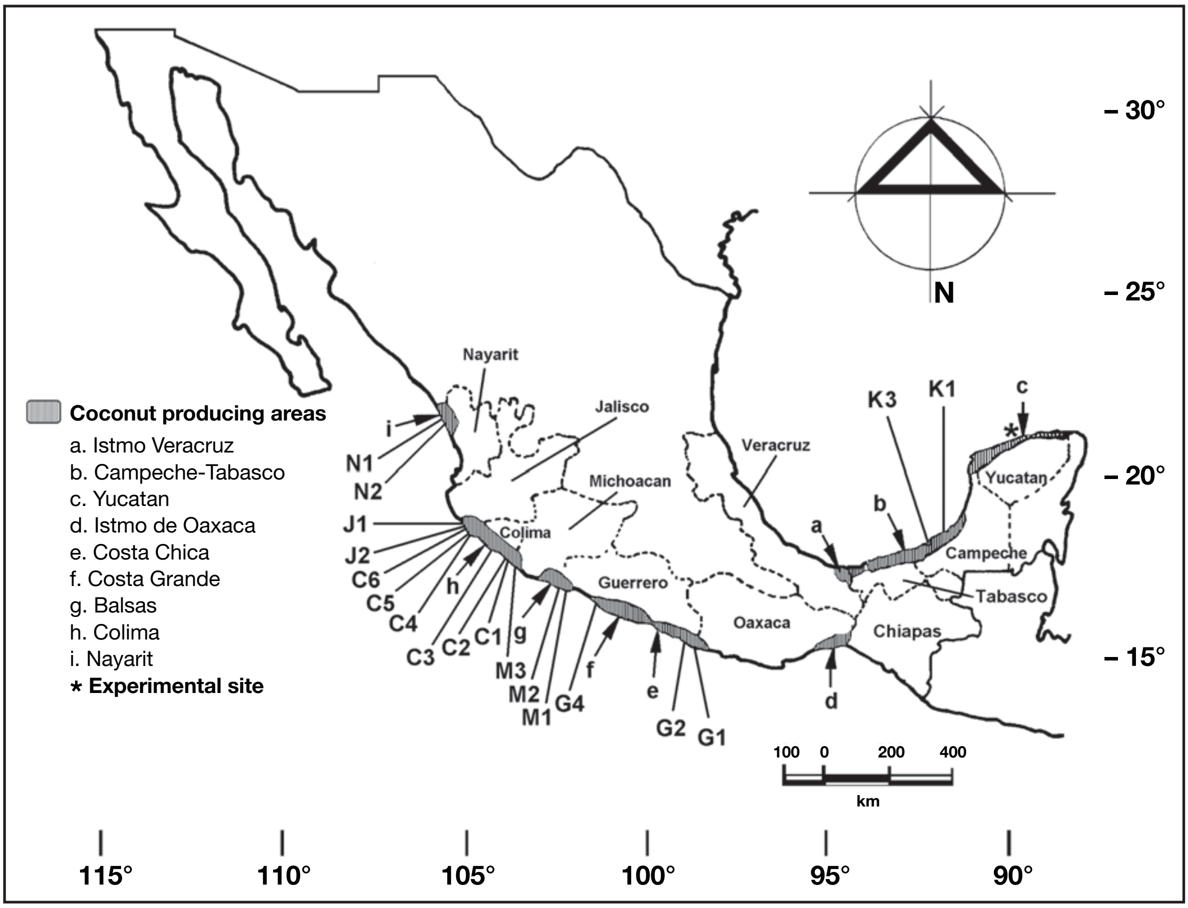

Figure 1. Collection sites of the 18 coconut populations tested and location of testing site (*). Numerical code of populations as in Table 1.

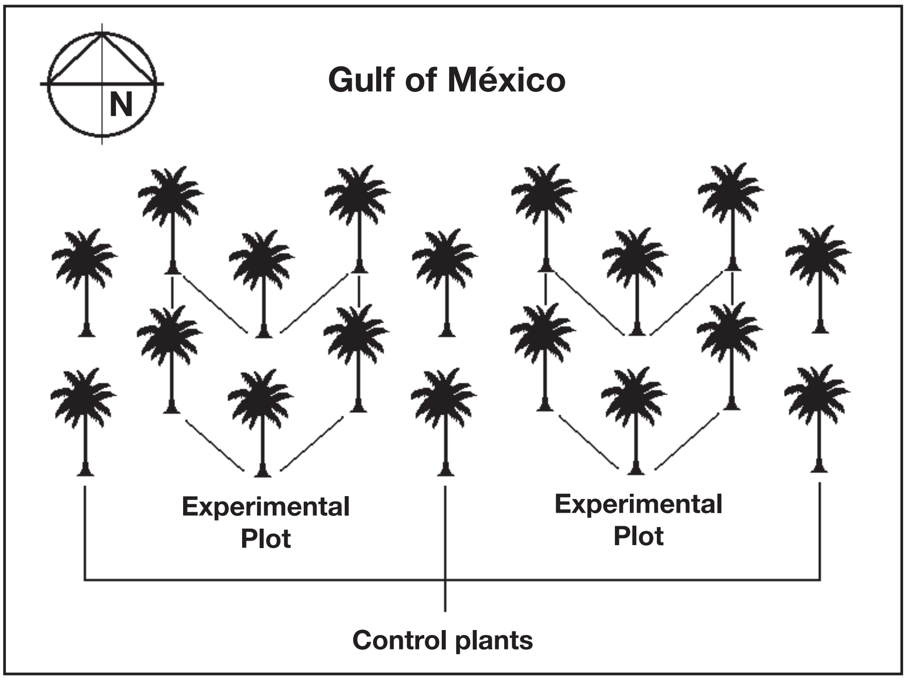

Figure 2. Diagram showing the spatial arrangement of palms in the trial at San Crisanto, Yucatan, Mexico.

Experimental design

The trial was established in a location severely affected by LY. The existing plants in the area were eliminated, with the exception of the bearing coconut palms. These were left untouched, as they were expected to be infected with the LY causal agent and hence would be a source of infection. The trial consisted of three blocks oriented north–south along a soil-moisture gradient. In each block 18 populations were established in a random distribution: 15 of PT, two of AT and one of MYD. Each population was represented by a plot of six palms (Figure 2). Rows of the two populations of MXAT, included as a susceptible control, were placed between plots. Each MXAT palm that died was substituted with another MXAT palm, in the expectation that, being a susceptible ecotype, the plant would become infected with the LY causal agent and promote the incidence of the disease. In addition, and for the same purpose, growth of grass was encouraged since grass favours the occurrence of homopterans, particularly *M. crudus* which was shown to be a vector of LY in Florida, USA (Howard et al. 1984b; Howard 1995). The trial consisted of 413 palms in total, including 304 MXPTs, 18 MYD and 91 MXAT.

Control of pests and diseases

Traps containing attractants and insecticide (90% Methomyl) were used for the preventive control of *Rhynchophorus palmarum* L., which is also a vector for *Rhadinaphelenchus cocophilus* (Cobb) Goodey, the causal agent of red ring disease. During 1991–1994, the attractant used was natural (pineapple or banana slices); subsequently a synthetic pheromone was used (Morín et al. 1986; Rochat et al. 1993). Three traps were placed per hectare in equidistant locations. Insects were removed weekly from the traps. Plot drainage was improved and a fungicide solution (50% Benomyl) was sprayed during the rainy season (June–November) to prevent establishment of *Phytophthora palmivora* Butl., which causes bud rot and thrives in wet conditions.

LY visual symptoms

Identification of LY on palms was based on symptoms reported by McCoy et al. (1983). The first symptom is premature nut fall, followed by inflorescence necrosis, which is readily observed on newly mature inflorescences as they emerge from the spathe. Normally light yellow to creamy white in colour, inflorescences instead are partly to totally blackened. As the disease progresses, additional emerging and unemerged inflorescences show more extensive necrosis and may be totally discoloured. Leaf yellowing usually begins once necrosis has developed on two or more inflorescences and is markedly more rapid than that associated with normal leaf senescence. Yellowing advances from the older, lower-most leaves to the younger, upper-most leaves. Affected leaves eventually turn brown, desiccate and often hang down around the trunk for several days before falling to the ground, leaving a bare trunk standing.

LY phytoplasma detection

Detection of LY phytoplasmas was carried out using various methods. During 1992–1993 these included staining with DNA binding probe DAPI (4'-6-diamidino-2-phenylindole; Sigma Laboratories, USA) and electron microscopy (Cardeña et al. 1991). During 1994–1996 detection was carried out using DNA dot hybridization with biotin-labelled probe LY143 (Harrison et al. 1992). Positive dot hybridization was equated with development of purple colour. After 1996, total DNA extracted from each sample was tested for LY phytoplasma by PCR employing the LY-specific non-ribosomal primer pair LYF1 and LYR1 (Harrison et al. 1994) or by nested PCR, using phytoplasma universal rRNA operon primer pair P1/P7 followed by LY group-specific 16S rRNA gene primer pair LY503f/LY16Sr (Harrison et al. 1999, 2002).

Sampling and DNA extraction

Tissue samples were collected from palms with LY symptoms and asymptomatic palms. The tissues were sampled from young leaves (spear leaf), the stem apex, inflorescences and trunk (shavings). DNA was extracted from 1 g of tissue using the cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1990), as modified by Harrison et al. (1994). The DNA was precipitated with ethanol, pelleted by centrifugation, resuspended in 100 µl of TE buffer (1 mM Tris, 0.1 mM EDTA, pH 8) and incubated with RNAse for 1 hr at 37 °C. Aliquots of final DNA preparations were used for DNA probing or as templates for PCR amplification.

Statistical analysis

Throughout 1992–2006, data were gathered on the number of palms dying of LY to calculate percentage of mortality relative to population and cumulative mortality. To conduct the statistical analyses, the populations were grouped within blocks by ecotype (as defined by Zizumbo-Villarreal and Colunga-GarciaMarin 2001) leaving an unbalanced test (Table 2). The plants of the MXAT ecotype were grouped every two or three rows to represent a plot in the corresponding block, resulting in plots with six plants each and one with eight (Figure 2).

Parametric and non-parametric analyses were conducted. For parametric analysis, we carried out one-way or two-way analysis of variance (ANOVA) with genotype–environment interaction and arcsin transformation of data using a generalized linear model (GLM) for unbalanced experiments (Zar 1984). Mean comparison analyses were carried out with LSD ($p<0.05$) using SAS for Windows, version 6.03 (SAS 1992). One-way non-parametric ANOVA was done with the Kuskal-Wallis test. Mean rank comparisons (Nei et al. 1975) were performed using the Mann-Whytney test ($p<0.05$) using SPSS for Windows, version 12.0.

Results

Mortality caused by factors other than LY

Throughout the duration of the trial, there were no deaths of coconuts due to *R. palmarum* and red ring diseases. Seven trees (1.7% of the total population in the trial) were killed by hurricane Isidore in 2002.

LY symptom development

During the first years of the trial, the existing bearing coconut palms that were left in the site developed LY symptoms and died, as expected. Some of the trial palms also died. As they were then non-bearing and lacking sexual organs, they showed only yellowing of the foliage (Figure 3A). From 1995 onwards, trial palms bearing nuts developed LY symptoms that were very similar to those observed in the bearing palms that were originally on the site. Symptoms started with most or all nuts falling within a few days (Figure 3B). This was followed by necrosis of the inflorescences; the first ones showed partial necrosis, while later ones showed more extensive necrosis (Figures 3C and D). Usually, after two or three necrotic inflorescences emerged, leaves turned yellow, then brown and died (Figures 3E and F). This occurred progressively from the older leaves (Figure 3E) to the younger ones (Figure 3F). After the death of the leaves, the crown fell, leaving the bare trunk (not shown). In some of the tested palms of the PT ecotypes, the leaves turned bronze instead of yellow.

Detection of LY phytoplasma DNA

All the palms showing symptoms of LY were sampled and analyzed to determine if phytoplasmas were present. During 1992–1993, this was carried out by DAPI staining, and all palms with symptoms were positive, showing epifluorescence within the phloem vessels (Figure 4A), whereas non-symptomatic MYD were negative. Palms developing LY symptoms in the period of 1994 to 1996 were analyzed using a DNA probe (LY143) and positive hybridization was observed in all palms with symptoms, whereas it did not occur in non-symptomatic control palms (Figure 4B). Symptomatic palms from late 1996 onwards were analyzed by PCR and positive amplification was obtained (Figure 4C) using either standard PCR (primer set: LYF1 / LYR1; amplicon size 1.07

Table 2. Accumulated mortality (1992–2006) due to lethal yellowing by ecotype and parametric and non-parametric ANOVA.

Ecotype	Populations	Plots	n	Parametric		Non-parametric
				M	SE	MR
MXAT	K1, K3	16	91	64.4 (a)	7.9	51.0 (a)
MXPT1	G4, M1, M2, M3, C4, C6, J1, J2	24	152	16.9 (b,c)	4.2	26.7 (b)
MXPT2	C1, C2, C3, N1, N2	15	90	14.4 (c)	4.3	25.6 (b)
MXPT3	G1, G2	12	62	35.7 (b)	7.8	39.9 (a,b)
MYD	C5	3	18	5.7 (c)	5.7	18.0 (b)

Notes: M = mean percentage; SE = standard error; MR = mean rank
Means and Mean rank followed by the same letter are not significantly different at $p<0.05$.

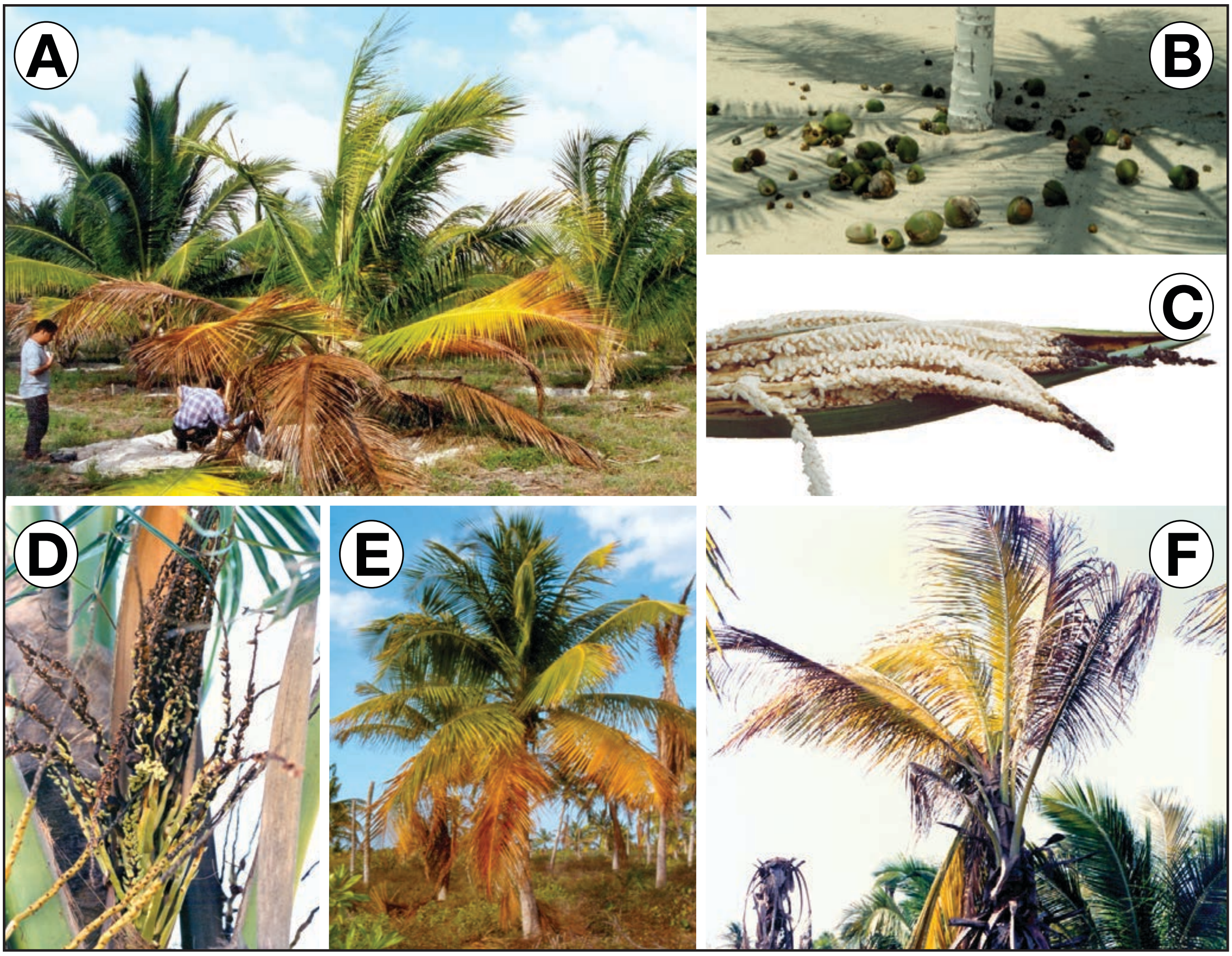

Figure 3. Symptoms of lethal yellowing. A. Non-bearing palms showing foliage yellowing. B. Bearing palms started showing nut fall (B). C and D. Inflorescence necrosis. Foliage yellowing started in older leaves (E) and continued with younger ones (F).

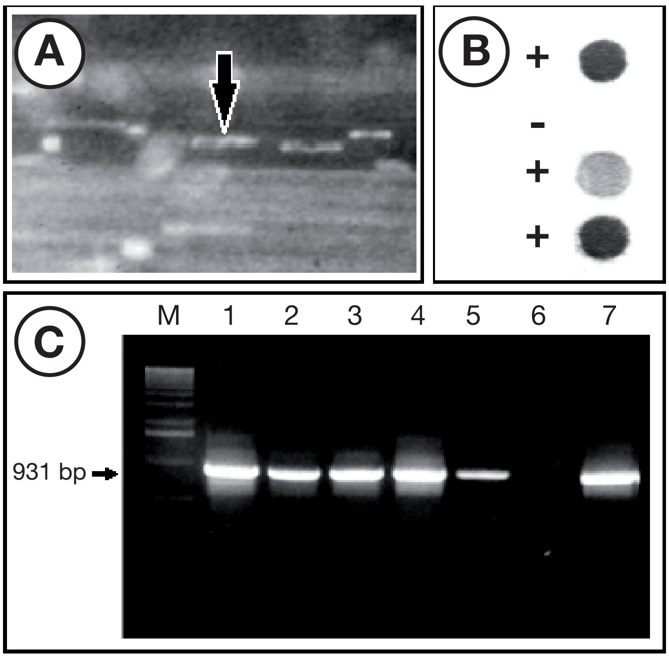

Figure 4. Methods used for the detection of lethal yellowing (LY) phytoplasmas. A. Detection of epifluorescence in phloem vessels (arrows) after DAPI staining of tissue samples of palms with LY symptoms. B. DNA probe (LY143) hybridization tested in extracts of palms: [+] positive control and symptomatic palms; [-] negative control. C. Amplicons obtained by PCR on plant extracts and developed by gel electrophoresis: [M], size markers; [1–5], samples from symptomatic palms; [6 and 7], negative and positive control samples, respectively.

Kb) or nested PCR (primer set: P1/P7 followed by LY503f/LY16Sr; amplicon size 0.93 Kb). The second test was used with samples that were negative with standard PCR and presumably with lower phytoplasma titre. No amplification occurred with either type of PCR in non-symptomatic palms, with the exception of one symptomless MXAT palm in 2001 (see below). With each methodology used, positive and negative control samples were analyzed and in all cases, the results were positive and negative, respectively. According to these results, obtained using a variety of methods, positive detection in symptomatic palms was considered indicative of the presence of LY phytoplasmas, and negative detection in the symptomless palms indicative of lack of infection.

Accumulated mortality due to LY (1992–2006)

LY started killing palms from the first year of the trial and the accumulated mortality increased rapidly during the first five years. Then it slowed, with the last palm death registered in 2003. Accumulated mortality of MXAT was 64% in the whole trial and 86% within block 3, which had the highest incidence of LY. In the LY-resistant ecotype MYD, accumulated mortality was 6% in the whole trial and 17% in block 3 (Figures 5 and 6). The accumulated mortality of the total population was 30% in the whole trial and 49% in block 3 (Figures 5 and 6). Positive PCR detection was obtained in one symptomless MXAT palm in 2001. Samples have been retaken and re-analyzed on several occasions since, with further positive results, but the palm is still alive.

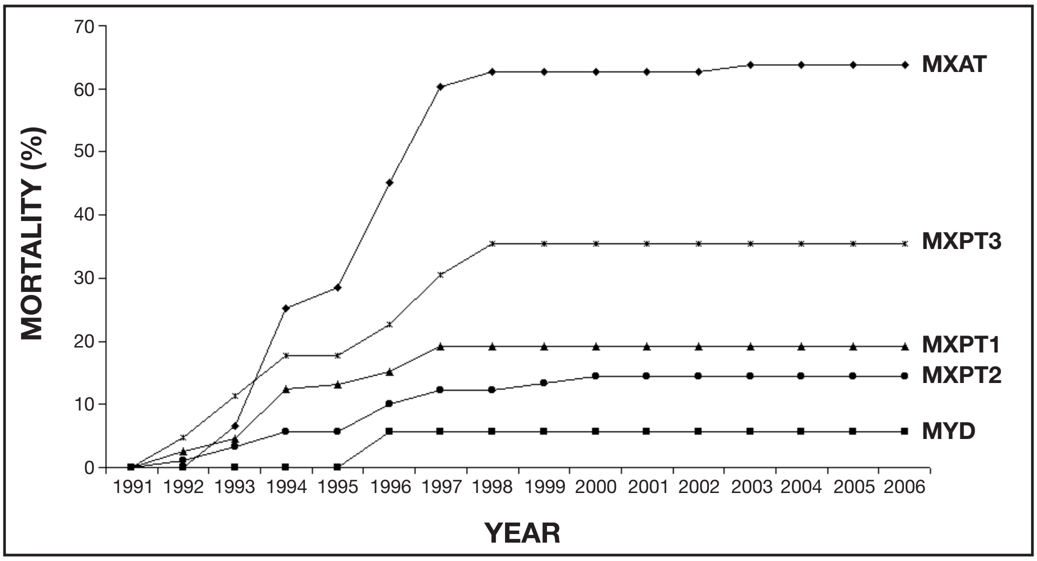

Figure 5. Total accumulated mortality due to lethal yellowing by ecotype in the trial San Crisanto, Yucatan, Mexico.

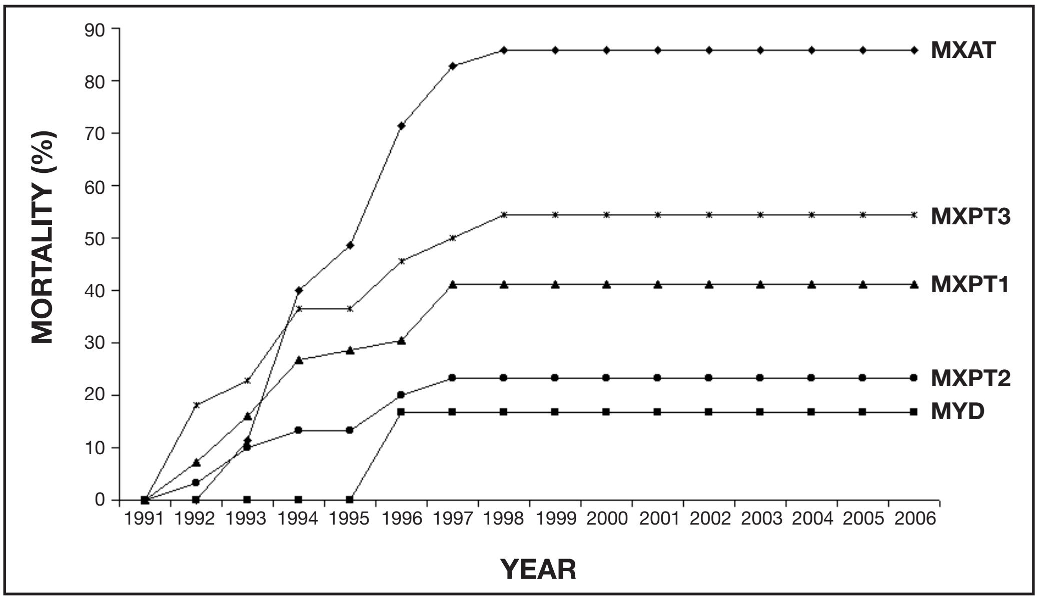

Figure 6. Accumulated mortality due to lethal yellowing by ecotype in block 3 of the trial established at San Crisanto, Yucatan, Mexico.

Mortality due to LY by ecotype

The two-way parametric ANOVA showed significant differences in mortality rate (*p*<0.0001) between ecotypes. Accumulated mortality was highest in MXAT and lowest in MYD, although not significantly less than that in MXPT1 and MXPT2 (Table 2). Non-parametric ANOVA showed similar results, although in this case the accumulated mortality of MXAT was significantly higher than that of other ecotypes with the exception of MXPT3 (Table 2).

Mortality due to LY per block

Accumulated mortality was significantly (*p*<0.0001) higher in block 3 than in blocks 1 and 2 (Figure 7) both in all ecotypes and in the susceptible control MXAT (Table 3). Block 3 is close to a lagoon ecosystem and blocks 2 and 1 are farther from it.

Genotype–environment interaction and LY mortality

There was no evidence of genotype–environment interaction (*p*= 0.75).

Mortality under high LY incidence (blocks 1 and 2)

Parametric ANOVA showed mortality was significantly greater in MXAT than in the other ecotypes in block 1, whereas in block 2 mortality was significantly higher in MXAT than in MXPT1 and MXPT2, but not significantly different to that in MXPT3 (Table 4). Non-parametric ANOVA also showed significant differences between ecotypes in block 1 (Chi-Square = 8.7; *p* = 0.03) and in block 2 (Chi-Square = 7.9; *p* = 0.05);

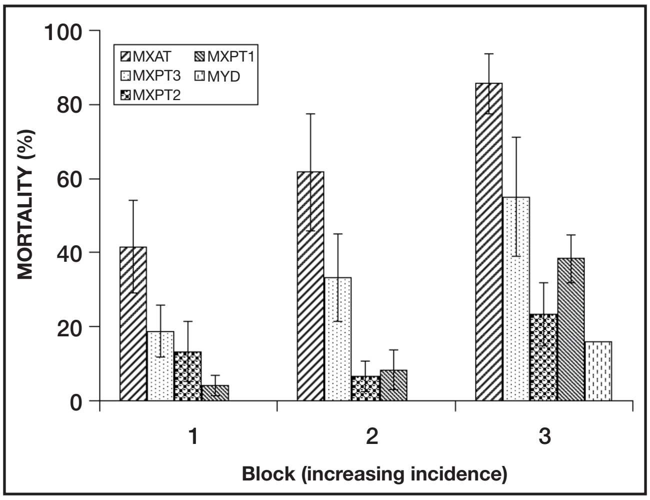

Figure 7. Accumulated mortality due to lethal yellowing by ecotype and block in the trial established at San Crisanto, Yucatan, Mexico.

Table 3. Accumulated mortality (1992–2006) due to lethal yellowing by block and parametric and non-parametric ANOVA analysis.

Block	Parametric				Non-parametric	
	All ecotypes		MXAT		All ecotypes	MXAT
	M	SE	M	SE	ME	ME
1	16.6 (a)	4.5	41.6 (a)	12.4	24.9 (a)	3.0 (a)
2	23.5 (a)	6.3	61.7 (a)	15.7	26.9 (a)	4.7 (a)
3	48.9 (b)	6.3	85.5 (b)	8.0	44.2 (b)	7.3 (b)

Notes: M = mean percentage; SE = standard error; MR = mean rank
Means and Mean rank followed by the same letter are not significantly different at *p*<0.05.

Table 4. Accumulated mortality (1992–2006) due to lethal yellowing by ecotype and block, and parametric and non-parametric ANOVA.

Ecotype	Populations	Plots	n	Parametric						Non-parametric		
				Block 1		Block 2		Block 3		Block 1	Block 2	Block 3
				M	SE	M	SE	M	SE	MR	MR	MR
MXAT	K1, K2	16	91	41.6 (a)	12.4	61.7 (a)	15.7	85.6 (a)	8.0	17.8 (a)	16.7 (a)	18.0 (a)
MXPT1	G4, M1, M2, M3, C4, C6, J1, J2	24	152	4.2 (b)	2.7	8.3 (b)	5.4	38.4 (b,c)	6.4	7.1 (b)	7.9 (b)	9.4 (b,c)
MXPT2	C1, C2, C3, N1, N2	15	90	13.3 (b)	8.2	6.7 (b)	4.1	23.3 (c)	8.5	10.3 (a,b)	8.3 (a,b)	6.0 (c)
MXPT3	G1, G2	12	62	18.8 (b)	7.1	33.3 (a,b)	11.8	55.0 (b)	16.1	12.3 (a)	13.9 (a)	12.8 (a,b)
MYD	C5	3	18	0		0		16.0				

Notes: M = mean percentage; SE = standard error; MR = mean rank
Means and Mean rank followed by the same letter are not significantly different at *p*<0.05.

mortality was significantly higher in MXAT than in MXP1 but not significantly different from that in MXPT2 and MXPT3 (Table 4).

Mortality under severe LY incidence (block 3)

Parametric ANOVA showed significant differences between ecotypes in block 3. Mortality was higher in MXAT than in the PT ecotypes (Table 4); and within the latter MXPT3 had the highest mortality but not significantly greater than that in MXPT1. Ecotype MXPT2 had the lowest mortality but this was not significantly different to that in MXPT1 (Table 4). Non-parametric ANOVA also showed significant differences between ecotypes (Chi-Square = 8.8; $p < 0.04$); mortality was higher in MXAT than in MXPT1 and MXPT2, but not significantly different to that in MXPT3 (Table 4). Ecotype MXPT2 had the lowest mortality but not significantly different to that in MXPT1 (Table 4).

Discussion

LY started killing some of the palms during the first year of the trial (1991–1992) when the palms were about 2.5 years old, still in the juvenile stage and about 2 years before the bearing stage. Previous studies (McCoy et al. 1983) had reported that LY affects primarily bearing palms or those about to reach this stage. In the present trial, the palms were much younger when LY started affecting them, probably because the preparation of the site for the establishment of the trial did not cause drastic changes in the soil and the vegetation, so the preparation did not prevent the occurrence of LY at the beginning of the trial.

Most of the losses due to LY occurred during 1991–1997, with fewer losses in 1997–1998; subsequently there was only a single diseased palm in 2003. There were no deaths after that, even within the susceptible MXAT palms. The reduced incidence of LY probably resulted from a decreased availability of inoculum since it coincided with the end of the massive loss of susceptible palms in the state of Yucatan and most of the Peninsula of Yucatan. In addition, the hurricanes Isidore in 2002 and Emily in 2005 severely affected the soil conditions of the trial site because of heavy rains and flooding. Several juvenile MXAT replanted palms were killed as a result. Habitat perturbations due to the hurricanes might have also hampered development of the vector.

There was a gradient of LY incidence across the three blocks of the trial, with significant differences of mortality between blocks. The average mortality for all the ecotypes was lowest in blocks 1 and 2 (nearer to the seashore), and highest in block 3 (nearer to the lagoon). These differences may be explained by higher soil moisture in block 3 and proximity to freshwater, environmental conditions that may be better for the vector development than those of blocks 1 and 2.

Cumulative mortality for the reference ecotypes ranged from 41.6 to 85.5% for MXAT and from 0% to 16.7% for MYD, similar to the mortality reported for Jamaica tall and MYD in Jamaica (Been 1981). The evaluation of the PT ecotypes showed mortality levels in between those of MYD and MXAT, as well as mortality differences between them, indicating that they probably have a different origin.

The significant differences in the mortality for each ecotype between the trial blocks indicate that even small differences in ecological conditions can influence LY incidence. This is consistent with previous observations for LY in Jamaica (Ashburner and Been 1997) and LYD in Africa (Sangare et al. 1992; Shuiling et al. 1992). For instance, in block 3, where incidence was the highest, there was a greater abundance of *Distichlis spicata* (L.) E. Greene, a grass species that hosts *M. crudus*, the homopteran that transmits LY phytoplasmas (Howard 1995). Indeed, a greater density of this insect, in both adult and larval stages, has been reported for this block (Piña-Quijano 1994). Thus, in block 3 there were ecological advantages for the persistence of the *M. crudus*, favouring incidence of LY. On the other hand, statistical analysis of the results showed that there is no ecotype–environment interaction and therefore the differential responses of the ecotypes to LY were independent of the environment.

The results presented here show that, together with MYD, coconut populations from the Pacific coasts of Mexico could be an important source of germplasm to deal with LY, particularly ecotypes MXPT2 and MXPT1 that had low levels of mortality even under severe disease pressure. Considering that a previous study in Jamaica on LY resistance showed that it has a high heritability (Ashburner and Been 1997), these two ecotypes could be recommended for: (a) replanting programmes; (b) as male parents for the production of dwarf × tall hybrids with the resistant MYD as female parent under a similar scheme to that previously used in Jamaica (Been 1995); and (c) for the selection of elite individuals with improved productivity for propagation purposes, including the use of micropropagation techniques (Pérez-Núñez et al. 2006).

There are several reasons to consider that ecotype MXPT2 could be a better choice for these purposes than MXPT1. The mortality of MXPT2 was consistently lower in all three blocks than that of the other two PT ecotypes, MXPT1 and MXPT3. Previous studies on morphological and physiological characterization of the five ecotypes, carried out under experimental conditions, indicated that MXPT2 was the best performer among the PT ecotypes in terms of: (1) early bearing; (2) high production of leaves and inflorescences; (3) high number of pistillated flowers per inflorescence and fruits per bunch; (4) high fruit content of solid endosperm; and (5) high fruit and oil yields (Zizumbo-Villarreal and Colunga-GarcíaMarín 2001; Zizumbo-Villarreal et al. 2005; Meléndez-Ramírez 2005). This ecotype was also found to have a high coefficient of variation of its characteristics within populations and high heritability values (Meléndez-Ramírez 2005; Zizumbo-Villarreal et al. 2005). Worldwide studies on genetic relations of coconut germplasm using molecular markers (SSR and ISSR) showed a small genetic distance between MYD and MXPT2 and that they grouped together (Baudouin and Lebrun 2002; Zizumbo-Villarreal et al. 2006). It is interesting to note that in the latter study a significant correlation was also found between mortality due to LY and genetic distance between MYD and the three MXPT ecotypes, suggesting that this correlation deserves to be explored more extensively.

Based on the present results, we anticipate that the impact of LY in coconut populations in Mexico will depend on the geographical distribution of the various ecotypes. Lower mortality may to be expected on the Pacific coasts where most of the germplasm is MXPT. In contrast, LY is likely to be more devastating in the Gulf of Mexico, as it has already been in the Yucatan Peninsula, since most of the germplasm cultivated in these two regions is the very susceptible MXAT. This loss is unfortunate because MXAT has very good yield of oil and also excellent fibre qualities (Meléndez-Ramírez 2005). It will be necessary to develop a conservation programme for MXAT and reduce the impact of loss due to LY.

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References

Ashburner GR, Been BO. 1997. Characterization of resistance to lethal yellowing in *Cocos nucifera* and implications for genetic improvement of this species in the Caribbean region. In: Eden-Green SJ, Ofori F, editors. International Workshop on Lethal Yellowing-Like Diseases of Coconut, Elmina, Ghana, November 1995. Natural Resources Institute, Chatham, UK, pp. 173–183.

Baudouin L, Lebrun P. 2002. The development of a microsatellite kit and dedicated software for use with coconuts. Burotrop Bulletin 17:16–20.

Been BO. 1981. Observations on field resistance to lethal yellowing in Jamaica. Oléagineux 36:9–11.

Been BO. 1995. Production and advantages of coconut hybrids. In: Oropeza C, Howard FW, Ashburner GR, editors. Lethal Yellowing: Research and Practical Aspects. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 187–194.

Broschat TK, Harrison NA, Donselman HM. 2002. Losses to lethal yellowing cast doubt on coconut cultivar resistance. Palms 46:185–189.

Cardeña R, Villanueva MA, Santamaría JM, Oropeza C. 1991. Presence in Yucatan of mycoplasma-like organisms in *Cocos nucifera* palms showing lethal yellowing disease symptoms. Canadian Journal of Plant Pathology 13:135–138.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13–15.

Eden-Green SJ. 1997. History, world distribution and present status of lethal yellowing-like diseases of palms. In: Eden-Green SJ, Ofori F, editors. Proceedings of an International Workshop on Lethal Yellowing-Like Diseases of Coconut, Elmina, Ghana, November 1995. Natural Resources Institute, Chatham, UK, pp. 9–25.

García E. 1988. Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía, UNAM, México, D.F.

Harries HC, Romney DH. 1974. Maypan: an F1 hybrid coconut variety for commercial production in Jamaica. World Crops 26:110–111.

Harrison NA, Barner R, Cox J, Tsai H, Richardson P. 1992. DNA probes for detection of mycoplasmalike organisms associated with lethal yellowing disease of palms in Florida. Phytopathology 82:216–224.

Harrison NA, Richardson P, Kramer B, Tsai H. 1994. Detection of mycoplasma-like organisms associated with lethal yellowing disease of palms in Florida by polymerase chain reaction. Plant Pathology 43:998–1008.

Harrison NA, Cordova I, Richardson P, DiBonito R. 1999. Detection and diagnosis of lethal yellowing. In: Oropeza C, Verdeil JL, Ashburner GR, Cardeña R, Santamaría JM, editors. Current Advances in Coconut Biotechnology. Kluwer Academic Publishers. Dordrecht, The Netherlands, pp. 183–196.

Harrison NA, Myrie W, Jones P, Carpio ML, Castillo MM, Doyle MM, Oropeza C. 2002. 16S rRNA interoperon sequence heterogeneity distinguishes strain populations of palm lethal yellowing phytoplasma in the Caribbean region. Annals of Applied Biology 141:183–193.

Howard FW. 1995. Lethal yellowing vector studies. I. Methods of experimental transmission. In: Oropeza C, Howard FW, Ashburner GR, editors. Lethal Yellowing: Research and Practical Aspects. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 43–57.

Howard FW, McCoy RE. 1980. Reduction in spread of mycoplasma-like organism-associated lethal decline of the palm, *Veitchia merrillii*, by use of insecticides. Journal of Economic Entomology 73:268–270.

Howard FW, Williams DS, Norris RC. 1984a. Insect transmission of lethal yellowing to young palms. International Journal of Entomology 26:331–338.

Howard FW, Tilmant JR, Burch DG, Hall D. 1984b. A field study to determine potential host plants of lethal yellowing agent. Research Report FL-84-1. Fort Lauderdale R&E Center, IFAS, University of Florida.

Howard FW, Atilano R, Barrant CL, Harrison NA, Theobald WF, Williams DS. 1987. Unusually high lethal yellowing disease incidence in Malayan Dwarf coconut palms in localized sites in Jamaica and Florida. Journal of Plantation Crops 15:86–100.

Johnston CF, Fielding WJ, Been B. 1994. Hurricane damage to different coconut varieties. Tropical Agriculture (Trinidad) 71:239–242.

McCoy RE, Howard FW, Tsai JH, Donselman HM, Thomas DL, Basham HG, Atilano RA, Eskafi FM, Britt L, Collins MB. 1983. Lethal yellowing of palms. Agricultural Experiment Station Bulletin No. 834. University of Florida, Florida, USA.

Meléndez-Ramírez V. 2005. Biología reproductiva y ecología de la polinización de la palma de coco (*Cocos nucifera* L.) en Yucatán, México. Doctoral Dissertation. Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México.

Morín JP, Lucchini F, Arauco JC, Ferrera JM, Fraga LS. 1986. Estudo de comportamento olfactivo de *Rhynchophorus palmarum* L. (Coleoptera, Curculionidae) no campo. Anais da Sociedade Entomológica do Brasil 18:267–273.

Nei N, Hull CH, Jenkins JG, Steinbrenner K, Bent DH. 1975. SPSS: Statistical Package for the Social Sciences (2nd ed.). McGraw-Hill, New York, USA.

Nienhaus F, Schuiling M, Gliem G, Schinzer D, Spittel A . 1982. Investigations on the etiology of the lethal disease of coconut palms in Tanzania. Journal of Plant Diseases and Protection 89:185–193.

Oropeza CM, Escamilla JA, Mora G, Zizumbo D, Harrison N. 2005. Coconut lethal yellowing. In: Batugal P, Rao R, Oliver J, editors. Status of Coconut Genetic Resources. IPGRI-APO, Serdang, Malaysia, pp. 349–363.

Pérez-Núñez MT, Chan JL, Saenz L, González T, Verdeil JL, Oropeza C. 2006. Improved somatic embryogenesis from coconut (*Cocos nucifera* L.) plumule explants cultivated *in vitro*. In Vitro Cellular and Developmental Biology – Plant 42:37–43.

Piña-Quijano PE. 1994. Estudio poblacional de *Myndus crudus* Van Duzee (Homopthera: Cixiidae) vector del amarillamiento letal del cocotero en el Estado de Yucatán. Bachelor Dissertation. Universidad Autónoma de Yucatán, Mérida, Yucatán, México.

Plavsic-Banjac B, Hunt P, Marasmorosh K. 1972. Mycoplasma-like bodies associated with yellowing disease of coconut palms. Phytopathology 62:298–299.

Reinert JA. 1977. Field biology and control of *Haplaxius crudus* on St. Agustine grass and Christmas palm. Journal of Economic Entomology 70:54–56.

Rochat D, Gonzalez VA, Mariau D, Villanuava A, Zagatti P. 1993. Evidence for male produce aggregation pheromone in American palm weevil *Rhynchophorus palmarum* Coleoptera, Curculionidae. Journal of Chemical Ecology 17:1221–1230.

Sangare A, De Taffin G, De Franqueville H, Arkhurst ED, Pomier M. 1992. Coconut lethal yellowing in Ghana: Initial results on planting material performance in the field. Oléagineux 47:699–704.

SAS. 1992. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute, Cary, NC, USA.

Shuiling M, Mpunami A, Kaiza DA, Harries HC. 1992. Lethal disease of the coconut palm in Tanzania: III. Low resistance of imported germplasm. Oléagineux 47:693–697.

Soil Survey Staff. 1999. Soil Taxonomy (2nd ed.). NRCS–USDA Agricultural Handbook 436. U.S. Government Printing Office, Pittsburgh, USA.

Zar JH. 1984. Biostatistical Analysis (2nd ed.). Prentice Hall, Englewood Cliffs, New Jersey, USA.

Zizumbo-Villarreal D. 1996. History of coconut in Mexico. Genetic Resources and Crop Evolution 43:505–515.

Zizumbo-Villarreal D, Colunga-GarcíaMarín P. 2001. Morpho-physiological variation and phenotypic plasticity in Mexican populations of coconut (*Cocos nucifera* L). Genetic Resources and Crop Evolution 48:547–554.

Zizumbo-Villarreal D, Hernández F, Harries HC. 1993. Coconut varieties in Mexico. Economic Botany 47:65–78.

Zizumbo-Villarreal D, Fernández-Barrera MA, Torres-Hernández N, Cardeña-López R. 1999. Lethal yellowing resistance germplasm from Mexico. In: Oropeza C, Verdeil JL, Ashburner GR, Cardeña R, Santamaría JM, editors. Current Advances in Coconut Biotechnology. Kluwer Academic Publishers. Dordrecht, The Netherlands, pp. 131–144.

Zizumbo-Villarreal D, Fernández-Barrera MA, Torres-Hernández N, Colunga-GarcíaMarín P. 2005. Morphological variation of fruit in Mexican populations of *Cocos nucifera* L. (Arecaceae) under *in situ* and *ex situ* conditions. Genetic Resources and Crop Evolution 52:419–432.

Zizumbo-Villarreal D, Ruiz-Rodríguez M, Harries HC, Colunga-GarcíaMarín P. 2006. Population genetics, lethal yellowing disease and relationships among Mexican and imported coconut ecotypes. Crop Science 46:2509–2516.

Ecogeographic genetic erosion, seed systems and conservation of plant genetic resources in Kabale highlands, Uganda

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Summary

Ecogeographic genetic erosion, seed systems and conservation of plant genetic resources in Kabale highlands, Uganda

The study examined the extent and underlying causes of genetic erosion, identified farmers' seed sources and exchange systems and documented conservation practices in the Kabale highlands of south-western Uganda. Data were collected using pre-tested structured questionnaires from a random sample of 120 farmers from six parishes. The analysis revealed substantial loss of traditional varieties of sweet potatoes (*Ipomea batatas*), potatoes (*Solanum tuberosum*), beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*). More than 18, 7, 9, and 3 varieties, respectively were reported to have been lost completely. The most frequently mentioned underlying cause of genetic erosion (cited by 93.7% of the farmers) was introduction of new varieties. Other causes were lack of market (68.8%), diseases (45.6%), shortage of land (41.9%), pests (33.4%), shortage of labour (23.5%), change in weather (19.6%) and loss of soil fertility (15.8%). Farmers relied mainly on their own seed for traditional varieties (81.5% of farmers), while nearly half of farmers obtained seed of new or modern varieties by cash purchase from the market. The exchange of seeds of modern varieties between the farmers was common practice. This has resulted in rapid and wide spread of modern varieties and has contributed to the abandonment of the traditional cultivars. Farmers maintained field stocks of vegetatively propagated crop species. For seed-propagated crops farmers mainly stored dried seeds in gunny sacks in their houses. Very few farmers employed traditional seed storage methods. The loss of landraces is a threat to national food security. Without adequate reservoirs of diverse genetic resources, future genetic improvement programmes will be jeopardized. There is therefore an urgent need to collect, document, conserve and utilize the traditional varieties and formulate policies that will protect them from further genetic erosion. Farmers and policy-makers should be sensitized on the value of maintaining crop genetic diversity.

Key words: Genetic erosion, plant genetic resources, Uganda.

Résumé

Érosion génétique écogéographique, gestion des semences et conservation des ressources phytogénétiques dans les hautes terres de Kabale, Ouganda

L'étude concerne l'importance et les causes de l'érosion génétique, les sources et les systèmes d'échange de semences parmi les agriculteurs ainsi que les pratiques de conservation dans les hautes terres de Kabale, au sud-ouest de l'Ouganda. Les données ont été collectées en utilisant un questionnaire structuré pré-testé à partir d'un échantillon aléatoire de 120 agriculteurs de six paroisses. L'analyse révèle une perte sensible de variétés traditionnelles de patate douce (*Ipomea batatas*), pomme de terre (*Solanum tuberosum*), haricot (*Phaseolus vulgaris*) et pois (*Pisum sativum*). Plus de 18, 7, 9 et 3 variétés respectivement sont définitivement perdues. La cause de la perte d'érosion génétique la plus fréquemment mentionnée (citée par 93,7 % des agriculteurs) est l'introduction de nouvelles variétés. D'autres causes sont l'absence de débouchés (68,8 %), les maladies (45,6 %), le manque de surfaces cultivables (41,9 %), les ravageurs (33,4%), le manque de main d'œuvre (23,5 %), le changement des conditions météorologiques (19,6 %) et la perte de fertilité du sol (15,8 %). Les agriculteurs utilisent principalement leurs propres semences pour les variétés traditionnelles (81,5 % des agriculteurs), tandis que près de la moitié d'entre eux achètent des semences de variétés nouvelles ou modernes sur le marché. L'échange de semences de variétés modernes entre agriculteurs est une pratique courante, ce qui a entraîné une extension rapide des variétés modernes et contribué à l'abandon des cultivars traditionnels. Les agriculteurs conservent des stocks en champs de plantes multipliées par voie végétative. Pour les plantes propagées sous forme de graines, les agriculteurs conservent principalement des graines séchées chez eux dans des sacs de jute. Très peu d'agriculteurs utilisent des méthodes traditionnelles de stockage de graines. La perte de variétés locales constitue une menace pour la sécurité alimentaire nationale. L'absence de systèmes appropriés de conservation de la diversité des ressources phytogénétiques menace les futurs programmes d'amélioration génétique. Il est donc urgent de collecter, documenter, conserver et utiliser les variétés traditionnelles et d'élaborer des politiques qui les protègent contre la poursuite de l'érosion génétique. Les agriculteurs et les décideurs doivent être sensibilisés à l'importance de la conservation de la diversité génétique des ressources agricoles.

Resumen

Erosión genética ecogeográfica, sistema de semillas y conservación de recursos fitogenéticos en Kabale Highlands, Uganda

Se examinó la amplitud y causas de la erosión genética, fuentes y sistemas de intercambio de semillas de los agricultores y prácticas documentadas de conservación en Kabale Highlands, Uganda sudoccidental. Los datos se recogieron en cuestionarios ya comprobados sobre una muestra aleatoria de 120 agricultores de seis parroquias. El análisis reveló una pérdida sustancial de variedades tradicionales de batatas (*Ipomea batatas*), papas (*Solanum tuberosum*), guisantes (*Phaseolus vulgaris*) y frijoles (*Pisum sativum*). Se han perdido más de 18, 7, 9 y 3 variedades respectivamente. La causa de erosión genética mencionada con más frecuencia fue la introducción de nuevas variedades (93,7 % de los agricultores). Otras fueron falta de mercados (68,8%) enfermedades (45,6%), escasez de tierras (41,9%), plagas (33,4%), escasez de jornaleros (23,5%), cambios climáticos (19,6%) y pérdida de fertilidad del suelo (15,8%). Para las variedades tradicionales los agricultores confiaban en sus propias semillas (81,5%), mientras que la mitad compraba de contado semillas de variedades nuevas. El intercambio de semillas de variedades modernas, práctica común entre los agricultores, provocó una rápida y amplia difusión de variedades modernas a expensas de los cultivares tradicionales. Los agricultores mantenían en el campo reservas de especies de propagación vegetativa. Para las propagadas por semilla conservaban en sus hogares semillas secas en sacos de arpillera. Pocos agricultores emplean métodos tradicionales de almacenamiento. La pérdida de variedades locales es una amenaza para la seguridad alimentaria nacional. Es urgente recoger, documentar, conservar y utilizar variedades tradicionales y formular políticas que las protejan de una ulterior erosión genética.

Introduction

Plant genetic resources include all plant species that contribute to peoples' livelihoods by providing food, medicine, shelter, fibre, and energy (Mooney 1997; Evenson et al. 1998; Hammer et al. 1999), whether cultivated or found in natural habitats as wild plants or relatives of crops. They are crucial to attempts to feed and sustain the steadily increasing global population (Arunachalam 1999). The need to maintain genetic diversity in agricultural systems to ensure that genetic resources continue to be available to support food security is widely accepted (FAO 1999).

The Kabale highlands of south-western Uganda are part of an intensively cultivated ecoregion with unique, diverse plant genetic resources (Crawley 2000; Africare 2001). These genetic resources are a reservoir of genetic adaptability that acts as a buffer against harmful environmental changes and economic challenges (Hammer et al. 1999; FAO 1999). These resources are now seriously threatened by genetic erosion (Jean-Mark 1999; Crawley 2000; Africare 2001).

Few national programmes have given high priority to quantification of genetic erosion, as apparent from the paucity of information in FAO (1997). However, the accurate documentation of genetic erosion of major agricultural crops is important both scientifically and socio-economically (FAO 1995; Smale 1996; Swanson 1996; Karp et al. 1997).

The objectives of this study were to i) determine the extent and underlying causes of genetic erosion of important food crops in south-western Uganda, ii) document farmers' seed sources and exchange systems and iii) to identify and document conservation practices.

Description of the study area

The study was conducted in Kabale District between June and December 2003. The District lies in the south-west of the Republic of Uganda between latitudes 1°S and 1°30'S and longitudes 29°18'E and 30°9'E. Altitude ranges from 1400 to 2500 metres above sea level. The District is divided into three counties (Rukiga, Ndorwa and Rubanda) and one municipality. It borders with Kisoro District in the west, Rukungiri District in the north, Ntungamo District in the east and the Republic of Rwanda in the south. It has a montane-type climate with a bimodal rainfall pattern, the short rains in March–May and the long rains in September–November. The mean annual rainfall is 1200 mm and mean annual temperature is 18°C. The relative humidity ranges between 90% and 100% in the mornings and between 50% and 60% throughout the year.

The average land area for agriculture is 2.06 hectares per household. The District is mountainous and has undulating hills with steep convex slopes of 10–60° and gentle slopes of 5–10° nearer the swampy valleys formerly occupied by papyrus swamps (Lindblade et al. 1996). The main soil types are volcanic, ferralitic and peat. Ferralitic soils are the most widespread type. Volcanic soils are found mainly in Muko subcounty. Peat soils are mainly found in papyrus swamps and produce the rich organic soils for agriculture; they dominate the valleys of Kabale District.

Important crops grown include sorghum (*Sorghum bicolor* L.), beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), potatoes (*Solanum tuberosum*), sweet potatoes (*Ipomea batatas*) and bananas and plantains (*Musa* spp.).

Materials and methods

Data were collected using pre-tested structured questionnaires (Appendix I) from a random sample of 120 farmers (20 farmers in each of the six parishes) (Table 1) and analyzed using SPSS for descriptive statistics. Percentages and cross tabulation techniques were used to examine the associations between factors investigated.

Results and discussion

Genetic erosion

Genetic erosion in bananas and plantains (*Musa* spp.)

There were no reports of banana and plantain cultivars being completely abandoned over the past 10–20 years. However, some cultivars were abandoned by some farmers but were still grown by others. For example, 'Gonja', 'Bogoya', 'Mabeere'/'Kitika', 'entundu' and 'engoote' were reported as still being grown by 7.7%, 20.4%, 33.3%, 19.5% and 29.9% of farmers while 46.4%, 31.7%, 24.3%, 19.6% and 17.4% of farmers reported abandoning them. The main reason given for abandoning cultivars was lack of a market. These cultivars were replaced with cooking types.

Genetic erosion in sorghum (*Sorghum bicolor* L.)

No sorghum variety was reported as being lost completely. However, some varieties are in danger of disappearing, mainly because of poor culinary quality and poor yields. These varieties include 'Mabeere' and 'Magune', which are still grown by 8.7% and 3.4%, respectively, of farmers interviewed but have been dropped by 43.8% and 72.5% of farmers.

Genetic erosion in peas (*Pisum sativum* L.)

Three pea varieties were reported as having been abandoned by all the farmers interviewed; varieties 'Kyambia', 'Rwantooro' and 'Nyakasaza' were lost completely and could not be traced from other farmers. A further two varieties, 'Misere' and

Table 1. Location of sample farmers, Uganda.

County/subcounty	Rukiga/Bukinda		Ndorwa/Buhara		Rubanda/Muko	
Parish	Nyakisiru	Karorwa	Bugarama	Rwene	Ikamiro	Butare

Table 2. Underlying causes of genetic erosion in south-western Uganda.

Cause	% of farmers interviewed
Introduction of new varieties	93.7
Lack of market	68.8
Diseases	45.6
Shortage of land	41.9
Pests	33.4
Shortage of labour	23.5
Change in weather	19.6
Loss of soil fertility	15.8
Others	10.6

'Amaharare', are still grown by a few farmers (7.0 and 2.4%, respectively) but were on the verge of extinction. This was mainly due to poor culinary quality and lack of market.

Genetic erosion in beans *(Phaseolus vulgaris* L.*)*

Nine bean varieties were reported as having been abandoned by all farmers interviewed: varieties 'Ntemeroruhanga', 'Kanyamunyu', 'Makara', 'Mugyerahansi', 'Kesharingwa', 'Kyinganente', 'Mirankwongyere', 'Murundi' and 'Kiribyonyami' were lost completely and could not be traced. A further five varieties were still grown by few farmers but are facing extinction: 'Ruhendamagari' (13.0%), 'Mwonyogwembeba' (8.5%), 'Kijunde' (5.6%), 'Rusavinyanza' (4.1%) and 'kikoti' (9.9%).

Genetic erosion in potatoes *(Solanum tuberosum* L.*)*

Seven potato varieties were reported as having been abandoned by all farmers interviewed: varieties 'Kashari', 'Magojo', 'Joseline', 'Ruranda', 'Kakwirwa', 'Rushwiga' and 'Kaposho' were lost completely and could not be traced. Five varieties were still grown by a few farmers but were at risk of extinction: 'Malierahinda' (5.8%), 'Katikamwe' (10.6%), 'Kisoro' (8.4%), 'Cruza' (44.4%) and 'Kabeera' (4.5%).

Genetic erosion in sweet potatoes *(Ipomea batatas* L.*)*

A lot of genetic erosion had occurred in sweet potatoes although there is still high diversity. Eighteen varieties were reported as having been abandoned: varieties 'Nyirasasi', 'Kakoba', 'Kikoyo', 'Magabari', 'Katere', 'Nshenhsera', 'Kashusha', 'Kahungyenzi', 'Kyitekamaju', 'Nkyiriza', 'Mukobwa', 'Nkijamundegye', 'Kyitambira', 'Kifefe', 'Nyinabusegyenyi', 'Kataikome', 'Nderera' and 'Ruranda' were lost completely and could not be traced from other farmers. A further eight varieties were still grown by some farmers but were on the verge of extinction: 'Kanyansi' (5.6%), 'Mulera' (6.7%), 'Sengamugabo' (5.3%), 'Rwampala' (4.8%), 'Ntegakatebe' (18.1%), 'Norah' (3.0%), 'Kyebandira' (45.5%) and 'Magumba' (3.5%).

Farmers' perceptions on causes of genetic erosion

The most frequently reported cause of genetic erosion, mentioned by 93.7% of farmers, was the introduction of new or modern varieties (Table 2). This is similar to findings reported by Zimmerer (1992), Charles and Weiss (1999), Kiambi (1998) and FAO (1999). Due to the superior qualities of modern varieties (especially higher yields and higher prices), farmers are increasingly replacing traditional varieties with modern varieties in many fields. The second most frequently cited cause of genetic erosion was lack of market (68.8%). Most of the farmers interviewed rely solely on agriculture for their entire livelihood and therefore give high priority to varieties that are in high demand and neglect or drop those with low demand. Diseases (45.6%) and pests (33.4%) were other major factors contributing to genetic erosion. The crop varieties that used to be resistant to most of the important diseases and pests in the District have lost resistance, resulting in decline in yield. Arunachalam (1999) also reported that natural disasters such as floods, droughts, diseases and pests contribute to genetic erosion.

Shortage of land (41.9%) and labour (23.5%) were other factors mentioned by farmers. As a result of high population pressure in the District, there is shortage of land and farmers have to utilize their meagre land holdings for the most productive and high-priced crop varieties. Lower-yielding varieties, which in most cases are traditional, are abandoned. The high rate of urban migration, especially among younger people, has reduced the labour force, resulting in the abandonment of crop varieties requiring high amounts of labour. This has also been reported by Charles and Weiss (1999) and Zimmerer (1992).

Changing climatic conditions have resulted in the loss of adaptation of some formerly high-yielding crop varieties, forcing farmers to shift to new, better-adapted varieties. The change in climate has also led to the introduction of new diseases and pests. A decline in soil fertility as a result of frequent cultivation of the land without furrowing was evident and some varieties have therefore been abandoned due to low productivity.

Other causes of genetic erosion (10.6%) included varieties dropped due to poor culinary qualities, undesirable seed colour (especially beans which were blue or black), seed size (small ones mostly dropped) and height (tall plants mostly dropped due to lodging).

Seed systems

Farmers' seed sources

The study revealed that farmers obtained seed from both informal and formal sources. This is in agreement with findings of others, including Pray and Ramasawmi (1991), Cromwell et al. (1992), Worede (1992), and Cromwell et al. (1993). Traditional varieties were mainly obtained through informal channels (especially own stock) while modern varieties were obtained from formal sources (Table 3). Informal seed supply is practised by farmers themselves in conservation and exchange of their landraces while the formal channel is used by non-governmental and research organizations to supply seed of modern varieties.

Table 3. Farmers' seed sources in south-western Uganda.

Seed source	Traditional varieties (%)	Modern varieties (%)
Own stock	81.5	31.3
Cash purchase from market	11.1	50.8
Cash purchase from shops	7.6	32.8
Cash purchase from other farmers	7.4	29.5
Exchange for other seeds	10.3	23.6
Free from other farmers	5.6	40.9
NGOs/CBOs/national/ international programmes	2.1	36.4

Table 4. Farmers' seed exchange systems in south-western Uganda.

	Traditional varieties (%)	Modern varieties (%)
Seeds given out to:		
None	41.7	3.1
One farmer	17.2	5.4
Two farmers	23.1	9.2
Three farmers	6.3	20.7
Four farmers	7.5	36.2
> four farmers	4.2	25.4
Seeds received from:		
None	46.1	5.3
One farmer	21.1	10.1
Two farmers	15.4	8.1
Three farmers	8.3	39.5
Four farmers	6.3	14.6
> four farmers	2.6	22.4

Seed exchange

As shown in Table 4, there was little exchange of seed of traditional varieties among the farmers in the District. In contrast, the exchange of seed of modern varieties among the farmers was more prevalent. This has resulted in a growing spread of the modern varieties, which has contributed to putting traditional varieties at risk of extinction. Widespread exchange of seed of new bean varieties among farmers in Kabale has also been reported by Mugisa-Mutetikka (1997), while David and Sperling (1997) reported similar behaviour elsewhere in eastern and central Africa.

Conservation practices

Farmers maintained field stocks of vegetatively propagated crop species such as bananas/plantains, sweet potatoes, pineapples (*Ananus comosus*), sugar-cane (*Saccharum officinarum*) and fruit trees. Potato seed tubers are kept in the dark, either on dry ground or raised platforms. For crop species propagated by seeds most farmers dried and stored seeds in their houses in gunny bags. Almost all farmers had abandoned traditional seed storage methods mainly because of theft of seed stocks.

When seeds are to be stored for long periods, they are treated with chemicals, especially Marathion, to control weevils. Some farmers were using wood ash but it was less effective.

Conclusions and recommendations

This study revealed that many traditional varieties or landraces have been lost and replaced with modern varieties. The most important cause of this erosion is the frequent introductions of modern varieties, which are now grown on a much larger area than are traditional varieties. There was also a lot of exchange of seed of modern varieties among the farmers, putting the traditional varieties at risk of extinction. The loss of these genetic resources is a threat to national food security. This results in reduction of the genetic base of the remaining varieties. Without adequate reservoirs of diverse genetic resources, future genetic improvement programmes will be jeopardized. There is therefore an urgent need to collect, document, conserve and utilize the traditional varieties and to formulate policies that will protect them from further genetic erosion. Farmers and policy-makers should be sensitized on the value of maintaining crop genetic diversity.

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References

Africare. 2001. Agroforestry development activities in Kabale district. A case of Africare's Uganda food security initiative (UFSI). Unpublished report. Africare, Washington DC, USA.

Arunachalam V. 1999. Conservation, genetic erosion and early warning system: key issues. In: Serwinski J, Faberova I, editors. Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, held at the Research Institute of Crop Production, Prague, Czech Republic, 21–23 June 1999. IPGRI, Rome, Italy.

Charles FH, Weiss E. 1999. Remote sensing contribution to an early warning system for genetic erosion of agricultural crops. In: Serwinski J, Faberova I, editors. Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, held at the Research Institute of Crop Production, Prague, Czech Republic, 21–23 June 1999. IPGRI, Rome, Italy.

Cromwell E, Friis-Hansen E, Turner M. 1992. The Seed Sector in Developing Countries: A Framework for Performance Analysis. ODI, London, UK.

Cromwell E, Wiggins S, Wentzel S. 1993. Sowing Beyond the State: NGOs and Seed Supply in Developing Countries. ODI, London, UK.

David S, Sperling L. 1997. Improving technology delivery mechanisms: Lessons from bean seed systems research in eastern and central Africa. African Crop Science Conference Proceedings 3:1341–1347.

Evenson RE, Gollin D, Santaniello G, editors. 1998. Agricultural values of plant genetic resources. CAB International, Wallingford, UK.

FAO. 1999. Early Warning System on Plant Genetic Resources. FAO, Rome, Italy.

FAO. 1997. The State of the World's Plant Genetic Resources for Food and Agriculture 1997. FAO, Rome, Italy.

FAO. 1995. Chapter 1: State of diversity. In: The State of the World's Plant Genetic Resources 1995. FAO, Rome, Italy.

Hammer K, Diederichsen A, Spahillari M. 1999. Basic studies toward strategies for conservation of plant genetic resources. In: Serwinski J, Faberova I, editors. Proceedings of the Technical Meeting on the Methodology of the FAO World Information and Early Warning System on Plant Genetic Resources, held at the Research Institute of Crop Production, Prague, Czech Republic, 21–23 June 1999. IPGRI, Rome, Italy.

Jean-Mark F. 1999. Mountain biodiversity at risk. Threats to knowledge from high places. IDRC Briefing No. 2. IDRC, Ottawa, Canada.

Karp A, Edwards KJ, Bruford M, Funk S, Vosman B, Morgante M, Seberg O, Kremer A, Boursot P, Arctander P, Tautz D, Hewitt GM. 1997. Molecular technologies for biodiversity evaluation: opportunities and challenges. Nature Biotechnology 15:625–628.

Kiambi D. 1998. Status of diversity, genetic erosion and indigenous knowledge of rice (*Oryza sativa*) in Tana River Delta, Kenya. Technical report. IPGRI, Rome, Italy.

Lindblade K, Tumuhairwe JK, Caswell G, Nkwiine C, Bwamiki D. 1996. More People, More Fallow: The Myth of Overcultivation in Kabale District, Uganda. Unpublished project report prepared for Rockefeller Foundation and CARE International, pp. 7–18, 29–56.

Crawley M. 2000. Improving Highland Farming in Africa. IDRC Bulletin. Available at: http://www.idrc.ca/en/ev-5337-201-1-DO_TOPIC.html.

Mooney PR. 1997. The Parts of Life: Agriculture Biodiversity, Indigenous Knowledge, and the Role of the Third System. Development Dialogue 1996 (1–2).

Mugisa-Mutetikka G. 1997. Factors influencing adoption and dissemination of new bean cultivars in Uganda. African Crop Science Conference Proceedings 3:1349–1355.

Pray CE, Ramaswami B. 1991. A Framework for Seed Policy Analysis. IFPRI, Washington DC, USA.

Smale M. 1996. Indicators of genetic diversity in bread wheats: selected evidence on cultivars grown in developing countries. Paper presented at a symposium, 'The Economics of Valuation and Conservation of Genetic Resources for Agriculture', Tor Vergata University, Rome, May 1996.

Swanson T. 1996. Global values of biological diversity: the public interest in the conservation of plant genetic resources for agriculture. Plant Genetic Resources Newsletter 105:1–7.

Worede M. 1992. A genebank working with farmers. In: Cooper D, Renee V, Hobbelink H, editors. Growing Diversity: Genetic Resources and Local Food Security. Intermediate Technology Publications, London, UK.

Zimmerer KS. 1992. The loss and maintenance of native crops in mountain agriculture. Geojournal 27(1):61–72.

Appendix I. Questionnaire on ecogeographic genetic erosion, seed systems and conservation of plant genetic resources in Kabale highlands, Uganda

1. Location

County…………………………. Sub-county…………………… Parish………………………….. Village………………………..

Altitude……………. Longitude…………….. Latitude……………… Date of interview……………………………

2. Identification of person interviewed

Name…………………………………………………….

Sex	Family size	Length of residence
a) Male	a) 1–5	a) 1–10
b) Female	b) 6–10	b) 11–20
	c) >10	c) 21–30
		d) >30 years
Age	**Marital status**	**Education level**
a) 20–30	a) Married	a) None
b) 31–40	b) Single	b) Primary
c) 41–50	c) Widowed	c) Secondary
d) 51–60	d) Separated	d) Tertiary
e) >60 years		
Ethnic group		**Land tenure**
a) Bakiga		a) Customary
b) Bafumbira		b) Mailo
c) Banyarwanda		c) Lease
d) Banyankole		d) Freehold

3. On-farm diversity

Do you grow crops on your farm? If yes, which ones?

Crop	Variety

4. In the last 10–20 years, the number of local varieties has:

In your farm	In your village
a) Increased	a) Increased
b) Decreased	b) Decreased
c) Stayed the same	c) Stayed the same

5. If the number has decreased, which local varieties/cultivars did you drop in the last few years?

Which?	When did it happen?	Why did it happen?

6. What do you think are the causes of genetic erosion?

a) Shortage of land b) Change in weather

c) Diseases d) Lack of market

e) Pests f) Introduction of new varieties

g) Loss of soil fertility h) Shortage of labour

i) Others (specify)

7. What are your sources for:

Traditional seeds?	Modern seeds?
a) Cash purchase from market	a) Cash purchase from market
b) Cash purchase from shops	b) Cash purchase from shops
c) Cash purchase from other farmers	c) Cash purchase from other farmers
d) Own stock	d) Own stock
e) Free from other farmers	e) Free from other farmers
f) Exchange for other seeds	f) Exchange for other seeds
g) NGOs/National/International programmes	g) NGOs/National/International programmes

8. How many farmers did you give seeds to during the past year?

Traditional seeds?	**Modern seeds?**
a) None	a) None
b) 1	b) 1
c) 2	c) 2
d) 3	d) 3
e) 4	e) 4
f) >4	f) >4

9. How many farmers did you receive seeds from during the past year?

Traditional seeds?	**Modern seeds?**
a) None	a) None
b) 1	b) 1
c) 2	c) 2
d) 3	d) 3
e) 4	e) 4
f) >4	f) >4

10. What is the impact of introduction of modern varieties on traditional ones?

11. Do you think it is possible to trace the lost varieties?

12. Does it matter to you that these varieties are lost?

13. How do you conserve your seeds for next planting?

Crop	Conservation method

Thank you very much for your information and cooperation.

Agricultural biodiversity in Grecìa and Bovesìa, the two Griko-speaking areas in Italy

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Summary

Agricultural biodiversity in Grecìa and Bovesìa, the two Griko-speaking areas in Italy

In September 2007 a collecting mission was carried out in Grecìa and Bovesìa, the two Griko-speaking areas in Italy. The primary objectives of the mission were to collect samples of local plant genetic resources together with data (e.g. ethnobotany, genetic and phenotypic variation, degree of genetic erosion) useful to breeders and genebank curators. A total of 293 accessions were collected from 57 sites, mainly cereals, pulses and vegetables. Uncommon landraces and relic crops were found, including 'mugnoli' (a botanical form of *Brassica oleracea* L. var. *italica* Plenck), 'meloncella' (a typical cucumber melon traditionally cultivated for its unripe fruits) and the very rare 'lenticchia nera' (*Vicia articulata* Hornem.). The results indicate strong genetic erosion of autochthonous landraces and the urgent need of their protection both *in situ* and *ex situ*.

Key words: Agricultural biodiversity, genetic erosion, collecting, linguistic enclaves, Grecìa, Bovesìa.

Résumé

Agro-biodiversité dans les deux régions italiennes où est parlé le griko : la Grèce salentine et Bovesia

En septembre 2007, une mission de collecte a été entreprise dans les deux régions italiennes où est parlé le griko (ou grico) : la Grèce salentine et Bovesia. La mission avait pour principaux objectifs de collecter des échantillons de ressources phytogénétiques locales ainsi que des données relatives notamment à l'ethnobotanique, à la variation génétique et phénotypique et au degré d'érosion génétique, utiles pour les sélectionneurs et les curateurs de banques de gènes. Un total de 293 accessions a été collecté dans 57 sites, principalement des céréales, des légumineuses et des légumes. Des variétés locales peu communes et des ressources agricoles reliques ont été trouvées, dont le « *mugnoli* », forme botanique de *Brassica oleracea* L. var. *Italica* Plenck, et le très rare « *lenticchia nera* » (*Vicia articulata* Hornem.). Les résultats révèlent une forte érosion génétique de variétés locales indigènes et souligne l'urgence de leur protection *in situ* et *ex situ*.

Resumen

Biodiversidad agrícola en Grecia y Bovesìa, las dos zonas de habla griega de Italia

En septiembre de 2007 se llevó a cabo una misión de recolección en Grecia y Bovesìa, las dos zonas de habla griega de Italia. Los objetivos primarios de la misión fueron recoger muestras de recursos genéticos de plantas locales junto con datos (por ejemplo etnobotánicos, genéticos y de variación fenotípica, grado de erosión genética) útiles para los fitomejoradores y encargados de bancos de genes. Se recogieron 293 accesiones de 57 sitios, sobre todo de cereales, legumbres y hortalizas. Se encontraron variedades locales no comunes y cultivos "reliquia", entre ellos "mugnoli" (una forma botánica de *Brassica oleracea* L. var. *italica* Plenck), "meloncella" (un pepino típico cultivado tradicionalmente por sus frutos sin madurar) y la muy rara "lenticchia nera" (*Vicia articulata* Hornem). Los resultados indican una fuerte erosión genética de las variedades locales autóctonas y la urgente necesidad de protegerlas tanto *in situ* como *ex situ*.

Introduction

Since 1996, scientists from the Plant Genetics Institute (IGV) of the National Research Council (CNR) of Bari (Italy) and the Crop Science Institute of Kassel University (Germany) have been studying the conservation and use of agrobiodiversity in Italian linguistic enclaves (Hammer et al. 1992; Laghetti et al. 1998; Hammer et al. 1999; Laghetti et al. 2003; Hammer et al. 2007a, 2007b, 2007c; Hammer-Spahillari et al. 2007; Laghetti et al. 2007a, 2007b; Miceli et al. 2007).

The results of these investigations pointed to a strong genetic erosion of autochthonous landraces and the urgent need of their protection both *in situ* and *ex situ*.

In September 2007 a new collecting mission was organized in Grecìa (Apulia region) and Bovesìa (Calabria region), the two Griko-speaking areas in Italy. The most important objectives of this mission were to collect samples of autochthonous plant genetic resources together with data (e.g. ethnobotany, genetic and phenotypic variation, degree of genetic erosion) useful to breeders and genebank curators.

The collecting and sampling strategies followed were the same as those used during previous similar missions (Hammer et al. 1991; Perrino et al. 1981).

Results and discussion

A total of 293 accessions were sampled from 57 sites. The detailed list of the material collected is given in Table 1.

Grecìa

'Grecìa' or 'Grecìa Salentina' (Salentinian Greece) is a Griko-speaking area in the peninsula of Salento in the Apulia region (southern Italy) (Figure 1). It consists of nine small towns: Calimera (population 7296), Martano (9573), Castrignano dei Greci (4107), Corigliano d'Otranto (5632), Melpignano (2209), Soleto (5537), Sternatia (2698), Zollino (2194) and Martignano (1770).

Figure 1. Location of Grecìa and Bovesìa in southern Italy.

Tremendous genetic erosion is evident since the 1980 collection mission of Perrino et al. (1981). The 2007 exploration and collecting mission included interviews, meetings and discussions with local public administrators, historians, agronomists, country people and city people. These indicated that in Salento the Greek tradition and language are quickly losing importance, especially among young people. This, together with the declining importance of agriculture and the emigration of people from Grecìa area, is eroding local cultural traditions.

The Grecìa's agriculture is characterized by a widespread cultivation of grapes, olives (e.g. the olive varieties 'Cellina di Nardò' and the almost extinct 'Butirra' introduced by ancient Greeks in Salento) and almond, and a lesser presence of rainfed herbaceous crops grown on winter rains. In irrigated areas vegetable and flower crops are more widespread (Marzi and Tedone 2007).

A total of 125 accessions were collected in 24 sites covering the most important and typical agricultural areas of Grecìa (Table 1).

Cereal production is less economically important than arboriculture. Durum wheat is the most important crop but minor cereals, such as oat and barley, are also grown. Common millet (*Panicum miliaceum* L.) and foxtail millet (*Setaria italica* (L.) P. Beauv.) have been grown since Roman times in this area (mainly at Melpignano) but now they are only rare relic crops and none were collected. Two samples of wild relatives of foxtail millet were found: *Setaria viridis* (L.) P. Beauv. and *S. glauca* (L.) P. Beauv. (Table 1).

Today pulses are very sporadically cultivated in Grecìa because, as in other Italian areas, it is cheaper to import them. However, they are still grown on small farms, mainly for the local market or for family consumption. Broad bean (*Vicia faba* L.) is the main pulse cultivated and five samples of local varieties were found. Seven landraces of pea (*Pisum sativum* L., 'alaco' in Griko) were also sampled. These included 'pisieddhu lundrejo' or 'svelto', characterized by very early flowering, and the famous dwarf type, 'pisello nano di Zollino'. Outside Grecìa four populations of the typical 'pisello riccio di Sannicola' pea were collected. This is an ancient 'curly pea' adapted to the very poor soils along the coast of west Salento. Nine samples of cowpea (*Vigna unguiculata* (L.) Walp.), known locally as 'pasuja metammai' (in Griko) or 'pasuli piccinni' (in Salentinian dialect), were collected, showing a wide variation of seed morphology. Some of these populations belong to *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc. Two landraces of common bean (*Phaseolus vulgaris* L.) were found, one of which was an old dwarf type from Cutrofiano used as dry seeds and called 'pasuli napoletani'. In the same area of Cutrofiano and in the surroundings of Soleto two samples of chickpea were collected; these are very similar to the black variety typical of Muro Leccese (outside Grecìa territory) that was collected in the Salento area together with seven other chickpea accessions. Four landraces of the 'tolica' grass pea (*Lathyrus sativus* L.) were also collected outside the Grecìa area.

Vegetables are economically very important in the irrigated agricultural areas of Grecìa. The most important intensive crops are artichoke, tomato and off-season potato (e.g. the 'patata novella Sieglinde' from Galatina, which is also exported to Germany). Other valuable vegetables include pepper, watermelon, melon and eggplant in the spring–summer season, often grown under tunnels; and green pea, Italian turnip broccoli (*Brassica rapa* L. subsp. *oleifera* (DC.) Metzg./*Brassica ruvo* Bailey; see Gladis and Hammer [1992]), salad crops and chicory during the winter season.

Eight landraces of tomato were gathered in the Grecìa area and seven outside (e.g. the well-known 'pomodoro di Morciano'; see Accogli et al. 2007a). A very-old-type tomato with yellow fruit (the trait of the first tomatoes introduced in Italy after Columbus) was found at Martano; it is named 'pummidora scimona' (in Griko) or 'pomodoro da serbo giallo' ('yellow tomato for storage'). Another characteristic landrace, 'pomodoro azekàlogeri' (in Griko) or 'pomodoro d'estate' ('summer tomato') was collected near Corigliano d'Otranto, while the 'pomodoro d'inverno' ('winter tomato') landrace was found at Soleto.

Among the other Solanaceae observed during the mission was an old type of eggplant that is particularly recommended for use in the famous local recipe 'Marangiana te Santu Ronzu', which was found at Martano. This was notable because today only modern cultivars are grown in the area.

The area of Salento (Grecìa included) is famous for cucumber melon 'meloncella' or 'menuceddha' (*Cucumis melo* L. subsp. *melo* convar. *adzhur* (Pang.) Grebenšč. var.

Table 1. Number of accessions collected in Salento (seven towns of Grecìa and other areas) and Bovesìa, 2007.

Species	Grecìa								Other areas (Salento)	Bovesìa	Grand total
	Martano	Zollino	Corigliano	Soleto	Cutrofiano	Sternatia	Melpignano	Total			
Aegilops geniculata										1	1
Avena sativa				1				1			1
Hordeum vulgare				1				1			1
Setaria viridis	1							1			1
Setaria glauca	1							1			1
Sorghum sp.									1		1
Zea mays										2	2
Cereals total	**2**	**–**	**–**	**2**	**–**	**–**	**–**	**4**	**1**	**3**	**8**
Cicer arietinum				1	1			2	8		10
Lathyrus sativus									4		4
Lens culinaris				1				1			1
Lupinus albus						1		1			1
Lupinus sp.										1	1
Phaseolus vulgaris				1	1			2	5	17	24
Phaseolus coccineus										2	2
Pisum sativum	2	1		2	1	1		7	4		11
Pisum elatius										1	1
Vicia articulata										1	1
Vicia faba	1	1		1	1	1		5	1	2	8
Vicia sp.										2	2
Vigna unguiculata	3		1		5			9	4	2	15
Pulses total	**6**	**2**	**1**	**6**	**9**	**3**	**–**	**27**	**26**	**28**	**81**
Allium cepa	1		1		1			3	1	1	5
Allium sp.										1	1
Beta vulgaris					4			4	1		5
Brassica oleracea	2			2	2			6	6	3	15
Brassica rapa	1			1	4		1	7	13	1	21
Brassica tournefortii										2	2
Brassica sp.										2	2
Capsicum annum					1			1	2	1	4
Capsicum sp.		1			1			2			2
Cichorium endivia					1			1	1		2
Cichorium intybus	1		1		6			8	7	1	16
Cichorium sp.				1	1			2	2		4
Citrullus lanatus										1	1
Cucumis melo	3	1	4	4	7			19	13		32
Cucumis sp.									1		1
Cucurbita moschata		1			2			3			3
Cucurbita pepo	1		1	2	2			6	4	2	12
Cucurbita sp.				1	1		1	3	4	5	12
Cynara cardunculus var. *sylvestris*										1	1
Daucus carota									2		2
Eruca sativa					1			1			1
Foeniculum vulgare			1		2			3	4		7

Table 1. (cont.) Number of accessions collected in Salento (seven towns of Grecìa and other areas) and Bovesìa, 2007.

Species	Grecìa								Other areas (Salento)	Bovesìa	Grand total
	Martano	Zollino	Corigliano	Soleto	Cutrofiano	Sternatia	Melpignano	Total			
Lactuca sativa			1		2			3	1	1	5
Lactuca serriola										2	2
Lactuca sp.									1		1
Lagenaria siceraria										1	1
Lycopersicon esculentum	1	1	1	1	3		1	8	7	5	20
Mentha sp.	1							1			1
Ocimum basilicum		1			2			3			3
Petroselinum crispum					1			1		1	2
Sinapis alba					1			1		1	2
Sinapis sp.									2		2
Solanum melongena	1							1		1	2
Spinacia oleracea					1			1	1		2
Vegetables total	**12**	**5**	**10**	**12**	**46**	**–**	**3**	**88**	**73**	**33**	**194**
Cardiospermum halicacabum										1	1
Gossypium hirsutum			1					1			1
Hedysarum coronarium				1				1		1	2
Linum usitatissimum									1		1
Luffa cylindrica									1		1
Nicotiana tabacum		1			1			2			2
Smyrnium olusatrum			1					1			1
Trifolium incarnatum				1				1			1
Other species total	**–**	**1**	**2**	**2**	**1**	**–**	**–**	**6**	**2**	**2**	**10**
Total	**20**	**8**	**13**	**22**	**56**	**3**	**3**	**125**	**102**	**66**	**293**

chate (Hasselq.) Filov). Traditionally the unripe fruits of this crop are eaten raw, as salad, with starters. Twelve samples of this crop were collected. Seven typical local varieties of melon (*C. melo*) were also collected, including the old 'minna de moneca' ('nun's breast'), 'melone bianco d'inverno' ('poponia' in Griko) and 'malone zuccarino' ('sweet melon'), a characteristic type with long and sweet-smelling fruits. Unfortunately no examples were found of the old landraces of watermelon (*Citrullus lanatus* (Thunb.) Matsumura et Nakai) formerly cultivated in this area and characterized by yellow flesh, and these may now be extinct.

Twelve populations of 'mugnoli' (also called 'cavuli pezzienti' or 'pezzentieddhi'), were gathered, six in Grecìa. This is a very typical race of *Brassica oleracea* L. traditionally grown in Salento, where the young inflorescences together with a few leaves are harvested for use as cooking vegetable (Accogli and Marchiori 2007). According to Laghetti et al. (2005), 'mugnoli' might be considered as an early step in the evolution of broccoli (*B. oleracea* L. var. *italica* Plenck).

Even if many modern cultivars of salad crops are grown in Grecìa it was still possible to find landraces. Three lettuce landraces (e.g. the 'lattuga giganta paesana d'inverno' named 'marudia' in Griko, usually sown in October), one of rocket (*Eruca sativa* Mill.) and three of fennel (*Foeniculum vulgare* Mill. subsp. *vulgare*, 'málafro' in Griko) were collected.

A rare seed sample of cultivated Alexander's or horse parsley (*Smyrnium olusatrum* L.) was found on a farm near Corigliano d'Otranto. This plant, well known and widely used in the Roman era, was commonly eaten in Salento until it was replaced by celery (*Apium graveolens* L.) (Palombi 2005). In the past local people both cultivated this plant and collected it from the wild, referring to it as 'zavìrna', 'ngirle', 'nzirnia' or 'svernì' ('svernìa' in Griko, 'smirnio' in Italian). Its shoots, roots, seeds, young leaves and inflorescences were used in a number of local recipes both raw and cooked (Maglie 1999). This plant was also used as a medicinal herb for its diuretic and depurative properties (Accogli et al. 2006).

Another member of the Umbelliferae traditionally cultivated in Salento is the carrot landrace 'Pastinaca de Santu Pati' or 'carota giallo-viola di Tiggiano' or 'carota di Sant'Ippazio', an uncommon long yellow–violet carrot

(*Daucus carota* L.). Two samples of this crop found in the Tiggiano–Tricase district, but its cultivation is almost unknown in other zones. It is sown in July and is ready during the winter (Accogli et al. 2007b). It is traditionally sold on 19 January (St Ippazio's Day) in the local market of Tiggiano, where the crop has a long-standing importance in rituals for protecting the male genitals, whose protector is St Ippazio. This root is much appreciated for its sweet, fresh and delicate taste and also for its softness.

Tobacco (*Nicotiana tabacum* L.) was until some years ago very widespread in Salento. However, it has fallen out of favour in Italy in recent years and is being replaced by alternative crops (Maglio 2007). A few plants of the cultivars 'Erzegovina' and 'Virginia bright' were found on two farms and were sampled. These cultivars were cultivated till 3–5 years ago, but today have disappeared.

An old population of flax (*Linum usitatissimum* L.) was also gathered in Salento but outside Grecìa; this is another traditional crop of south Italy already grown since classical times but today very rare (Hammer et al. 1986).

Bovesìa

Bovesia, otherwise known as 'Area Grecanica' or 'Grecìa Calabra' ('Calabrian Greece'), is one of the two remaining Griko-speaking areas in southern Italy. It is part of the cultural and linguistic heritage of the *Magna Graecia* (8th century BC) and the later Byzantine Empire. The area is located at the tip of Calabria, near Reggio Calabria city (Figure 1), and consists of eleven villages: Bagaladi, Bova, Bova Marina, Brancaleone, Condofuri (with Condofuri Marina and Gallicianò), Melito di Porto Salvo (with Pentedattilo), Palizzi (with Palizzi Marina), Roccaforte del Greco, Roghudi, San Lorenzo and Staiti. This linguistic enclave (in all ca. 460 km^2) is mainly located along the valleys of Amendolea, Siderone and San Pasquale rivers, in an inaccessible mountainous area. As a result the area has maintained much of its original culture until recently. The communities in the coastal areas of Bovesìa have lost more of their Greek roots than those in the mountains. In light of this, the collecting mission focused on the inner montane area.

Today, only about 2000 people speak and understand 'Grecanico' ('Greek language of Calabria'), which is also known as 'Calabrian-Greek dialect' or 'Greek-Bovesian'. Of these, about 50 are less than 35 years old. The language was spoken throughout the whole of south Calabria until the 15th to 16th century, when it was gradually replaced by a Romance dialect (Calabrian).

Germplasm collecting missions were conducted in Bovesia in 1984 (Perrino and Hammer 1985) and 1986 (Hammer et al. 1987). The authors highlighted the impact on agriculture of large-scale emigration from rural areas of Calabria, especially its south-eastern part, where Bovesia is located. In particular, they noted extensive genetic erosion especially in mountain areas, affecting primarily cereals; the effect on vegetable genetic resources was less, possibly because vegetables were often cultivated in the small gardens of the farmers (Hammer et al. 1987).

The current collecting mission collected 66 accessions in 17 agriculture sites in Bovesìa (Table 1).

This region has never been known for widespread cereal cultivation, and only two landraces of maize (called 'panniculu') were collected (Table 1).

In the 18th century broad beans, peas, grass peas, lentils and lupins were abundantly cultivated in the area of Bova while common beans and chickpeas were absent (Alagna and Tuscano 2005). In contrast, only 28 samples of autochthonous pulses were gathered during this collecting mission, and more than half of these were of common bean. Strong genetic erosion was observed for all local pulses; only one population of the so called 'lenticchia nera' ('black lentil', *Vicia articulata* Hornem.) was found. Samples of this crop were last collected in 1950 in neighbouring parts of Sicily (Hammer et al. 1992, p. 8) and in 1998 in Sardinia (Laghetti et al. 1999).

A total of 33 accessions of vegetables were found and sampled mainly from family gardens, including a wild garlic probably escaped from cultivation close to the abandoned town of Roghudi, six cultivated *Brassica* species that are yet to be classified (locally named 'làcana'), two wild populations of *B. tournefortii* Gouan formerly cultivated as an oil plant (Alam 1945) gathered in the uninhabited village of Pentedattilo, and one accession of wild artichoke (*Cynara cardunculus* L. var. *sylvestris* Lam.). Among the five local 'tomate' (*Lycopersicon esculentum* Mill.) accessions collected was one type with fruit weighing more than 1.5 kg/fruit.

Other areas

A total of 92 accessions of various plants were collected in 16 sites of Salento outside the Grecìa area, mainly in the area of Tricase (Table 1).

Conclusions

Crop genetic resources can survive longer in linguistic enclaves than elsewhere, but they are still threatened by the loss of the importance of traditional crops and the decline of culture and language that are the foundations of the enclaves.

The south of Italy is still a centre of diversity for several crops but modern cultivars are progressively replacing its landraces. Another threat facing traditional crops is their exploitation by seed companies in other areas. For example, some seed firms from northern Italy are selling seeds of cucumber melon, or 'meloncella', called 'Cetriolo Carosello Spuredda Leccese'. However, according to our information this variety of meloncella is selected and multiplied in central–north Italy and does not perform well in southern Italy, where the crop originates. For this reason the genebank of IGV at Bari holds a representative collection of cumber melon germplasm from Apulia and is implementing a strategy for its characterization and safeguarding.

Further collecting and activities to safeguard germplasm of local crops is necessary and urgent.

Availability of germplasm

The material collected has been deposited in the genebank of Bari for further classification and characterization. After their multiplication, the accessions collected will be available for distribution to the scientists.

Further data and details about this collecting mission are reported in the exploration registers at IGV.

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References

Accogli R, Marchiori S. 2007. Verifica agronomica di *Brassica oleracea* L. var. *botrytis* subvar. *cimosa*. Distribuzione e consumo nel Salento. In: Maglio D, editor. Le colture alternative al tabacco nel Salento (Puglia) e nelle province campane di Benevento e Salerno. Risultati finali del Progetto Co.Al.Ta. Tipografia Cooperate, Santa Severa, Rome, Italy, pp. 431–435.

Accogli R, Lattanzi E, Medagli P, Di Pinto G. 2006. Piante spontanee alimentari e aromatiche nella tradizione popolare del Salento e della Ciociarìa. Editrice Frusinate s.r.l., Frosinone, Italy, 84 pp.

Accogli R, Lombardi D, Marchiori S. 2007a. Reperimento di germoplasma e verifica agronomica di cultivar locali di pomodoro attribuibili alla varietà 'pomodoro di Morciano'. In: Maglio D, editor. Le colture alternative al tabacco nel Salento (Puglia) e nelle province campane di Benevento e Salerno. Risultati finali del Progetto Co.Al.Ta. Tipografia Cooperate, Santa Severa, Rome, Italy, pp. 447–457.

Accogli R, Negri C, Tommasi L, De Bellis L, Miceli A, Marchiori S. 2007b. Conservazione e valorizzazione di *Daucus carota* L. varietà locale 'pistanaca de Santu Pati': verifica agronomica e caratterizzazione molecolare. In: Maglio D, editor. Le colture alternative al tabacco nel Salento (Puglia) e nelle province campane di Benevento e Salerno. Risultati finali del Progetto Co.Al.Ta. Tipografia Cooperate, Santa Severa, Rome, Italy, pp. 437–446.

Alam Z. 1945. Nomenclature of oleiferous brassicas cultivated in Punjab. Indian Journal of Agricultural Science 15(4):173–181.

Alagna D, Tuscano F. 2005. Bova – Città nel Regno di Napoli nella Calabria Ulteriore (1775). Nuove Edizioni Barbaro di Caterina Di Pietro, Delianuova (RC), Italy, 283 pp.

De Lucia M. 1988. Il ruolo della coltivazione e della manifattura del cotone in Terra d'Otranto nel secolo diciannovesimo. Piero Lacaita Editore, Manduria, Italy, 35 pp.

Gladis Th, Hammer K, 1992. Die Gaterslebener *Brassica*-Kollektion – *Brassica juncea*, *B. napus*, *B. nigra* und *B. rapa*. Feddes Repertorium 103:469–507.

Hammer K, Cifarelli S, Perrino P. 1986. Collection of landraces of cultivated plants in south Italy, 1985. Kulturpflanze 34:261–273.

Hammer K, Perrino P, Pignone D. 1987. Collection of plant genetic resources in south Italy, 1986. Kulturpflanze 35:389–399.

Hammer K, Laghetti G, Cifarelli S, Perrino P. 1991. Collecting in north-eastern Italy using the indicator-crop method. Plant Genetic Resources Newsletter 86:39–40.

Hammer K, Knüpffer H, Laghetti G, Perrino P. 1992. Seeds from the past. A catalogue of crop germplasm in South Italy and Sicily. Istituto del Germoplasma, CNR, Bari, Italy, II + 173 pp.

Hammer K, Knüpffer H, Laghetti G, Perrino P. 1999. Seeds from the past. A catalogue of crop germplasm in Central and North Italy. Istituto del Germoplasma, CNR, Bari, Italy, IV + 254 pp.

Hammer K, Cifarelli S, Laghetti G. 2007a. Linguistic islands and plant genetic resources – the case of German-speaking villages in northern Italy. In: Hauptvogel P, Benediková D, Hauptvogel R, editors. Plant Genetic Resources and their Exploitation in the Plant Breeding for Food and Agriculture. Book of Abstracts of the 18th Eucarpia Genetic Resources Section Meeting, Piešt'any, Slovak Republic, 23–26 May 2007. SARC – Research Institute of Plant Production Piešt'any, Piešt'any, Slovak Republic, 32 pp.

Hammer K, Miceli F, Cifarelli S, Laghetti G. 2007b. Sauris (Zahre), a cultural and linguistic island in Carnia (Italy). In: Bullitta S, editor. Plant Genetic Resources of Geographical and Other Islands. (Conservation, Evaluation and Use for Plant Breeding). Proceedings of the XVII Eucarpia Genetic Resources Section Meeting, Castelsardo, Italy, 30 March–2 April 2005. CNR-ISPAAM, Sassari, Italy, pp. 185–192.

Hammer K, Laghetti G, Pignone D, Psarra E, Samaras S. 2007c. Safeguarding agro-biodiversity of three Ionian Greek islands: Lefkada, Ithaca and Kefalonia. International Journal of Island Affairs 16(1):24–36.

Hammer-Spahillari, M, Laghetti G, Cifarelli S, Hammer K. 2007. Ethnographic islands: the case of the Arbëreshë. In: Bullitta S, editor. Plant Genetic Resources of Geographical and Other Islands. (Conservation, Evaluation and Use for Plant Breeding). Proceedings of the XVII Eucarpia Genetic Resources Section Meeting, Castelsardo, Italy, 30 March–2 April 2005. CNR-ISPAAM, Sassari, Italy, pp. 173–183.

Laghetti G, Xhuveli L, Perrino P, Olita G, Hammer K. 1998. Collecting crop genetic resources in Italian towns of Albanian origin: Basilicata region. Plant Genetic Resources Newsletter 114:29–34.

Laghetti G, Perrino P, Cifarelli S, Bullitta S, Hammer K. 1999. Collecting crop genetic resources in Sardinia, Italy and its islands, 1998. Plant Genetic Resources Newsletter 120:30–36.

Laghetti G, Perrino P, Cifarelli S, Bullitta S, Hammer K. 2003. Collecting plant genetic resources in Italy, 2001. Plant Genetic Resources Newsletter 136:23–30.

Laghetti G, Martignano F, Falco V, Cifarelli S, Gladis Th, Hammer K. 2005. "Mugnoli": a neglected race of *Brassica oleracea* L. from Salento (Italy). Genetic Resources and Crop Evolution 52(5):635–639.

Laghetti G, Miceli F, Cifarelli S, Hammer K. 2007a. Collecting crop genetic resources in Italy, 2004. Plant Genetic Resources Newsletter 152:82–87.

Laghetti G, Pignone D, Cifarelli S, Hammer K, Skoula M. 2007b. Collecting agro-biodiversity on the island of Crete (Greece). International Journal of Island Affairs 16(1): 6–18.

Maglie L. 1999. Piante spontanee commestibili nella tradizione del Capo di Leuca. Editrice Salentina, Galatina, Italy, 143 pp.

Maglio D, editor. 2007. Le colture alternative al tabacco nel Salento (Puglia) e nelle province campane di Benevento e Salerno. Risultati finali del Progetto Co.Al.Ta. Tipografia Cooperate, Santa Severa, Rome, Italy, 769 pp.

Marzi V, Tedone L. 2007. La Puglia: il Salento. In: Maglio D, editor. Le colture alternative al tabacco nel Salento (Puglia) e nelle province campane di Benevento e Salerno. Risultati finali del Progetto Co.Al.Ta. Tipografia Cooperate, Santa Severa, Rome, Italy, pp. 93–127.

Miceli F, Dalla Costa L, Laghetti G, Hammer K. 2007. Resia: A deep-rooted Slavic Community in the Italian Alps and its Garlic Landrace. In: Bullitta S, editor. Plant Genetic Resources of Geographical and Other Islands. (Conservation, Evaluation and Use for Plant Breeding). Proceedings of the XVII Eucarpia Genetic Resources Section Meeting, Castelsardo, Italy, 30 March–2 April 2005. CNR-ISPAAM, Sassari, Italy, pp. 257–259.

Palombi D, 2005. Erbario Salentino. Erbe e piante medicinali del territorio di Martano e zone limitrofe. Edizioni del Grifo, Lecce, Italy, 527 pp.

Perrino P, Hammer K. 1985. Collection of land-races of cultivated plants in South Italy, 1984. Kulturpflanze 33:225–236.

Perrino P, Hammer K, Hanelt P. 1981. Report of travels to South Italy 1980 for the collection of indigenous material of cultivated plants. Kulturpflanze 29:433–442.

Characterization of total salt soluble seed storage proteins of grain legumes using SDS-PAGE

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Summary

Characterization of total salt soluble seed storage proteins of grain legumes using SDS-PAGE

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to characterize the total salt soluble seed storage proteins of 149 different accessions of nine grain legumes, viz. *Cajanus cajan*, *Vigna radiata*, *Vigna mungo*, *Vigna unguiculata*, *Phaseolus vulgaris*, *Pisum sativum*, *Cicer arietinum*, *Lens culinaris* and *Glycine max*. Each of the species showed a distinct banding pattern. Based on banding pattern, *Vigna radiata* showed the highest intraspecific variation, followed by *Vigna unguiculata*, *Pisum sativum* and *Phaseolus vulgaris*. Variations among accessions of *Cajanus cajan*, *Vigna mungo*, *Cicer arietinum*, *Lens culinaris* and *Glycine max* were not significant. Further protein bands was scored for presence or absence and genetic distance was calculated using the UPGMA procedure and a dendrogram was constructed. The dendrogram resulted in three distinct clusters representing three different tribes as revealed by conventional classification based on morphological characters.

Key words: Characterization, SDS-PAGE, seed storage proteins, grain legumes, phylogeny.

Résumé

Caractérisation par SDS-PAGE de protéines de réserve totales de graines de légumineuses solubles en présence de sels

L'électrophorèse sur gel de polyacrylamide – dodécylsulfate de sodium (SDS-PAGE) a été utilisée pour caractériser les protéines de réserve totales solubles en présence de sels dans des graines de149 accessions différentes appartenant à neuf légumineuses : *Cajanus cajan*, *Vigna radiata*, *Vigna mungo*, *Vigna unguiculata*, *Phaseolus vulgaris*, *Pisum sativum*, *Cicer arietinum*, *Lens culinaris* et *Glycine max.* Chacune des espèces présente un profil de bandes distinct. Sur la base de ces profils, *Vigna radiata* présente la variation intraspécifique la plus élevée, suivie par *Vigna unguiculata*, *Pisum sativum* et *Phaseolus vulgaris.* Les variations parmi les accessions de *Cajanus cajan*, *Vigna mungo*, *Cicer arietinum*, *Lens culinaris* et *Glycine max* ne sont pas significatives. En outre, la présence ou l'absence des bandes protéiques a été consignée et la distance génétique a été déterminée en utilisant la méthode UPGMA et un dendrogramme a été construit. Le dendrogramme fait apparaître trois groupes distincts représentant trois tribus différentes, identifiées par la classification conventionnelle basée sur des caractères morphologiques.

Resumen

Caracterización del almacenamiento de proteínas de semillas de gramíneas en solución salina total empleando SDS-PAGE

Se empleó electroforesis en sodio dodecilsulfato-poliacrilamida (SDS-PAGE, sigla en inglés) para caracterizar el almacenamiento de proteínas de semillas de gramíneas en solución salina total, obtenidas de 149 accesiones diferentes de nueve gramíneas: *Cajanus cajan*, *Vigna radiata*, *Vigna mungo*, *Vigna unguiculata*, *Phaseolus vulgaris*, *Pisum sativum*, *Cicer arietinum*, *Lens culinaris* y *Glycine max*. Cada una de las especies mostró una pauta de bandeo distintiva. Sobre la base de la pauta de bandeo, *Vigna radiata* presentó la mayor variación intraespecífica, seguida de *Vigna unguiculata*, *Pisum sativum* y *Phaseolus vulgaris.* Las variaciones entre accesiones de *Cajanus cajan*, *Vigna mungo*, *Cicer arietinum*, *Lens culinaris* y *Glycine max* no eran significativas. Se clasificaron ulteriormente las bandas de proteínas respecto de su presencia o ausencia, y se calculó la distancia genética empleando el procedimiento UPGMA (método de grupos de pares no ponderado con media aritmética, sigla en inglés) y se elaboró un dendrograma que produjo tres conglomerados distintos que representaban tres tribus diferentes, como ya lo revelaba la clasificación convencional basada en los caracteres morfológicos.

Introduction

With ca. 20 000 species the legumes are the third largest family of higher plants. Grain legumes provide about one-third of all dietary protein nitrogen and one-third of processed vegetable oil for human consumption (Graham and Vance 2003). Seeds of grain legumes contain at least 20% to 40% of protein. In many parts of the world legumes complement cereals or root crops, the primary source of carbohydrates, in terms of amino acid composition. While cereal seed proteins are deficient in lysine, legume seed proteins are deficient in S-containing amino acids and tryptophan (Wang et al. 2003). This situation may explain why legumes and cereals have been domesticated together in most centres of crop domestication (Gepts 2004).

Using conventional methods based on morphological traits, the legume family has been divided into three subfamilies: Caesalpinieae, Mimosoideae and Papilionateae. The Papilionateae have been further subdivided into five tribes: Phaseoleae, Vicieae, Cicereae, Aeschynomenae and Genistae (Summerfield and Roberts 1985).

There have been a substantial number of studies that have used sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to profile seed storage proteins in legumes (Ladizinsky 1975, 1979; Ladizinsky and Hymowitz 1979; Ahmad and Slinkard 1992; Fotso et al. 1994; Ahmad et al. 1996; Limongelli et al. 1996; Ahmad 1997; El-Shanshoury 1997; Krochko and Bewley 2000; Valizadeh 2001; Mallick and Sawhney 2002; Asghar et al. 2003). Other studies have investigated isozyme variations (Erskine and Muehlbauer 1991; Labdi et al. 1996; Pasquet 2000).

Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species, generating pertinent information to complement evaluation and passport data (Sammour 1991).

In the present study, total salt soluble protein of 149 accessions representing 9 different species and 3 tribes of grain legumes were resolved on SDS-PAGE. The total number of bands and their respective molecular weights of nine grain legumes were analyzed. Relationship among nine species of grain legumes were analyzed using UPGMA (Unweighted Pair Group Method Using Arithmetic Averaging) cluster analysis.

Material and methods

Plant material

The seeds of 149 accessions representing 9 different species (*Cajanus cajan*, *Vigna radiata*, *Vigna mungo*, *Vigna unguiculata*, *Phaseolus vulgaris*, *Pisum sativum*, *Cicer arietinum*, *Lens culinaris* and *Glycine max*) and 3 tribes of grain legumes were obtained from the Indian Institute of Pulse Research, Kanpur, the Indian Vegetable Research Institute, Varanasi, the Punjab Agricultural University, Ludhiana, G.B. Pant University of Agriculture and Technology, Pantnagar, Annamalai University, Chennai and Narendra Dev Agriculture University, Faizabad (Table 1).

Electrophoretic analysis (SDS-PAGE)

Total salt soluble proteins were extracted by putting 30 mg of ground seeds in 1 ml of 50 mM tris-HCl (pH 7.5) and 0.5 M NaCl and keeping the mixture at 4°C for 60 minutes. The mixture was then frozen at -20°C and thawed three times over 24 hrs to disrupt the tissue and release the proteins. It was finally centrifuged at 10 000 G for 15 minutes (Miller et al. 1972).

Samples were prepared for electrophoresis by mixing 10 µl of extracted protein, 2.5 µl of 2-mercaptoethanol and 7.5 µl of 0.002% bromophenol blue in 0.0625 M tris-HCl (pH 6.8) containing 10% glycerol and 2% SDS (Valizadeh 2001). The SDS-PAGE was carried out according to Laemmli (1970) and protein staining was performed using Coomassie Blue according to Hames and Rickwood (1990). SDS-PAGE was performed with 10–12% separating gel containing

Table 1. List of grain legumes studied using SDS-PAGE for protein profiling.

Sl No.	Common name	Latin binomial	Tribe	Accession	Number of accessions
1	Pigeon pea	*Cajanus cajan*	Phaseoleae	Pusa 9, Bahar, T7, Narendra1, Al201, IPA337, IPA204, IPA61, IPA20, IPA24, IPA3088, IPA341, IPA242, IPA234, IPA98-3, IPA2013, IPA385, UPAS120	18
2	Mung bean	*Vigna radiata*	Phaseoleae	UPM98-1, PDM139, PDM54, MUM2, PUSABOLD2, PM4, EC393407, EC30400, EC393410, ML843, AUGG56, AUGG16, AUGG66, AUGG13, AUGG52, AUGG19, AUGG2005, AUGG15, AUGG12, AUGG14, AUGG18, AUGG57, AUGG65, AUGG17, PDM11, SML668, SML134, PM2	28
3	Cowpea	*Vigna unguiculata*	Phaseoleae	IC9883, IC97704, VRC32, VRC7, PIC246, IC9739, IC2875, VRC11, N106, PG1	10
4	Black gram	*Vigna mungo*	Phaseoleae	AUBG4, AUBG1, AUBG6, AUBG8, AUBG12, IPU2001, IPU94-1, DPU88-31, UL-338, AUBG9, AUBG14, AUBG2, AUBG11, AUBG13, KU96-3, UK17,GREEN SEED 1, KU99, T9, UG218, AUBG3, AUBG7, AUBG5, AUBG15, NPU99-2, GREEN SEED	26
5	Common bean	*Phaseolus vulgaris*	Phaseoleae	IIHR909, MFB2, IIFB1, ARKAKOMAL, IIHR108, IVRFB1, RAJMA DESI, RAJMA KASHMIRI	8
6	Soya bean	*Glycine max*	Phaseoleae	SL 295, VLS21, PS 1092, PS 1241, PS 1042, PS 1024, PK 472, PK 416, PS 1029	9
7	Lentil	*Lens culinaris*	Vicieae	LP12, T36, PL407, PL639, B77, LL147, L4147, NDL1, DPL15, LL699, DPL58, NDL96-12, DPL62, NDL4696	14
8	Pea	*Pisum sativum*	Vicieae	KPMR522, IPFD99-3, SWATI IPF99-25, RACHANA, VRPM9, VRP22, VRP5, VRP6, KPMR400, HUP2, HFP8909, JAYANTI, KFP103, IM9102, DMR7, VRP7, DPL62, NDL4696	18
9	Chickpea	*Cicer arietinum*	Cicereae	IPC98-12, JGK1, C214, JG74, KAK2, BG 212, PG 114, CHANA AVARODHI, JG62, BG396, C235, PANT114, H208, BG1053, PBG5, PBG1, PUSA362, PG186	18

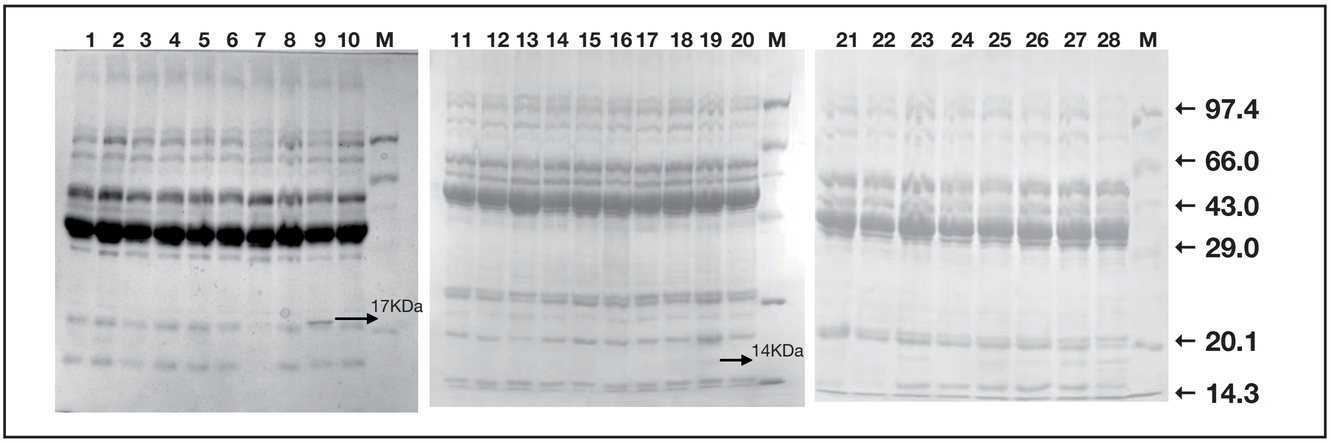

Figure 1. Ten percent SDS-PAGE showing total salt soluble seed storage proteins of different accessions of *Vigna radiata*. Lane 1–28: UPM98-1, PDM139, PDM54, MUM2, PUSABOLD2, PM4, EC393407, EC30400, EC393410, ML843, AUGG56, AUGG16, AUGG66, AUGG13, AUGG52, AUGG19, AUGG2005, AUGG15, AUGG12, AUGG14, AUGG18, AUGG57, AUGG65, AUGG17, PDM11, SML668, SML134, PM2. M: medium-range protein marker.

acrylamide/bis solution (30% total monomer, 2.67% cross-linking monomer), 1.5 M tris-HCl (pH 8.8) and 10% SDS. The stacking gel (4%) contained acrylamide/bis solution (30% total monomer, 2.67% cross-linking monomer), 0.5 M tris-HCl (pH 6.8) and 10% SDS. Ten percent APS and an appropriate amount of TEMED (5–10 µl for 10 ml of monomer solution) were added prior to pouring the gel. The electrode buffer contained 0.025 M tris-HCl (pH 8.3), 0.192 M glycine and 0.1% SDS. A vertical slab gel electrophoresis system (Bangalore Genei, India) was used for SDS-PAGE analysis.

The relative mobility (Rf) value of each protein band was calculated using software of Gel Documentation System (Alpha Imager™, USA). A standard molecular weight marker (medium range) consisting of phosphorylase b (97.4 kDa), bovine serum albumin (66.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (29.0 kDa), soya bean trypsin inhibitor (20.1 kDa), and lysozyme (14.3 kDa) (Bangalore Genei, India) was used for calculating the molecular weights of different protein bands.

Data analysis

The UPGMA method was used for cluster analysis. Jaccard's similarity index (S) was calculated for all possible pairs using the formula S=W/A+B-W where W is the number of bands of common mobility, A and B are the number of bands in the two species. The similarity matrix thus obtained was converted to dissimilarity matrix (1-S) and used to construct a dendrogram by the UPGMA using arithmetic means (Sneath and Sokal 1973). The software used for this analysis was SPSS version 12.0 for Windows.

Results and discussion

No significant variation was seen in protein profiles among accessions of pigeon pea (*Cajanus cajan*), soya bean (*Glycine max*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*), irrespective of the source of germplasm, although variation was observed in the density or sharpness of a few bands. This indicates that, in addition to total seed storage protein, other proteins, such as 11S, 2S globulins, should be used for studying polymorphism within varieties or within populations of these grain legumes.

Intraspecific variation in protein profiles was observed in green gram (*Vigna radiata*), cowpea (*Vigna unguiculata*), pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris*). Substantial protein polymorphism was found in green gram accessions EC393410, with a unique band at 17 kDa, and AUGG12, with a unique band at 14 kDa (Figure 1). In cowpea accessions VRC7 and PG1 bands of molecular weights 50.1, 35.1, 26.6 and 24.3 kDa were absent and in IC9883, IC97704,

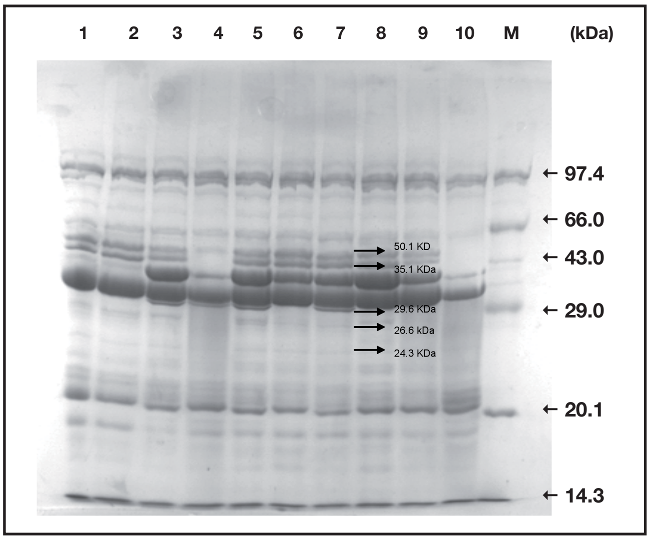

Figure 2. Ten percent SDS-PAGE showing total salt soluble seed storage proteins of different accessions of *Vigna unguiculata*. Lane 1–10: IC9883, IC97704, VRC32, VRC7, PIC246, IC9739, IC2875, VRC11, N106, PG1. M: medium-range protein marker.

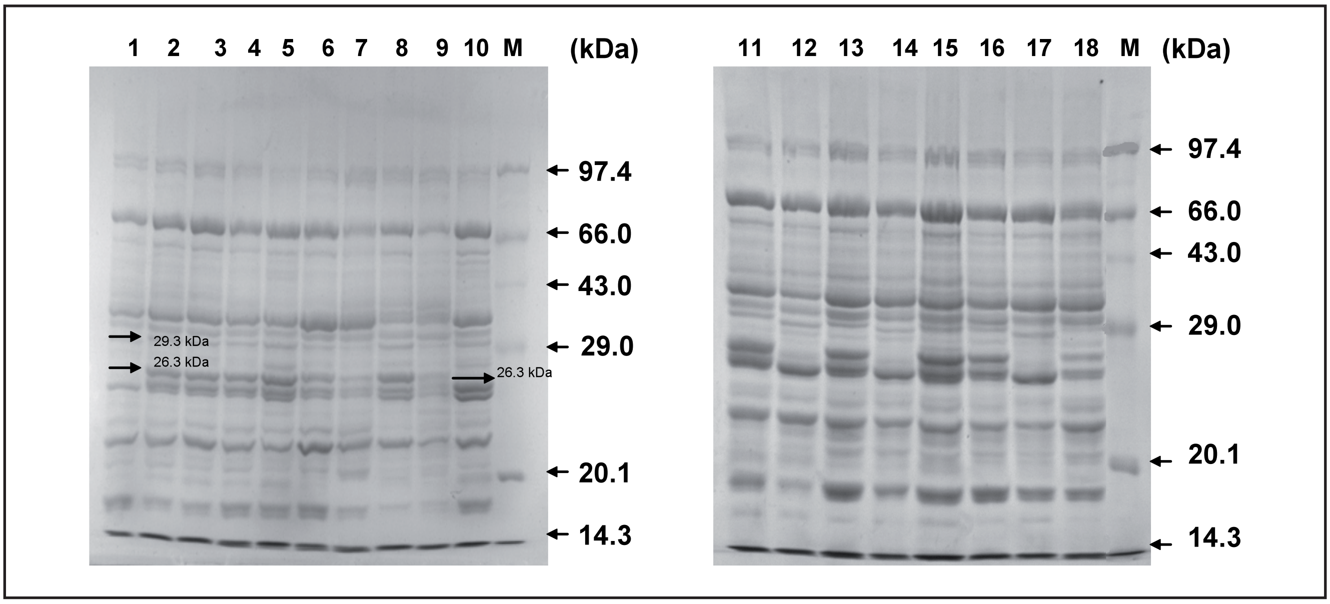

Figure 3. Ten percent SDS-PAGE showing total salt soluble seed storage proteins of different accessions of *Pisum sativum*. Lane 1–18: KPMR522, IPFD99-3, SWATI IPF99-25, RACHANA, VRPM9, VRP22, VRP5, VRP6, KPMR400, HUP2, HFP8909, JAYANTI, KFP103, IM9102, DMR7, VRP7, DPL62, NDL4696. M: medium-range protein marker.

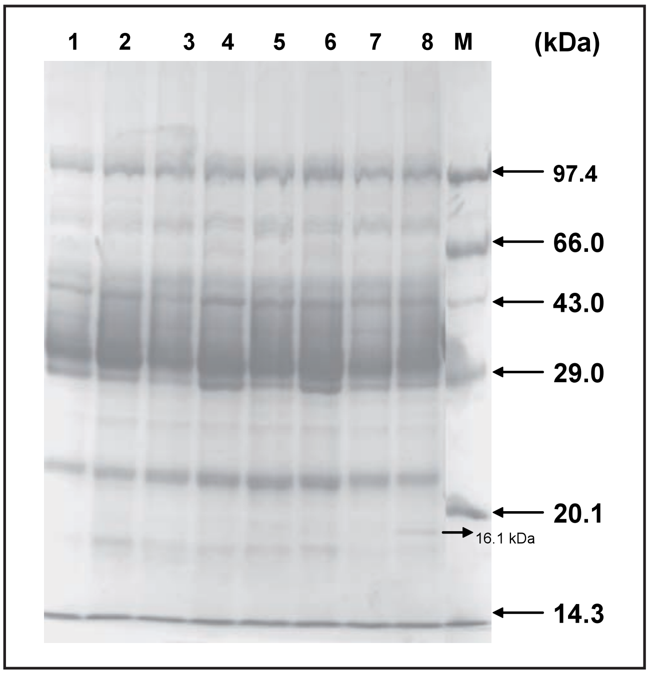

Figure 4. Ten percent SDS-PAGE showing total salt soluble seed storage proteins of different accessions of *Phaseolus vulgaris*. Lane 1–8: IIHR909, MFB2, IIFB1, ARKAKOMAL, IIHR108, IVRFB1, RAJMA DESI, RAJMA KASHMIRI. M: medium-range protein marker.

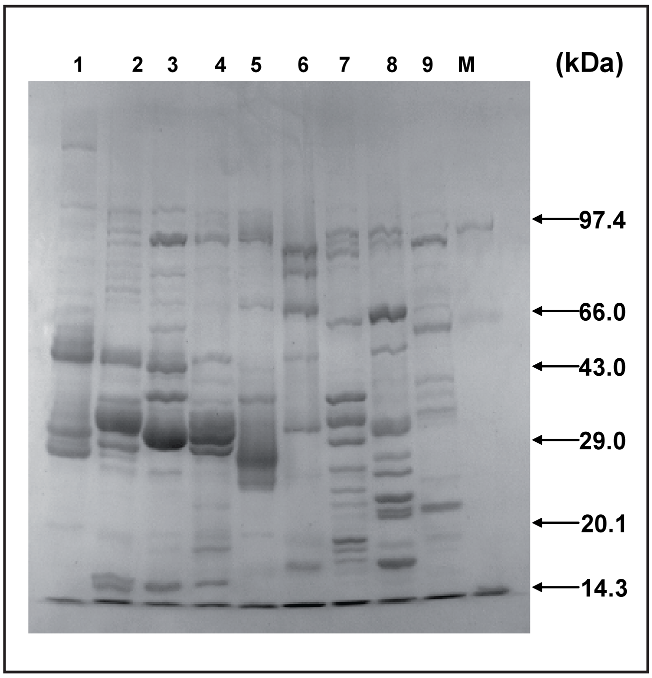

Figure 5. SDS-PAGE of seed storage proteins in nine grain legume species. 1 – *Cajanus cajan*; 2 – *Vigna radiata*; 3 – *Vigna unguiculata*; 4 – *Vigna mungo*; 5 – *Phaseolus vulgaris*; 6 – *Glycine max*; 7 – *Lens culinaris*; 8 – *Pisum sativum*; 9 – *Cicer arietinum*. M: molecular weight marker.

IC9739, VRC11, N106 and PG1 the band of molecular weight 29.6 kDa was absent (Figure 2). In the case of pea accessions KPMR522 and P5 the band of molecular weight 26.3 kDa was absent while the band of 29.3 kDa was also absent in accession KPMR522 (Figure 3). One accession of common bean, 'Rajma Kashmiri', lacked a band of 16.1kDa that was present in other accessions (Figure 4). The total number of bands resolved on SDS-PAGE for each of the nine legume species and their molecular weights are listed in Table 2.

The SDS-PAGE banding patterns from one accession of each species were compared and cluster analysis was performed using the UPGMA method. The banding patterns

Table 2. Characterization of total seed storage proteins of nine grain legumes as resolved on SDS-PAGE: number of bands, molecular weights of bands, Rf values and intensity of bands.

Legume	Total no. of protein bands	Molecular weight of bands (kDa)	Corresponding Rf value	Intensity of bands		
				dense	medium	light
Pigeon pea	15	89.8, 83.9, 78.3, 54.9, 41.2, 38.2, 30.5, 28.9, 26.1, 24.5, 23.5, 21.0, 14.5, 12.9, 11.6.	0.128, 0.156, 0.183, 0.325, 0.440, 0.470, 0.560, 0.582, 0.623, 0.648, 0.664, 0.710, 0.858, 0.904, 0.945,	3	4	8
Mung bean	22	84.8, 78.8, 69.1, 62.9, 53.6, 47.7, 40.3, 38.9, 35.6, 34.6, 29.2, 27.8, 25.8, 24.7, 23.8, 22.3, 21.0, 19.3, 16.5, 14, 12.3, 7.0	0.131, 0.161, 0.215, 0.254, 0.319, 0.367, 0.436, 0.451, 0.487, 0.499, 0.567, 0.588, 0.618, 0.636, 0.651, 0.678, 0.701, 0.737, 0.800, 0.869, 0.922, 0.788	7	5	10
Cowpea	21	89.3, 81.2, 73.3, 66.2, 58.2, 52.9, 50.1, 45.5, 40.0, 37.9, 35.1, 29.6, 26.6, 24.3, 23.5, 21.6, 20.6, 17.9, 16.4, 15.8, 14.6	0.222, 0.257, 0.295, 0.332, 0.380, 0.416, 0.436, 0.471, 0.519, 0.539, 0.567, 0.630, 0.670, 0.703, 0.715, 0.746, 0.763, 0.816, 0.849, 0.861, 0.892	6	7	8
Black gram	23	97.4, 91.7, 84.3, 78.1, 75.2, 70.7, 65.5, 53.3, 49.4, 44.7, 41.4, 38.1, 36.1, 34.8, 33.2, 29.8 26.6, 23.7, 21.5, 19.9, 19.0, 16.8, 14.8	0.203, 0.226, 0.257, 0.286, 0.300, 0.323, 0.351, 0.429, 0.457, 0.494, 0.523, 0.554, 0.574, 0.589, 0.606, 0.646, 0.689, 0.731, 0.769, 0.797, 0.814, 0.860, 0.909	6	5	12
Common bean	22	96.1, 89.2, 76.9, 68.9, 61.4, 52.5, 48.4, 42.3, 34.5, 32.7, 30.5, 28.1, 26.8, 25.9, 20.9, 19.5, 18.5, 16.8, 16.1, 14.8, 13.9, 12.9	0.168, 0.195, 0.248, 0.287, 0.328, 0.384, 0.414, 0.462, 0.535, 0.555, 0.579, 0.608, 0.625, 0.637, 0.715, 0.740, 0.759, 0.793, 0.808, 0.837, 0.861, 0.888.	3	4	15
Soya bean	14	99.5, 88.3, 78.4, 69.1, 51.1, 41.5, 36.6, 33.0, 30.5, 28.1, 16.4, 24.6, 22.5, 13.8	0.178, 0.223, 0.268, 0.316, 0.431, 0.509, 0.557, 0.597, 0.627, 0.657, 0.681, 0.708, 0.741, 0.928.	5	3	6
Lentil	22	85.7, 80.5, 66.3, 60.1, 56.5, 50.7, 45.4, 41.7, 39.7, 36.9, 33.8, 31.1, 28.9, 27.2, 26.2, 23.4, 22.1, 21.0, 20.0, 19.0, 15.7, 15.1	0.277, 0.290, 0.371, 0.406, 0.429, 0.465, 0.509, 0.540, 0.558, 0.585, 0.616, 0.647, 0.674, 0.696, 0.710, 0.750, 0.772, 0.790, 0.808, 0.826, 0.897, 0.911	4	7	11
Pea	23	88.0, 62.7, 56.7, 54.6, 51.7, 50.2, 46.8, 44.0, 40.1, 37.4, 35.2, 31.6, 29.3, 27.3, 26.5, 24.5, 22.7, 20.5, 19.3, 18.3, 17.1, 15.0, 14.2	0.206, 0.335, 0.374, 0.388, 0.409, 0.421, 0.447, 0.471, 0.506, 0.532, 0.556, 0.597, 0.626, 0.653, 0.665, 0.694, 0.724, 0.762, 0.785, 0.806, 0.832, 0.882, 0.903	3	7	13
Chickpea	25	108.3, 98.8, 92.5, 81.2, 75.0, 68.9, 65.0, 60.4, 58.5, 53.0, 48.7, 47.4, 43.8, 41.9, 40.3, 37.5, 34.6, 29.4, 27.3, 25.3, 23.1, 21.5, 20.5, 19.6, 14.9	0.134, 0.169, 0.194, 0.245, 0.275, 0.308, 0.331, 0.359, 0.371, 0.409, 0.442, 0.452, 0.482, 0.500, 0.515, 0.543, 0.573, 0.636, 0.664, 0.694, 0.730, 0.758, 0.775, 0.793, 0.896	7	5	13

were quite distinct from each other and showed variations in terms of number of bands of different molecular weights (Figure 5). Thus, SDS-PAGE profiling of total seed storage proteins can be used for differentiating grain legumes at species level. UPGMA cluster analysis (Figure 6) grouped the nine grain legumes into three distinct groups. The species from the Phaseoleae tribe (pigeon pea, mung bean, cowpea, black gram [*Vigna mungo*], common bean and soya bean) formed one group, while those from the Vicieae tribe (lentil and pea) formed a second. Chickpea, the sole representative of the tribe Cicereae, occupies its own group in the dendrogram. The maximum genetic dissimilarity of 5.29 was observed between lentil and mung bean and cowpea, while the minimum genetic disparity of 2.0 was seen between mung bean and cowpea (Table 3). This is quite evident as cowpea and mung bean belong to Phaseoleae tribe while lentil is member of Vicieae tribe.

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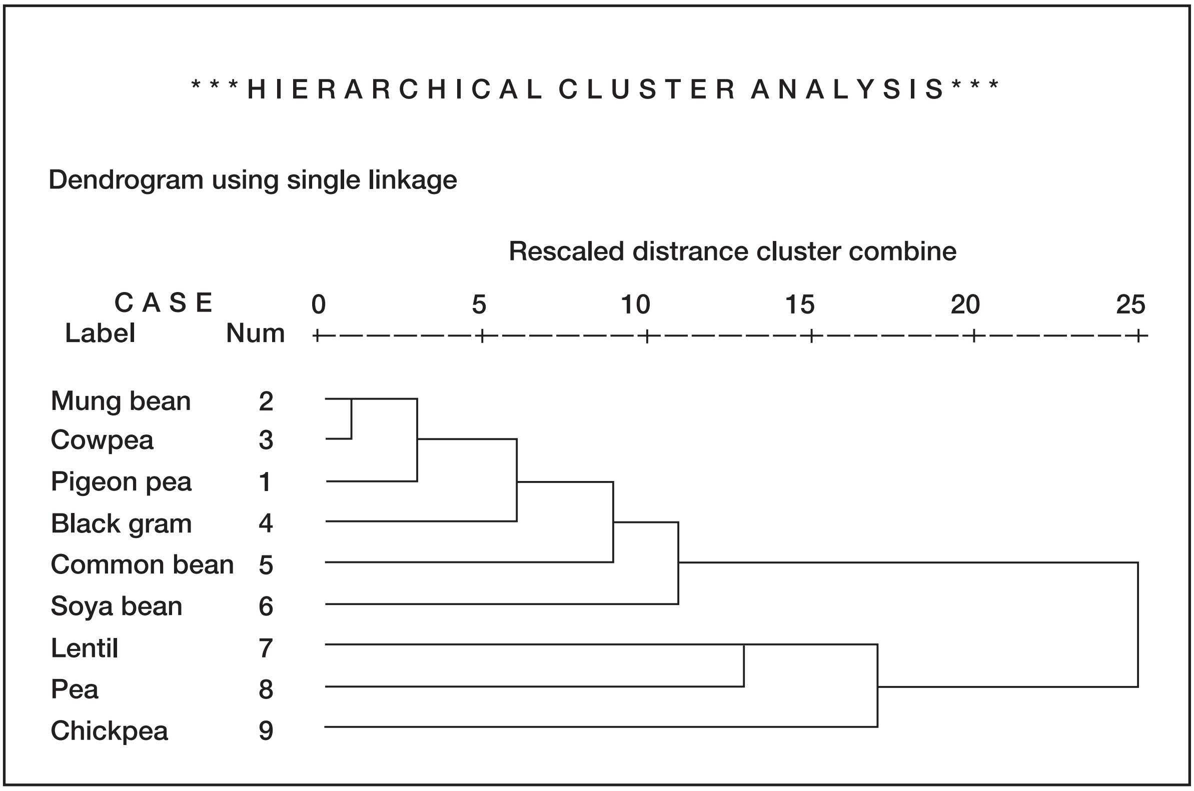

Figure 6. Cluster analysis of nine grain legumes using the UPGMA method.

Table 3. Dissimilarity matrix based on observed seed storage protein banding patterns among all possible pairs of nine grain legumes.

	Pigeon pea	Mung bean	Cowpea	Black gram	Common bean	Soya bean	Lentil	Pea	Chickpea
Pigeon pea	0.000	2.646	2.236	3.317	2.828	3.162	5.196	5.099	4.359
Mung bean		0.000	2.000	2.449	3.000	3.606	5.292	5.196	4.690
Cowpea			0.000	3.162	3.000	3.606	5.292	5.196	4.472
Black gram				0.000	2.646	3.317	4.690	4.796	4.472
Common bean					0.000	2.828	4.796	4.690	4.123
Soya bean						0.000	4.359	4.000	3.873
Lentil							0.000	3.000	4.000
Pea								0.000	3.606
Chickpea									0.000

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References

Ahmad M. 1997. Genetic diversity and relationships in *Lens* species and their F_1 interspecific hybrids as determined by SDS-PAGE. New Zealand Journal of Crop and Horticultural Sciences 25:99–108.

Ahmad F, Slinkard AE. 1992. Genetic relationships in the genus *Cicer* L. as revealed by polyacrlyamide gel electrophoresis of seed storage proteins. Theoretical and Applied Genetics 84:688–692.

Ahmad M, David L, Niel MC. 1996. Comparison of crossability, RAPD, SDS-PAGE and morphological markers for revealing genetic relationships within and among *Lens* species. Theoretical and Applied Genetics 93:788–793.

Asghar R, Siddique T, Afzal M. 2003. Inter and intra-specific variation in SDS-PAGE electrophoregrams of total seed protein in chickpea (*Cicer arietinum* L.) germplasm. Pakistan Journal of Biological Sciences 6(24):1991–1995.

El-Shanshoury AR. 1997. The use of seed proteins revealed by SDS-PAGE in taxonomy and phylogeny of some *Lathyrus* species. Biologia Plantarum 39(4):553–559.

Erskine W, Muelhlbauer FJ. 1991. Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm. Theoretical and Applied Genetics 83:119–125.

Fotso M, Azanza JL, Pasquet R, Raymond J. 1994. Molecular heterogeneity of cowpea (*Vigna unguiculata* Fabaceae) seed storage proteins. Plant Systematics and Evolution 191:39–56.

Gepts P. 2004. Domestication as a long-term selection experiment. Plant Breeding Reviews 24:1–44.

Graham PH, Vance CP. 2003. Legumes importance and constraints to greater use. Plant Physiology 131:872–877.

Hames BO, Rickwood D. 1990. Gel Electrophoresis of Proteins, a Practical Approach (2nd ed.). Oxford University Press, Oxford, UK.

Krochko JE, Bewley JD. 2000. Seed storage proteins in cultivars and subspecies of alfalfa (*Medicago sativa* L.). Seed Science Research 10:423–434.

Labdi M, Robertson LD, Singh KB, Charrier A. 1996. Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism. Euphytica 88:181–188.

Ladizinsky G. 1975. Seed protein electrophoresis of the wild and cultivated species of *Vicia faba*. Euphytica 24:785–788.

Ladizinsky G. 1979. Species relationships in the genus *Lens* as indicated by seed protein electrophoresis. Botanical Gazette 140(4):449–451.

Ladizinsky G, Hymowitz. 1979. Seed protein electrophoresis taxonomic and evolutionary studies. Theoretical and Applied Genetics 54:145– 151.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Limongelli G, Laghetti G, Perrino P, Piergiovanni AR. 1996. Variation of seed storage proteins in landraces of common bean (*Phaseolus vulgaris* L.) from Basilicata, Southern Italy. Euphytica 92:393–399.

Miller MK, Schonhorst MH, McDaniel RG. 1972. Identification of hybrids from alfalfa crosses by electrophoresis of single seed proteins. Crop Science 12:535–537.

Mallick DK, Sawhney S. 2002. Seed protein profile and phylogenetic relationships in the genus *Lens*. Physiology and Molecular Biology of Plants 8(2):279–284.

Pasquet RS. 2000. Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp. Theoretical and Applied Genetics 101:211–219.

Sammour RH. 1991. Using electrophoretic techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. Journal of the Islamic Academy of Sciences 4(3):221–226.

Sneath PHA, Sokal RR. 1973. Numerical Taxonomy. W.H. Freeman, San Francisco, USA.

Summerfield RJ, Roberts EH. 1985. Grain Legume Crops. Collins Publications, London, UK.

Valizadeh M. 2001. Seed storage protein profile of grain legumes grown in Iran, using SDS-PAGE. Journal of Agricultural Science and Technology 3:287–292.

Wang TL, Domoney C, Hedley CL, Casey R, Grusak MK. 2003. Can we improve the nutritional quality of legume seeds? Plant Physiology 131:886–891.

Variability in seed and seedling traits of *Celtis australis* Linn. in Central Himalaya, India

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Summary

Variability in seed and seedling traits of *Celtis australis* Linn. in Central Himalaya, India

This investigation deals with genetic variability in seed and seedling traits of different populations of *Celtis australis*. Significant (p<0.01) provenance variations were recorded for the traits except for seed germination. On average, seed weight, shoot dry weight, shoot length and root dry weight exhibited more variability. Heritability and genetic gain were higher for seed weight and seed length than other traits. For seedling traits, heritability was moderately higher, except for seed germination in nursery. Wide variability observed in seed traits could be utilized for genetic improvement in this promising agroforestry tree crop of Central Himalaya, India.

Key words: *Celtis australis*, genetic advance, genetic gain, heritability, seed and seedling traits, seed source.

Résumé

Variabilité des caractères des graines et des plantules de *Celtis australis* Linn. dans le centre de l'Himalaya, Inde

L'étude a pour objet la variabilité génétique des caractères des graines et des plantules de différentes populations de *Celtis australis*. Des variations significatives (P< 0,01) en fonction de l'origine ont été observées pour tous les caractères excepté la germination des graines. En moyenne, la plus grande variabilité est observée pour le poids des graines, le poids sec des pousses, la longueur des pousses et le poids sec des racines. L'héritabilité et le gain génétique sont supérieurs pour le poids des graines et la longueur des graines par rapport aux autres caractères. L'héritabilité est un peu plus élevée pour les caractères des plantules, sauf en ce qui concerne la germination en pépinière. La grande variabilité observée pour les caractères des graines pourrait être utilisée dans des programmes d'amélioration génétique de cet arbre de l'Himalaya central (Inde), prometteur pour l'agroforesterie.

Resumen

Variabilidad de los rasgos de semillas y plantines de *Celtis australis* Linn. en Himalaya Central, India

El tema de esta investigación es la variabilidad genética de semillas y plantines de diferentes poblaciones de *Celtis australis*. Se registraron variaciones de proveniencia significativas (p>0,01) respecto de todos los rasgos excepto la germinación de las semillas. En promedio, el peso de semillas, el peso de brotes secos, el largo de los brotes y el peso de las raíces secas mostraron más variabilidad. La heredabilidad y la ganancia genética fueron más elevadas respecto del peso y el largo de las semillas que de otros rasgos. En cuanto a los rasgos de los plantines, la heredabilidad fue moderadamente más alta, excepto para la germinación de semillas en el vivero. La amplia variabilidad observada en los rasgos de las semillas puede utilizarse para el mejoramiento genético de este promisorio cultivo arbóreo agroforestal del Himalaya Central en la India.

Introduction

Celtis auatralis Linn. (Family Ulmaceae) is a promising indigenous agroforestry tree species of Central Himalaya, India, extending eastwards to Nepal (Gaur 1999). It is a moderate sized deciduous tree, commonly cultivated in Jammu and Kashmir, Himachal Pradesh, Uttaranchal and parts of Eastern Himalaya (Luna 1996). *Celtis australis* has a wide range of ecological adaptation and is found distributed from about 500 to 2500 masl in agrisilviculture, silvipastoral and agrihortisilvipastoral agroforestry systems throughout the hills (Singh 1982). It plays a vital role in the livelihoods of the hill people by supplying highly palatable, nutritious and tannin-free fodder for livestock, particularly during the lean period (Singh 2004). Its timber is excellent and used for making tool and whip handles, cups, spoons, agricultural implements, etc. (Bhatt and Verma 2002).

Altitudinal variations in seed and seedling characters of tree species have been reported by various workers (Dhillon and Khajuria 1994; Gera et al. 2000; Saklani 1999). However, for the utilization of observed variation in species, it is a prerequisite to know the extent of variation and also whether it is due to genetic or environmental factors (Burton and Devane 1953). Hence information on variation in the desirable parameters and their correlation is vital for any breeding programme (Johnson et al. 1955). A species exhibiting a wide range of variability in terms of mean value and high standard deviation, variance, coefficient of variation and genotypic, phenotypic and environmental coefficients of variation provides ample scope for undertaking screening for the desired traits (Gwaze 1997).

Variations refer to the measurable differences in individuals for a particular trait and may partly be due to genotypic (heritable) and partly to environmental (non-heritable) effects. The relative magnitude of these components determines the genetic properties of any particular species (Jain 1982). The proportion of total heritable variation is termed the broad sense heritability (Lush 1937). The heritability provides a measure of genetic variations upon which all the possibilities of changing the genetic composition of the species depend. In the case of *C. australis*, significant altitudinal variations in seed and seedling characteristics have been recorded (Singh et al. 2004, 2006) including nutritional composition of the foliage (Singh 2004). The present study deals with genetic variability in seed and seedling traits of *C. australis*. The objective of the study is to utilize the desired traits for further genetic improvement in this potential tree crop.

Materials and methods

The investigation studies the genetic variability in seed and seedling traits of selected populations of *C. australis* L. in Central Himalaya, India. Across an altitudinal gradient, thirteen seed sources were identified and seeds were harvested from each source to establish a seed population trail. The seed sources ranged from 29°37′ to 32°12′ N latitude and 76°53′ to 79°38′ E longitude, and from 550 to 1980 m altitude (Table 1). Geographical locations of sampling population of *C. australis* are shown in Figure 1.

For each site, 2 kg of seeds were collected (between December 2000 and February 2001) from ten candidate trees, which were 100 to 300 m apart to avoid narrowing down the variation in the sample due to relatedness or inbreeding (Wang et al. 1982). The bulk sample from each site was given an accession number (provenance title) before entering the laboratory. Ripe drupes of *C. australis* were allowed to dry in the sun for 24 hr. Thereafter, seeds were soaked in cold water overnight, macerated on a wire mesh and rinsed with water to remove the pulp. Seeds tended sink to the bottom while the soft pulp material tended to float. The seeds were again dried in the sun for 24 hr. Seed moisture content was determined on a fresh weight basis by drying the seeds at 103±3 °C as per ISTA rules (ISTA 1999).

Table 1. Geographical parameters of seed collection sites of *Celtis australis*.

Area (State)	Provenance	District	Altitude (masl)	Latitude (N)	Longitude (E)
Garhwal Hills (Uttaranchal)	Srinagar	Pauri	550	30°13'	78°48'
	Khandah srikot	Pauri	750	30°10'5"	78°47'8"
	Gajeli	Pauri	960	30°14'8"	78°37'4"
	Agroda	Pauri	1180	30°06'	78°42'
	Guptakashi	Rudraprayag	1350	30°34'02"	79°15'
	Badiyargaon	Rudraprayag	1980	30°25'	78°52'
	Kandikhal	Tehri	1550	30°20'40"	78°38'05"
Kumaon Hills (Uttaranchal)	Garud	Bageshwer	900	29°56'20"	79°34'4"
	Tallimari	Almora	1250	29°45'	79°25'
	Koshi	Almora	1000	29°37'	79°38'
Himachal Hills (Himachal Pradesh)	Mandi	Mandi	1050	31°42'	77°45'
	Palampur	Kangra	1100	32°12'	76°53'

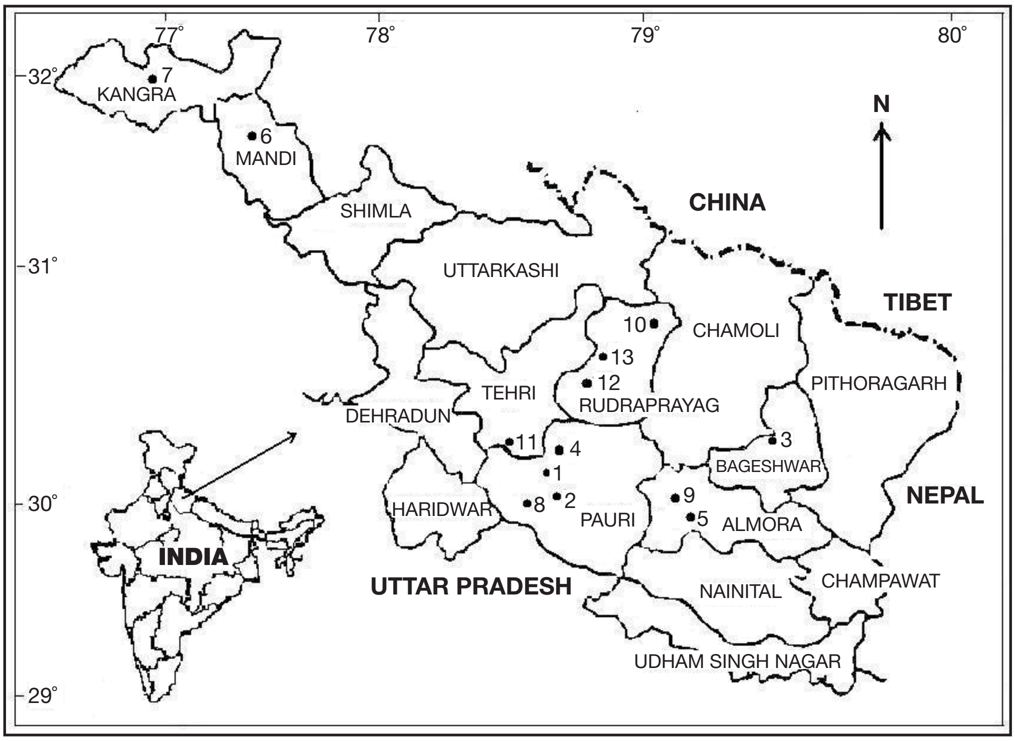

Figure 1. Seed collection sites (provenances) of *Celtis australis*:
1. Srinagar;
2. Khandah srikot;
3. Garud;
4. Gajeli;
5. Koshi;
6. Mandi;
7. Palampur;
8. Agroda;
9. Tallimari;
10. Guptakashi;
11. Kandikhal;
12. Jakholi;
13. Badiyargaon.

Five replicate samples, each consisting of 20 seeds, were randomly selected from each seed lot to record the morphological variability in seed characteristics (seed length, breadth, and seed weight). Seed length and breadth were measured using a micrometer (Besto make). The seed weight of five replicate samples (each of 1000 seeds) was obtained as per ISTA (1999) rules, using an electronic top pan balance. The seeds of each provenance were stored in seed containers at room temperature from March to July 2001, and sowing was done in first week of August (i.e. at the onset of the monsoon).

Seed germination and seedling growth in the nursery was observed in the experimental garden of the Forestry Department, Srinagar, Uttaranchal, India (30°13′ N; 78°48′ E; 530 masl). The average minimum and maximum temperatures for the site were 19.7 and 37.5 °C, respectively. Seeds were soaked in distilled water for 24 hrs before sowing in 1×1 m, well prepared nursery beds in a randomized block design with four replicates. Seed germination was recorded for 60 days, or until there was no further germination for a few days. Surviving plants in each bed were counted after 12 months of growth.

To assess the growth, 10 seedlings of each population in the four replicates were randomly tagged and plant height, diameter, number of branches and number of leaves per seedling recorded until one-year old. Thereafter, tagged seedlings were uprooted to record the biomass yield and root length. The data was subjected to statistical analysis using mean values, ANOVA and correlation coefficient (Gupta 1981); genotypic, phenotypic and environmental variances, and their coefficient and heritability; genetic gain and genetic advance were estimated (Burton and Devane 1953; Johnson et al. 1955).

Results

Variability estimation for seed traits

Variability estimates and genetic parameters were calculated for seed traits, namely length, breadth, weight, moisture and germination percentage. Among various traits, seed weight showed the highest range (47.80–83.09 g/1000 seed) with highest mean value (66.86 g/1000 seed) and maximum value for standard deviation (11.09). Seed breadth, however, exhibited the minimum range (4.01–4.79 mm) with minimum mean value (4.44 mm) and standard deviation (0.25). Coefficient of variance (which helps to compare the variability for different characters) showed that all the characters were not equally variable and it was highest for seed germination in the laboratory at 25 °C (19.24%) and lowest for seed breadth (5.64%). Analysis of variance showed significant ($p<0.01$) provenance variations for seed traits, including germination percentage in the laboratory. Further, the germination percentage exhibited maximum phenotypic (173.0) and environmental variance (116.14) with coefficients of variation of 30.86, 17.69 and 25.29, respectively, for phenotypic, genotypic and environmental variation. Seed weight ranked after seed germination with respect to phenotypic and environmental variance, whereas genotypic variance was higher for seed weight (122.10). Among various parameters, seed breadth exhibited the lowest values for phenotypic, genotypic and environmental variance, including phenotypic and genotypic coefficient of variation. Seed weight exhibited the lowest value (3.03) for environmental variation.

On average, seed length and seed weight had almost equal values for phenotypic and genotypic variance and coefficient of variation, indicating that they are under equal influence of genetic interaction. Thus, all the morphological traits (except germination percentage) showed higher values for genotypic

Table 2. Estimation of variability in different seed characteristics of *C. australis.*

	Seed length	Seed breadth	Seed weight	Seed moisture	Laboratory seed germination
Range	4.46–6.31	4.01–4.79	47.80–83.09	6.00–9.20	33.00–57.00
Mean	5.41	4.44	66.86	7.79	42.62
SD	0.52	0.25	11.09	0.94	8.20
CV%	9.61	5.64	16.59	12.07	19.24
F value	35.69**	17.91**	149.8**	7.69**	3.45**
Phenotypic variance	0.29	0.09	126.20	1.45	173.00
Genotypic variance	0.25	0.07	122.10	0.83	56.86
Environmental variance	0.036	0.02	4.10	0.62	116.14
PCV	9.89	6.80	16.80	15.46	30.86
GCV	9.24	5.98	16.53	11.69	17.69
ECV	3.51	3.25	3.03	10.11	25.29
H%	87.41	76.92	96.75	57.24	32.86
GA	0.96	0.48	22.38	1.42	8.90
Genetic gain	17.74	10.84	33.47	18.23	20.89

Key to abbreviations: SD = Standard derivation; CV% = Coefficient of variation; F = Analysis of variation; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; ECV = Environmental coefficient of variation; H = Heritability; GA = Genetic advance; **Significant at $p<0.01$.

variance and genotypic coefficient of variation compared with environmental variance and coefficient of variation, indicating that all the morphological characters were strongly influenced by phenotypic and genetic interaction. However, seed germination showed the higher values of phenotypic and environmental variance and coefficient of variance compared to genotypic variance and coefficient of variation, indicating that germination percentage was influenced by both phenotypic and environmental interactions (Table 2).

Heritability values for seed weight and seed length were high, i.e. 9.68 and 8.74, respectively, compared with moderately high values for seed breath (7.72) and seed moisture (5.72). Genetic gain was, however, highest for seed weight (3.35%), followed by seed germination (20.89%) and seed moisture (18.23%). Moreover, genetic advance was also highest (22.38) for seed weight, followed by seed germination (8.90) (Table 2).

Variability estimation for seedling traits

Among various parameters, shoot dry weight varied widely with the widest range. A wide range among sources was also found for survival percentage, number of branches per plant, nursery germination percentage, shoot length and root dry weight. The range due to seed source in most of the seedling parameters were found to be wide and differences were significant (p<0.01) except for nursery germination. The coefficient of variation percentage among seed sources was found to be maximum for shoot weight (40.09%), followed by seedling weight (32.73%), root weight (31.71%), number of branches per seedling (30.95%), leaf weight (29.36%), nursery germination (27.95%), number of leaves per plant (24.56%) and shoot length (22.65%). Root:shoot ratio of seedlings showed minimum variability (13.11%), followed by collar diameter (14.33%).

Seedling survival percentage showed maximum value for phenotypic (357.85) and environmental (253.63) variance, while shoot length had maximum value (165.32) for genotypic variance, indicating that seedling survival was influenced by phenotypic and environmental interaction, whereas shoot growth was governed more by phenotypic and genetic interactions. While number of leaves per plant was influenced by both genetic and environmental interaction, shoot weight exhibited maximum values for phenotypic (46.03) and genotypic (36.99) coefficient of variation. Nursery germination percentage showed the maximum value for environmental variation (41.17). Shoot length and shoot weight showed maximum values for genotypic variance and coefficient of variation compared with the environmental variance and coefficient of variation. At the same time, survival percentage, number of leaves per plant and nursery germination percentage exhibited maximum values for environmental variance and coefficient of variation compared with genotypic variance and coefficient of variation (Table 3).

Broad sense heritability was moderate to high for all the growth attributes, except for nursery germination. However, heritability values for shoot length, shoot dry weight, total

Table 3. Estimation of variability in different seedling characteristics of *C. australis*.

	Range	Mean	SD	CV %	F value	Phenotypic variance	Genotypic variance	Environmental variance	PCV	GCV	ECV	H%	GA	Genetic gain
NG%	19.00–44.00	29.0	8.28	27.95	1.59ns	170.80	28.26	142.54	45.07	18.33	41.17	16.55	4.46	15.38
Surv.%	48.58–89.11	69.94	12.95	18.50	2.64**	357.85	104.12	253.63	27.04	14.59	22.77	29.10	11.34	16.21
SL	37.89–79.24	61.05	13.83	22.65	7.61**	240.36	165.32	75.04	25.39	31.06	14.19	68.78	21.99	36.02
RT	20.23–38.16	30.47	5.51	18.08	3.38**	50.84	22.71	28.67	23.40	15.56	17.57	44.67	6.56	21.53
CD	4.66–7.15	5.93	0.85	14.33	4.28**	1.15	0.60	0.55	18.08	13.09	12.52	52.43	1.16	19.56
NOB	1.82–6.15	4.33	1.34	30.95	4.64**	2.59	1.42	1.17	37.16	27.52	24.98	54.83	1.82	42.03
NOL	37.80–74.58	55.98	13.75	24.56	3.84**	291.01	141.59	149.42	30.47	21.26	21.84	48.65	17.09	30.53
R/S	0.39–0.75	0.52	0.10	19.23	3.34**	0.015	0.006	0.009	23.55	14.90	18.24	40.00	0.01	19.23
RDW	1.41–3.77	2.46	0.78	31.71	4.63**	0.77	0.42	0.347	35.67	26.28	23.95	54.29	0.98	39.84
SDW	2.10–7.30	4.24	1.70	40.09	6.45**	3.81	2.46	1.35	46.03	36.99	27.40	64.57	2.59	61.08
LDW	1.81–4.37	3.27	0.96	29.36	2.67**	1.71	0.61	1.10	39.99	23.88	32.07	35.67	0.96	29.36
R/SW	0.50–0.79	0.61	0.08	13.11	2.51**	0.015	0.004	0.011	20.08	10.37	17.19	26.67	0.07	11.48
TDW	5.32–15.44	10.02	3.28	32.73	4.78**	16.16	9.01	7.15	40.12	29.96	26.69	55.75	4.62	46.11

Key to abbreviations: NG% = Nursery germination percentage; Surv.% = Survival percentage; SL = Shoot length; RL = Root length; CD = Collar diameter; NOB = Number of branches per plant; NOL = Number of leaves per plant; R/S = Root to shoot length ratio; RDW = Root dry weight; SDW = Shoot dry weight; LDW = Leaf dry weight; R/SW = Root/shoot dry weight; TDW = Total dry weight; SD = Standard deviation; CV% = Coefficient of variation; F = Analysis of variation; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; ECV = Environmental coefficient of variation; H = Heritability; GA = Genetic advance; NS = Non-significant; **Significant at p<0.01.

dry weight, number of branches per seedling and root dry weight were 6.88, 6.46, 5.58, 5.43 and 5.48, respectively, compared with the moderately high values for other seedling traits. Lowest value was, however, recorded for nursery germination (16.55%). While genetic advance exhibited highest (21.99) and lowest (0.070) values, respectively, for shoot length and root:shoot ratio, genetic gain was recorded highest (61.08%) for shoot weight, followed by seedling weight (46.11%) and number of branches per plant (42.03%). This implies that seed weight was strongly governed by genotypic variance. Genetic gain was also highest for seed weight compared to other traits.

Discussion

Seed and seedling characteristics of *C. australis* exhibited a wide range of variation among populations. Coefficient of variation also indicated that all the characters were not equally variable. On average, shoot weight, seedling weight, root weight and branch number exhibited maximum value for coefficient of variation. Analysis of variance showed significant ($p<0.01$) variations for different seed and seedling parameters, except for nursery germination. Seedling survival, number of leaves per plant and nursery germination exhibited maximum phenotypic and environmental variance, indicating that these parameters were not under genetic control. In contrast all seed traits, shoot length, collar diameter of seedlings, number of branches per plant and root weight exhibited maximum value for genetic variation and coefficient of variation as compared with the environmental variance and coefficient of variation, indicating that these traits were under strong genetic influence. Wang et al. (1982) also reported that seeds of many tree and shrub species exhibit genetic influence over hardseedcoatedness. In another study, Uniyal (1998) reported that seed weight and seed germination of *Grewia optiva* was under strong genetic control. Similarly, seed weight and root length in *Quercus leucotrichophora* was reported to be under strong genetical control (Saklani 1999). Our findings in *C. australis* are in conformity with these research findings.

In general, heritability estimates along with genetic gain were reported more useful than heritability alone in predicting the resultant effect of selecting the best genotypes for a given trait (Volker et al. 1990; Singh and Chaudhary 1993). In the present investigation, genetic gain was highest for seedling weight, followed by seed weight. High heritability for cone weight and number of seeds per cone in *Pinus roxburghii* has also been recorded by Sharma et al. (1999). Dhillon and Khajuria (1994) also recorded more than 75.0% heritability for various seed traits of *Acacia nilotica*. Moderately high heritability estimates with moderate genetic advance has earlier been reported for plant height of *Terminalia arjuna* (Srivastva et al. 1993); plant height and stem diameter of *Grewia optiva* (Sharma and Sharma 1995), *Eucalyptus grandis* (Subramanian et al. 1995) and *Dalbergia sissoo* (Gera et al. 2000).

In *C. australis*, seed weight, shoot length, shoot weight, number of branches and seedling weight exhibited high heritability and genetic gain, indicating that these characteristics are most suitable for selecting the plant material for further planting stock improvement of *C. australis,* which supports the earlier findings of Franklin (1989), Gwaze (1997) and Srivastava et al. (1993).

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References

Bhatt BP, Verma ND. 2002. Some multipurpose tree species for agroforestry systems. ICAR Research Complex for NEH Region, Umiam, Meghalaya, India.
Burton GW, Devane EW. 1953. Estimating hereditary in *Festuca arundinacea* gomenticated clonal material. Agronomy Journal 45: 78–81.
Dhillon GPS, Khajuria HN. 1994. Genotypic variation in germination potential in *Acacia nilotica* (C.) seeds. Indian Journal of Forestry 17(4): 345–347.
Franklin EC. 1989. Theoretical and applied aspects of short-term progeny testing in Loblolly pine. In: Proceedings of the 20th Southern Forest Tree Improvement Conference, Charleston, South Carolina, USA, 26–30 June 1989. pp. 259–268.
Gaur RD. 1999. Flora of the District Garhwal: Northwest Himalaya with Ethnobotanical Notes. Transmedia Publication Centre, Srinagar Garhwal, Uttaranchal, India.
Gera MN, Gera B, Gupta BN. 2000. Preliminary observations on genetic variability and character association in *Dalbergia sissoo* Roxb. Indian Forester 134: 608–615.
Gupta SP. 1981. Statistical Methods. 15th Edition. Sultan Chand & Sons, New Delhi, India.
Gwaze DP. 1997. Genetic parameter estimate for height and stem straightness in *Pinus taeda* L. and implications for breeding. PhD Thesis, University of Edinburgh, UK.
ISTA. 1999. Tropical and Sub-Tropical Tree and Shrub Seed Handbook. International Seed Testing Association, Zurich, Switzerland.
Jain SK. 1982. Statistical Techniques in Quantitative Genetics. Indian Agricultural Statistical Research Institute, New Delhi, India.
Johnson HW, Robinson HF, Combstock RE. 1955. Estimates of genetic and environmental variability in soybean. Agronomy Journal 47: 314–318.
Luna RK. 1996. Plantation Trees. International Book Distributors, Dehradun, Uttaranchal, India.
Lush JL. 1937. Animal Breeding Plan. Iowa State College Press, Ames, Lowa, USA.
Saklani KP. 1999. Altitudinal and seasonal variation in relation to fodder quality of oak (*Quercus leucotrichophora* A. Camus ex. Bahadur) in Garhwal Himalaya. DPhil Thesis, H.N.B. Garhwal University, Srinagar, Uttaranchal.
Sharma R, Bhalaik RR, Tembhune BV. 1999. Estimates of genetic parameters of some cone and seed characters of *Pinus roxburghii* Sargent. Journal of Tropical Forestry 15: 108–111.
Sharma RK, Sharma SS. 1995. Heritability and association analysis in *Grewia optiva*. Indian Forester 121(4): 18–32.
Singh B. 2004. Altitudinal variation in relation to seed, seedling and fodder quality of *Celtis australis* L.: A promising agroforestry tree-crop of Central Himalaya (Garhwal & Kumaon), India. DPhil Thesis, H.N.B. Garhwal University, Srinagar Garhwal, Uttaranchal, India.
Singh B, Bhatt BP, Prasad P. 2004. Effect of seed source and temperature on seed germination of *Celtis australis* L. A promising agroforestry tree-crop of Central Himalaya, India. Forest Trees and Livelihoods 14: 53–60.
Singh B, Bhatt BP, Prasad P. 2006. Variation in seed and seedling traits of *Celtis australis*, a multipurpose tree in Central Himalaya, India. Agroforestry Systems 67: 115–122.

Singh NB, Chaudhary VK. 1993. Variability, heritability and genetic gain in cone and nut characters of Chilgoza Pine (*Pinus gerardiana* Wall). Silvae Genetica 42: 2–3.
Singh RV. 1982. Fodder Trees of India. Oxford and IBH Publication Co., New Delhi, India.
Srivastva DP, Srivastava PK, Goal AK, Thangavelu K. 1993. Genetic variability in *Terminalia arjuna* Bedd. Indian Journal of Forestry 16(3): 223–225
Subramanian KN, Mandal AK, Nicodemus A. 1995. Genetic variability and character association in *Eucalyptus grandis.* Annals of Forestry 3(2): 134–137.
Uniyal AK. 1998. Provenance variation in seed and seedling of *Grewia optiva* Drumm. DPhil Thesis, H.N.B. Garhwal University, Srinagar Garhwal, Uttaranchal, India.
Volker PW, Dean CA, Tibbits WN, Ravenwood IC. 1990. Genetic parameters and gain expected from selection in *Eucalyptus globulus* in Tasmania. Silvae Genetica 39(1): 18–21.
Wang BSP, Pitel JA, Webb DP. 1982. Environmental and genetic factors affecting tree and shrub seeds. In: Thompson JR, editor. Advances in Research and Technology of Seed. Part 7. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.

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Wild edible tubers (*Dioscorea* spp.) and their contribution to the food security of tribes of Jeypore tract, Orissa, India

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Summary

Wild edible tubers (*Dioscorea* spp.) and their contribution to the food security of tribes of Jeypore tract, Orissa, India

The study examined the conservation and utilization of wild edible tubers (*Dioscorea* spp.) in Jeypore tract, Orissa, India. Data were collected from three tribal communities residing in 15 villages, using a pre-structured questionnaire, joint forest visits, focus group discussions and market surveys. It was found that nine tuber species contributed to household diets during time of both normal food availability and during food shortages days. They also contributed to enhanced household income. Collecting, processing and marketing of tubers were found to be gender and age specific. The study shows that overexploitation due to population growth and reduced forest cover is one of the major threats to the wild tubers. The findings suggest that: a) public awareness and community based management is the appropriate approach for conservation; b) investigation of the phytochemical analysis of the tubers can be done to learn their nutritional and medicinal properties; and c) dissemination of knowledge is necessary for *ex situ* conservation, domestication and enhancement of these wild edible tubers.

Key words: Wild edible tubers, tribes, conservation, food security, Jeypore tract.

Résumé

Tubercules comestibles sauvages (Dioscorea spp.) et leur contribution à la sécurité alimentaire de tribus du « Jeypore tract », Orissa, Inde

L'étude a pour objet la conservation et l'utilisation des tubercules comestibles sauvages (*Dioscorea* spp.) du « Jeypore tract », dans l'État d'Orissa, en Inde. Les données ont été collectées auprès de trois communautés tribales résidant dans 15 villages, en s'appuyant sur un questionnaire pré-structuré, des visites conjointes en forêt, des discussions de groupe ciblées et des études de marché. On a observé que neuf espèces de tubercules contribuent à l'alimentation domestique tant pendant les périodes où les aliments sont normalement disponibles que pendant les périodes de pénurie. Ils contribuent également à l'amélioration des revenus des ménages. La collecte, la préparation et la commercialisation des tubercules sont des activités spécifiquement liées au sexe et à l'âge. L'étude montre que la surexploitation due à l'accroissement de la population et à la réduction du couvert forestier est l'une des principales menaces pour les tubercules sauvages. Les observations montrent que : a) la prise de conscience collective et la gestion fondée sur la communauté constituent une bonne approche pour la conservation ; b) l'analyse phytochimique des tubercules est une approche permettant de connaître leurs propriétés nutritionnelles et médicinales ; et c) la diffusion des connaissances est nécessaire pour une conservation *ex situ*, la domestication et l'amélioration de ces tubercules comestibles sauvages.

Resumen

Tubérculos comestibles silvestres (Dioscorea spp.) y su contribución a la seguridad alimentaria de tribus de la región de Jeypore en Orissa, India

El estudio examinó el método de conservación y utilización de tubérculos silvestres comestibles (*Dioscorea* spp.) por parte de tribus de la región de Jeypore en Orissa, India. Se recogieron datos en tres comunidades tribales residentes en 15 aldeas empleando un cuestionario preestructurado, visitas conjuntas a los bosques, debates en grupos focales e inspección de mercados. Se constató que nueve especies de tubérculos forman parte de las dietas domésticas tanto en tiempos de normal disponibilidad de alimentos como durante los días de escasez. Esto también contribuye a mejorar los ingresos hogareños. Se informó que la recolección, procesamiento y comercialización de los tubérculos era ocupación específica según la edad y el sexo. El estudio mostró que la sobreexplotación debida al crecimiento de la población y a la reducción de la cubierta forestal era una de las mayores amenazas para los tubérculos silvestres. Los hallazgos sugieren que: a) el método apropiado para la conservación es la concientización y el ordenamiento basados en la comunidad; b) se debe efectuar el análisis fitoquímico de los tubérculos a fin de conocer sus propiedades nutricionales y medicinales; c) es necesario difundir conocimientos relativos a la conservación *ex situ*, domesticación y mejoramiento de estos tubérculos silvestres comestibles.

Background

Millions of people in many developing countries do not have enough food to meet their daily requirements and many more are deficient in one or more micronutrients (FAO 2004). In many cases rural communities depend on wild resources, including wild edible plants, to meet their food needs in periods of food shortage. The diversity in wild species offers variety in the diet and contributes to household food security. India holds rich genetic diversity in tropical root and tuber crops, particularly aroids, yams and several minor tuber crops.

Wild edible tuber species are an important source of food in India and have a significant place in the dietary habits of small and marginal farm families and forest-dwelling communities during periods of food scarcity (Roy et al. 1988; Arora and Pandey 1996). Edible tubers not only enrich the diet of the people but also possess medicinal properties. Many tropical tuber species are used in the preparation of stimulants, tonics, carminatives and expectorants. These properties need to be documented to validate, quantify and spread this valuable knowledge (Edison et al. 2006).

Indigenous knowledge on wild tubers is an integral part of the traditional and sociocultural lives of people in India. Historically, tribal and rural people identified and collected wild tubers from the forests and developed a range of processing methods in accordance to their needs. Now, however, this knowledge is being lost as a result of the spread of modern technologies in rural and tribal areas. A harmonious blend of indigenous knowledge with modern science is essential to promote sustainable development and utilization of wild edible tubers.

The Jeypore tract

The Jeypore tract of Orissa, India, is a particular case in point. The Jeypore tract, administratively known as the Undivided Koraput district, is situated in the southern part of Orissa, India, between 17°50′ and 20°03′ North and 81° 27′ and 84°01′ East (Figure 1). It spans the Eastern Ghats mountain range, and is extensively forested. The tract is characterized by scattered, sharp, isolated hills with an altitudinal rage of 150–1000 m. The climate is warm and humid, with mean maximum and minimum temperatures of 30.6 °C and 17.3 °C respectively. The average rainfall of the district is 1521.8 mm over 82 rainy days. Forest covers 24% of the area, down from 70% about 50 years ago (Rath 2002). Indiscriminate felling of trees for timber and the paper industry has resulted in large-scale deforestation. This rapid depletion of forest cover has resulted in the virtual disappearance of traditional roots, herbs and tubers. These changes pose serious threats not only to the tribal societies but also to the flora and fauna of the tract, including wild edible tubers (Gowtham Shankar 2005).

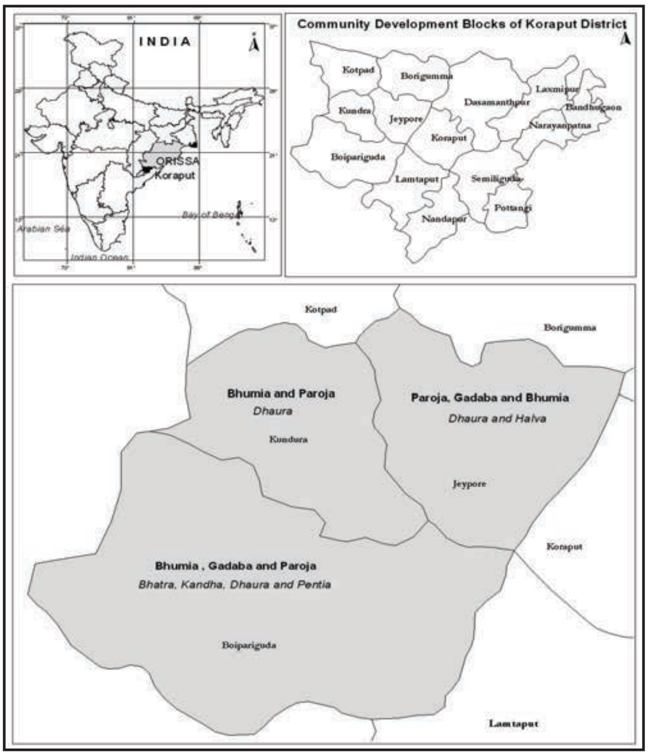

Figure 1. Location of the Jeypore tract, Orissa, India.

In 2001 55% of the population of the Jeypore tract were tribals, down from 61% in 1961. The total population of the district more than doubled between 1961 and 2001, from 1 561 051 in 1961 to 3 541 710 in 2001. The 29 tribes living in Jeypore tract belong to the Proto-Australoid ethnic group and speak Austro-Asiatic languages. Nine tribal groups—Bhatra, Bhumia, Bonda, Gadaba, Gond, Kondh, Koya, Paroja and Saora—each with more than 100 000 people (except Bonda), dominate the tract. The tribal economy survives on agriculture and use of forest resources. The three main activities are food gathering, shifting agriculture and settled agriculture. Agriculture is the mainstay of the economy, which in most of the cases is below subsistence level. Small and marginal land holders (those with less than one hectare) constitute 69% of the total farming community but possess only 34% of the land. Agricultural labourers constitute 31.26% of the total workers in study area.

Nine different species of edible tuber grow wild in the forests of Jeypore and are familiar to the area's tribal communities. Only one domesticated species—*Dioscorea alata* (known locally as Khamba Alu)—is grown in the Jeypore tract.

Food is usually in short supply during summer and monsoon seasons, before the *kharif* (rainy season) crops are harvested. In the past, in the tribal areas wild edible tubers provided food for 20–30 days to needy families during the four months of the food crisis period. However, population growth has led to overexploitation of the wild resources and reduced the availability of tubers in the forest. Wild tubers are mainly collected and sold by tribal landless families and farmers with small and marginal land holdings. Villagers living near the forests also collect wild edible tubers, while people living further from the forests prefer to buy tubers from local markets.

Wild tuber species have not been systematically documented and studied in Jeypore tract. There is a need to document the existing species of wild edible tubers to enable specialized research on the nutritional and medicinal values of these tubers and to disseminate that knowledge among the tribal people to generate interest in conservation and domestication of wild tuber species.

Methodology

Information on wild edible tubers was collected from members of three tribal communities—Paroja, Gadaba and Bhumia—in 15 villages in the Jeypore tract. The study used a pre-structured questionnaire through focus group discussions. Tribal people aged between 15 and 60 years shared their knowledge on different tuber species gathered by them. Field visits were arranged to document the collecting process and photographs were taken. The tuber species were identified taxonomically by referring to *The Flora of Orissa* (Saxena and Brahmam 1995) using the characteristics provided by the tribal people. A special meeting was organized with a group of women to record food-processing procedures. Weekly markets in three places—Doraguda on Mondays, Baligaon on Wednesdays and Boipariguda on Fridays—were surveyed to gather information on the market potential of the wild tubers, including their availability and buyer preferences.

Results and discussion

Most of the villages visited are small, with an average of 65 households. Households consisted of five to seven members. Average land holding was 1.88 hectares. Most of the people live in thatched houses close to the forest.

The staple food is rice, supplemented with millets, pulses and vegetables. Wood for fuel, house construction, making agricultural implements and timber and medicinal herbs are gathered from the forest. The food products collected from the forest include roots, tamarind, tamarind seeds, leaves, jackfruit and seeds and mango stones. Tribals living closest to the forest depend most on forest produce and wild edible tubers are a vital food for them.

Diversity of wild edible tubers

Nine wild tuber species were found across the district: *Dioscorea oppositifolia*, *D. glabra*, *D. tomentosa*, *D. wallichi*, *D. hamiltonii*, *D. bulbifera*, *D. puber*, *D. pentaphylla* and *D. hispida* (Table 1).

Tubers are collected and consumed from time to time by tribal and rural people over a period of 4–5 months from May–June to September–October. The availability of individual tuber species varies from one to three months. For example, *D. oppositifolia* is available at the onset of pre-monsoon rains in May/June, while *D. hispida* is available at the end of the monsoon in September/October. In between, other species are available intermittently. Staggered collecting provides food over a considerable period of time.

Tubers are locally named according to their taste, shape and texture and are identified on the basis of their colour, size and direction of their growth, fibre content and cooking properties. Tubers are eaten as a main course or included while preparing curries or sour dishes.

Three species are widely consumed: *D. oppositifolia* is the most widely consumed, followed by *D. hamiltonii* and *D. wallichi.* The food characteristics and uses of all nine species are given in Table 2. The least used species is *D. hispida* which has an intoxicating effect similar to alcohol. Tribes avoid consuming this tuber.

Collecting

The indigenous knowledge held by the different tribal communities helps in conserving the tuber genetic resources in their natural habitats, as well as in selecting the preferred tubers depending upon availability, taste, size, shape, market and medicinal values. Men and women, both young and old, have profound knowledge about the wild tuber species, but women have more practical knowledge of them. They can identify a tuber species by looking at the leaf sheath colour

Table 1. Description of wild edible tubers by tribal people in the Jeypore tract, Orissa, India.

Scientific name	Local name	Young shoot colour	Leaf shape	Tuber shape	Tuber size (length/diameter)	Skin colour	Tuber colour	Tuber surface	Growth
Dioscorea oppositifolia	Pit kanda	White	Oblong/heart-shaped and glossy	Cylindrical	60 cm/7–8 cm	Brown	White	Smooth	Downward
Dioscorea glabra	Soronda kanda	White/thorny	Ovate tripartite leaves, glabrous	Cylindrical	30 cm/25–30 cm	Dark brown	Light brown/red	Hairy	Downward
Dioscorea tomentosa	Taragai kanda	Black	Heart shaped and glossy	Resembles the fingers of a hand	30 cm/5–7 cm	White	White	Hairy	Lateral branching
Dioscorea wallichi	Cherenga kanda	Light red	Long, but resemble beetle leaves	Cylindrical	60 cm/5 cm	Dark brown	White	Hairy	All directions
Dioscorea hamiltonii	Sika kanda	Blackish	Lanceolate to ovate small leaves but resembles beetle leaves	Cylindrical	30–45 cm/5–8 cm	White	White	Smooth	All directions
Dioscorea bulbifera	Pita kanda	Red	Broadly ovate, resemble beetle leaves	Spherical	75 cm/7–12 cm	Yellow	Yellow	Hairy	Downward
Dioscorea puber	Kasha kanda	Black	Ovate/round leaves	Cylindrical and long	90 cm/30–38 cm	Brown	Light yellow	Hairy	Downward
Dioscorea pentaphylla	Mitni kanda	Thorny and red	Orbicular/beetle leaves	Long and cylindrical	45–60 cm/6–8 cm	Dark brown	Pale white	Smooth	Lateral growth
Dioscorea hispida	Kulia kanda	Light grey	Trifoliate	Irregular/subglobose	12–26 cm/30 cm	Yellowish	Yellowish white	Hairy	Bulbous/corm-like

Table 2. Cooking quality, cooking methods and consumption of wild edible tubers collected in Jeypore tract, Orissa, India.

Scientific name	Local name	Cooking quality (as perceived by the tribal women interviewed)	Cooking method/ consumption
Dioscorea oppositifolia	Pit kanda	Non-sticky, sweet and very tasty	Boil/whole meal
Dioscorea glabra	Soronda kanda	High fibre and not tasty. Consuming large quantities can cause diarrhoea, heaviness of stomach	Curry/side dish
Dioscorea tomentosa	Taragai kanda	High fibre, sticky, no taste	Curry/side dish
Dioscorea wallichi	Cherenga kanda	Sweet, non-sticky, fibrous but cooks well. Causes itching sensation on hand while cutting	Curry/side dish
Dioscorea hamiltonii	Sika kanda	Tasty and sweet, non-fibrous	Boil and curry/whole and side dish
Dioscorea bulbifera	Pita kanda	Bitter before processing. After processing taste changes to sweet like potato, non-fibrous	Boil and curry/whole and side dish
Dioscorea puber	Kasha kanda	Not tasty. Non-fibrous and boiled easily	Boil and curry/whole and side dish
Dioscorea pentaphylla	Mitni kanda	Medium fibrous, sweet taste	Boil and curry (curry is more common)
Dioscorea hispida	Kulia kanda	Causes nausea	Used for medicinal purposes only

and for the presence or absence of thorns. They decide on the best time for collecting tubers based on leaf characteristics: they collect tubers either before the leaves are formed or after the leaves become dry. According to the women, the tubers do not boil properly after leaves have grown.

Women take the lead in the identification, collection, transportation, processing and marketing of all the tuber species except *D. oppositifolia*. Harvesting *D. oppositifolia* involves digging down at least 1 m to find the thread-like stem and 1.25–1.5 m to get to the tuber. On locating the plant, men do the labour-intensive work of digging down 0.6–1.0 m and then leave to look for the next tuber; women then dig out the tuber. This complementary process helps in collecting more tubers. For the other species, tubers are accessible after two to three showers of rain, which is the beginning of the agricultural season. Collecting is done during breaks from fieldwork. Normally the collecting group consists of men and women of different ages, varying from 15–60 years with the majority between 20 to 40 years old; at times older men also go to collect tubers that are easily located and less labour intensive to meet family needs in times of food scarcity. The group leaves around 10 am and returns by 4 pm, regardless of the amount they collect. The decision on the make up of the groups is based on the distance to the forest and availability of tubers. Women take the lead in collecting of tubers that are available in abundance, in close proximity and those that can be easily dug out. Four to 10 collecting trips may be made for a particular tuber species. The quantity of tubers collected per visit varies from 3 to 12 kg. Tubers are stored in bamboo baskets.

Food processing

Women look after the entire process of food preparation, including washing, peeling, cutting, cooking and distributing tubers among the family members. After examining the quantity and palatability of the tuber, they decide the method of cooking (Table 2). If the quantity is large and the taste is good, they boil the tubers to be consumed as main food with finger millet gruel. If the quantity is less and the taste is average, they prepare curry to be eaten along with rice. Cooking the tubers takes about 30 minutes. One family can consume about 1.5 kg of tubers in a meal.

If the skin of the tuber is smooth it is removed by scraping on the storage basket, otherwise a knife is used to remove the skin. To prepare the curry, the tuber is sliced into 25 mm cubes and seasoned with garlic, chilli, turmeric and other spices and sometimes mixed with pulses, brinjal and onion. Preparing *D. bulbifera* requires a different process to make the tuber edible; this takes 24 hours. First the tuber is washed and boiled along with the skin. The skin is then peeled off and the tuber is cut into slices and kept over night in a basket in running water. The next day the tubers are boiled again, the water is thrown away and the tubers are eaten along with salt and chilli.

Economic significance and market value

Wild tubers are available seasonally in the local weekly markets and fetch only low prices (Table 3). Only *D. oppositifolia* has a strong market demand, and this is reflected in a higher price. Women carry loads of up to 10 kg of tubers to market on their head, walking a maximum of 10 km.

Away from the weekly markets and in inaccessible areas, tubers are bartered for other commodities within villages. One kilogram of tubers may be bartered for 500–700 g of rice or finger millet; more people prefer finger millet to tubers because the tubers are more difficult to digest. Tubers are also given to friends and neighbours as gifts if there is a surplus. Alternatively, a group of collectors may share the collected tubers among themselves.

Table 3. Market price of wild edible tubers collected in Jeypore tract, Orissa, India.

Scientific name	Local name	Market price (rupees per kg)
Dioscorea oppositifolia	Pit kanda	8–12
Dioscorea glabra	Soronda kanda	2–3
Dioscorea tomentosa	Taragai Kanda	2–5
Dioscorea wallichi	Cherenga kanda	2–5
Dioscorea hamiltonii	Sika kanda	7–8
Dioscorea puber	Kasha kanda	5
Dioscorea bulbifera	Pita kanda	Not traded
Dioscorea pentaphylla	Mitini kanda	Exchanged for finger millet
Dioscorea hispida	Kulia kanda	Not traded

US$ 1 = 48 Indian Rupees.

Medicinal and sacred value

Some species of *Dioscorea* also possess medicinal properties and are used by local traditional healthcare practitioners as stimulants, tonics, carminatives and expectorants. Some examples of these uses are given below.

About 250 g of *D. oppositifolia* is boiled with 100–150 g of horse gram (*Dolichos uniflorus*) and given to women once a day for nearly a month after giving birth to revive their strength. Lactating mothers are given 100–150 g of *D. puber* boiled with 250 g of black taro *(Colocasia esculenta)* to increase their milk flow. All the other wild edible tubers are considered beneficial to both men and women. *Dioscorea glabra* is considered a general tonic. Boiled *D. hamiltonii* is consumed to get relief from piles. *Dioscorea tomentosa* works as a tonic and provides strength to work. Consumption of burnt *D. bulbifera* enhances appetite. One tuber of *D. hispida* weighing around 2 kg has an intoxicating effect equivalent to five bottles of beer, i.e. 900 ml. At times people eat this tuber to forget their sorrows, but eating too much causes diarrhoea and vomiting. Local traditional healthcare practitioners use it to treat aliments such as vomiting and dysentery. As *D. glabra* resembles the wild boar, tribal priests substitute it in ritual sacrifices while praying for childless couples.

Conclusion

The study revealed that different tribal communities continue to have and use knowledge about the wild tuber species, including their habitat, collecting period, sustainable collecting, mode of preparation and consumption and marketing. To date this knowledge appears to be fairly well conserved and used as a result of continued reliance of local communities on the wild edible tubers. Analysis of the results showed that most of the edible plants are used mainly by tribal and poor families both during normal and difficult times. Utilization of the wild edible plants by the younger generations ensures the maintenance of knowledge associated with the different species.

Traditional healthcare practitioners appear to make considerable use of the *Dioscorea* species studied. This suggests that further investigation is needed into the pharmacological properties of all these species.

Safe conservation, sustainable use and in-depth study of wild tuber diversity is essential to the continued use of this diversity for meeting the present and future food needs of the tribal and rural people during periods of food scarcity, as well as their use in traditional medicine. This will require active community involvement in the process and sharing of benefits.

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References

Arora RK, Pandey A. 1996. Wild Edible Plants of India, Diversity, Conservation and Use. National Bureau of Plant Genetic Resources, New Delhi, India.

Edison S, Unnikrishnan M, Vimala B, Pillai SV, Sheela MN, Sreekumari MT, Abraham K. 2006. Biodiversity of tropical tuber crops in India. NBA Scientific Bulletin No. 7. National Biodiversity Authority of India, Chennai, Tamilnadu, India. 60 pp.

FAO. 2004. The State of Food Insecurity in the World. Monitoring the progress towards the world food summit. FAO, Rome, Italy.

Gowtham Shankar KJN. 2005. Strengthening tribal economies through endogenous development. COMPASS Magazine, February 2005. pp. 32–35.

Rath B. 2002. People–Forest–State. A Statistical Review of the Triangular Relationship in Orissa. Vasundhara, Bhubaneswar, Orissa, India. Available from: http://www.vasundharaorissa.org/download22/People%20%E2%80%93%20Forest%20%E2%80%93%20State%20A%20Statistical%20review%20of%20the%20Triangular%20Relationship%20in%20Orissa..pdf. Date accessed: 22 July 2009.

Roy B, Halder AC, Pal DC. 1988. Plants for Human Consumption in India. Botanical Survey of India, Calcutta, India. pp. 63–65.

Saxena HO, Brahmam M. 1995. The Flora of Orissa, Vol. I–IV. Orissa Forest Development Corporation, Bhubaneswar, India.

Genetic variation within and among the populations of *Podophyllum hexandrum* Royle (Podophyllaceae) in western Himalaya

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Summary

Genetic variation within and among the populations of *Podophyllum hexandrum* Royle (Podophyllaceae) in western Himalaya

Podophyllum hexandurm Royle (Himalayan mayapple) is an important medicinal plant growing in higher regions of western Himalaya. Due to overexploitation, the populations of *P. hexandrum* are decreasing in size. This could lead to loss of genetic diversity and compromise species survival. This study used RAPD analysis to estimate genetic variation and determine genetic structure in five geographically distinct populations of *P. hexandrum*. The analysis revealed high molecular diversity within populations as well among populations. The Pangi and Kukumseri populations were geographically isolated from the other three populations and were subject to less anthropogenic pressure, but showed little within-population genetic diversity. Populations from the Great Himalayan Nation Park and Chamba were subject to greater anthropogenic pressure but showed high within-population genetic diversity. Gene flow between the populations was evident from the RAPD analysis; populations exposed to higher anthropogenic pressure showed higher gene flow and greater genetic similarity with nearby populations. The more-isolated populations, Pangi and Kukumseri, showed less genetic similarity with other populations and less gene flow. Pangi and Kukumseri populations should be investigated for their genetic isolation.

Key words: *Podophyllum hexandrum*, population structure, genetic variation, western Himalaya.

Résumé

Variation génétique intra- et inter-populations de *Podophyllum hexandrum* Royle (Podophyllaceae) de l'Himalaya occidental

Podophyllum hexandrum Royle (pomme de mai) est une plante médicinale importante des hautes régions de l'Himalaya occidental. En raison de la surexploitation, la taille des populations de *P. hexandrum* a régressé, ce qui peut entraîner une perte de diversité génétique et compromettre la survie de l'espèce. Cette étude utilise l'analyse RAPD pour estimer la variation génétique et déterminer la structure génétique dans cinq populations géographiquement distinctes de *P. hexandrum*. L'analyse révèle une importante diversité moléculaire au sein des populations et entre populations. Les populations de Pangi et de Kukumseri sont géographiquement isolées des trois autres populations et ont été moins soumises à la pression anthropogène, mais elles présentent une moindre diversité génétique intra-population. Les populations du Great Himalayan Nation Park et de Chamba ont subi une pression anthropogène plus forte mais leur diversité génétique intra-population est importante. Le flux de gènes entre les populations est mis en évidence par l'analyse RAPD : le flux de gènes est plus important dans les populations soumises à une plus forte pression anthropogène et celles-ci présentent une plus grande similitude génétique avec les populations voisines. Le flux de gènes est moindre dans les populations plus isolées (Pangi et Kukumseri) qui présentent une moindre similitude génétique. L'isolement génétique de ces deux populations justifie leur étude.

Resumen

Variación genética en y entre poblaciones de *Podophyllum hexandrum* Royle (Podophyllaceae)

Podophyllum hexandrum Royle (podofilo, manzana de mayo del Himalaya) es una importante planta medicinal que crece en las alturas del Himalaya occidental. El tamaño de las poblaciones de *Podophyllum hexandrum* está disminuyendo debido a la sobreexplotación. Esto puede conducir a la pérdida de diversidad genética y comprometer la supervivencia de la especie. Para evaluar la variación genética y determinar la estructura genética de cinco poblaciones geográficamente diferentes de *Podophyllum hexandrum* se utilizó en el presente estudio análisis RAPD (polimorfismo de DNA amplificado al azar, sigla en inglés). El análisis reveló una gran diversidad molecular en y entre las poblaciones. Las poblaciones Pangi y Kukumseri estaban geográficamente aisladas de las otras tres poblaciones y sujetas a menor presión antropogénica, pero mostraron poca diversidad genética dentro de la población. Las poblaciones del Parque Nacional del Gran Himalaya y las de Chamba sufrían una mayor presión antropogénica y mostraron elevada diversidad genética dentro de ellas. El análisis RAPD evidenció flujo de genes entre las poblaciones: las expuestas a mayor presión antropogénica mostraron mayor flujo de genes y mayor similitud genética con las poblaciones vecinas. Las poblaciones más aisladas, Pangi y Kukumseri, mostraron menor similitud genética con las demás poblaciones y menor flujo de genes. Se deberían examinar las poblaciones Pangi y Kukumseri respecto de su aislamiento genético.

Introduction

Podophyllum hexandrum Royle is an herbaceous, rhizomatous species of medicinal importance that is now endangered in India (Nayar and Sastry 1990). The rhizomes and roots of *P. hexandrum* contain lignans with anti-tumour properties, such as podophyllotoxin, 4'-demethyl podophyllotoxin and podophyllotoxin 4-o-glucoside (Tyler et al. 1988; Broomhead and Dewick 1990).

Among these, podophyllotoxin is the most important for its use in the partial synthesis of the anti-cancer drugs etoposide and teniposide (Issell et al. 1984). The podophyllotoxin content of Himalayan mayapple is high (4.3%) compared with other species of *Podophyllum*, notably *P. peltatum* (0.25%), the most common species in the American subcontinent (Jackson and Dewick 1984).

There is considerable variation in morphological characters such as plant height, leaf characteristics, fruit weight, seed weight and seed colour and in chemical characters such as podophylloresin and podophyllotoxin content in rhizomes (Airi et al. 1997; Purohit et al. 1999). Esterase isozyme analysis has indicated the existence of high inter- and intrapopulation variation in *P. hexandrum* from Garhwal Himalaya (Bhadula et al. 1996). At least four distinct morphological variants, with 1, 2, 3 and 4 leaves, have been reported (Purohit et al. 1998). Polypeptide profiles in seeds of these variants indicate that they may be genetically distinct from each other.

The population of *P. hexandrum* in western Himalaya is declining, and in some areas the plant has almost disappeared as a result of anthropogenic activities and overexploitation (Bhadula et al. 1996). Thus, there is a pressing need to understand and conserve the genetic diversity of this important medicinal plant.

Attempts have been made to conserve this plant through *in vitro* propagation and artificial breaking of seed dormancy (Nautiyal et al. 1987; Nadeem et al. 2000). Efforts to collect and maintain germplasm have been mainly centred on clearly defined characters recognizable in a phenotype. However, there is now a paradigm shift in looking for characters or genes using molecular markers (Tanksley and McCouch 1997). Such data help to provide bases for decisions about allocation of resources towards maintenance and utilization of genetic diversity. In western Himalaya, two populations of *P. hexandrum* have been characterized for their inter- and intrapopulation genetic variation using RAPD analysis (Sharma et al. 2000). Considering the wide distribution of *P. hexandrum* throughout western Himalaya, the above analysis did not reflect the genetic diversity of the species. Hence, it is necessary to assess genetic diversity of *P. hexandrum* at inter- and intrapopulation level in disturbed and undisturbed habitats. This paper attempts to describe variation in the genetic diversity of populations collected from five geographical locations.

Materials and methods

Plant material

Five populations of *P. hexandrum* Royle were collected from five sites in the western Himalayan region. Nine or ten plants were collected at random at each site. The rhizome bud was taken and grown in the glasshouse. Table 1 summarises the characteristics of the populations, including size, geographic coordinates and altitude.

DNA isolation and RAPD analysis

DNA was isolated from tender leaves of the plants growing in the glasshouse using a modified version of the protocol followed by Saghai-Maroof et al. (1984). Polymerase chain reaction for RAPD profiling was carried out in 25 µl volume. A reaction tube contained 25 ng of genomic DNA, 0.3 unit of Taq DNA polymerase, 100 µM of each dNTPs, 1.5 M $MgCl_2$ and 5 pmol of the decamer primer. Amplification was carried out at 94 °C, 36 °C and 72 °C for 35 cycles. The amplified products were separated on 1.6% agarose gel containing 0.5 µg/ml of ethidium bromide and photographed with Fotodyne MP-ST photographic system equipped with an MP4+ Polaroid instant camera. Random decamer primer kits A, D, G and E (Operon Tec. Inc., USA) were used for amplification.

Data analysis

RAPD profiles were analyzed by scoring the presence and absence of bands from the amplified product of different accessions and similarity indices were generated after multivariate analysis using Nei and Li's (1979) coefficient. The degree of RAPD polymorphism was also quantified using the Shannon index (Sh) (Weaver and Shannon 1949) to calculate allelic richness. The percentage of polymorphic loci (P% = polymorphic loci/total loci) index was calculated using POGENE 1.31 software. Genetic structure and degree of genetic differentiation were estimated using molecular variance analysis (AMOVA). This statistical analysis is recognized as an effective tool to define population structure and the degree of genetic differentiation (Excoffier et al. 1992). Total genetic diversity in variance components was calculated among and within individuals of each population. The Euclidean genetic distances constructed for AMOVA were also used for principal coordinate analysis for grouping individuals of the populations. The significance of estimates was obtained through 999 data replications (see http://www.anu.edu.au/BoZo/GenAlEx/). The average similarity matrix between the populations was used to generate a tree for cluster analysis by UPGMA (Unweighted Pair Group

Table 1. Location and size of *P. hexandrum* populations sampled in western Himalaya, India.

Geographical location of populations	No. of samples	Total no. of individuals	Altitude (m asl)
Chamba	10	600	2200
Pangi	10	150	3600
Koksar	9	250	3200
Kukumseri	10	800	2800
Great Himalayan Nation Park (GHNP)	9	40	2400

Method with Arithmetic average) method using NTSYS 2.1. Genetic distances between the populations (Nei 1972) were calculated based on binary RAPD data.

Results

Out of the 80 primers screened, 11 produced 76 reproducible polymorphic bands. These were used for genetic diversity analysis. Each band produced was considered a locus. The Chamba population had the highest Shannon index (0.41) and the highest DNA polymorphism (80.20%), while the Kukumseri population had the lowest Shannon index (0.20) and the lowest DNA polymorphism (42.11%) (Table 2).

The Kukumseri and Pangi populations showed clear clustering in the principal coordinate analysis, indicating a narrow genetic base at these two sites (Figure 1). Although the individuals of Chamba population form a distinct group, the looseness of the grouping indicates a broad genetic base. Most individuals of the Koksar population formed a tight cluster but two plants did not fall within the cluster. The individuals of the GHNP population were randomly scattered, suggesting a broad genetic base.

AMOVA values obtained from RAPD data show that 74% of total variation can be attributed to the individuals within population while 26% is due to differences among populations (Table 3).

The maximum genetic similarity was observed between Pangi and Kukumseri populations, followed by GHNP and Chamba population (Table 4). Highest gene flow was found between GHNP and Chamba populations (Nm=1.510), while the lowest was between the Koksar and Pangi populations (0.376) (Table 4).

Table 2. Diversity index and DNA polymorphism in five *P. hexandrum* populations collected in western Himalaya, India.

Geographical location of populations	Shanon index	DNA polymorphism (%)
Chamba	0.4109	80.20
Pangi	0.2394	47.37
Koksar	0.3067	59.21
Kukumseri	0.2042	42.11
Great Himalayan Nation Park (GHNP)	0.3329	60.53

Discussion

Podophyllum hexandrum is an herbaceous medicinal plant of great value. It is distributed along the western Himalaya in the Greater Himalayan and Trans-Himalayan regions. Five geographically distinct populations were collected from a variety of habitats ranging from grassy meadows to undergrowth of pine forest to rocky cold desert and analyzed for genetic diversity and their relationships. Two of the populations, from Kukumseri and Pangi, showed a narrow genetic base and appeared to be distinct from the other three populations. High genetic variability was found within the Chamba and GHNP populations. However, molecular variation among the populations was very low.

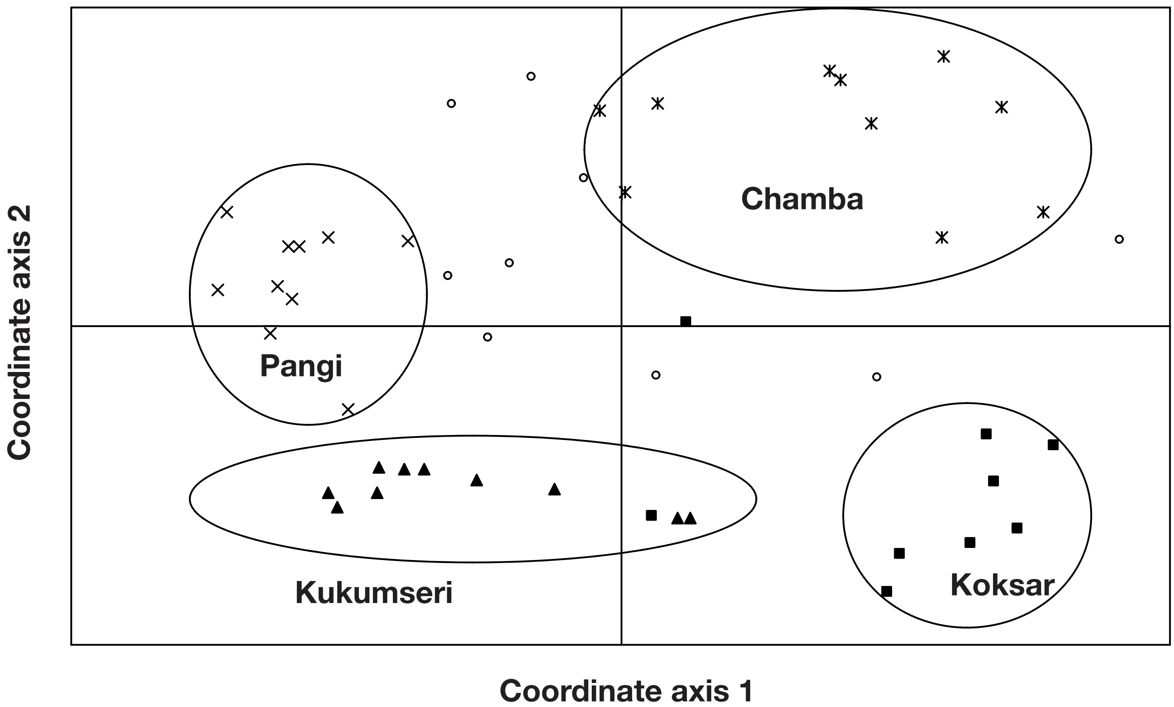

Figure 1. Principal coordinate plot showing grouping of *P. hexandrum* populations in western Himalaya, India.

Table 3. Analysis of molecular variance (AMOVA) in 48 individuals among five populations of *P. hexandrum*.

Source of variation	d.f.	SS	MS	Est. variance	% of variation	*p*
Among population	4	145.767	36.442	2.930	26	0.001
Within population	43	358.233	8.331	8.331	74	0.001

d.f. – degrees of freedom; SS – sum of squares; MS – mean squared deviation; *p* – probability level.

Table 4. Estimates of Nei Genetic distances and gene flow (Nm) between the populations of *P. hexandrum* in western Himalaya, India.

No.	Population	Population	Genetic distance (Nei)	Gene flow (Nm)
1	GHNP	Koksar	0.125	0.903
2	GHNP	Kukumseri	0.101	0.827
3	Koksar	Kukumseri	0.122	0.598
4	GHNP	Pangi	0.109	0.849
5	Koksar	Pangi	0.207	0.376
6	Kukumseri	Pangi	0.081	0.794
7	GHNP	Chamba	0.105	1.510
8	Koksar	Chamba	0.141	0.913
9	Kukumseri	Chamba	0.166	0.547
10	Pangi	Chamba	0.167	0.602

The AMOVA analysis indicated a risk of genetic erosion in the Pangi and Kukumseri populations that may lead to genetic drift. The close genetic relationship between the Pangi and Kukusmseri populations may be related to the close geographical proximity of these two sites. However, the Koksar population was genetically distinct from the Kukumseri population despite its close geographic proximity; both sites are in the Lahul valley of the Trans-Himalayan region. The Chamba and GHNP populations, which represented the Greater Himalayan region, also showed high genetic similarity and evidence of gene flow between them. The tribes of western Himalaya carry the pods and plants with them along their grazing routes, thus resulting in the gene flow.

The sites at Pangi and Kukumseri were relatively undisturbed compared with the other sites. However, the GHNP population has suffered greatly from grazing. Moreover, the gene flow indicated between the GHNP population and other populations is related to the accessibility and exploitation level of the population.

Analysis of genetic variation within the taxon is crucial for conservation purposes because of its implication for long-term survival and continuous evolution of species (Young and Clarke 2000). The reduced genetic variability in the Pangi and Kukumseri population may be the result of genetic isolation as a result of habitat fragmentation and anthropogenic pressure. The genetic variability detected among and between populations can be used to propose appropriate management actions. These include identifying populations and sites that should be protected and conserved. Ideally, populations should be selected that have the greatest genetic diversity and are free from the effects of anthropogenic pressure.

Based on our findings, the populations from Pangi and Kukumseri should be investigated in detail to determine the factors causing their narrow genetic diversity so as to plan conservation measures.

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References

Airi S, Rawal RS, Dhar U, Purohit AN. 1997. Population studies on *Podophyllum hexandrum* Royle—a dwindling medicinal plant of the Himalaya. Plant Genetic Resources Newsletter 110:29–34.

Bhadula SK, Singh A, Lata H, Kuniyal CP, Purohit AN. 1996. Genetic resources of *Podophyllum hexandrum* Royle, an endangered medicinal species from Garhwal Himalaya, India. Plant Genetic Resources Newsletter 106:26–29.

Broomhead AJ, Dewick PM. 1990. Tumor inhibitory aryltetralin lignans in *Podophyllum versipelle*, *Diphylleia cymosa* and *Diphylleia grayi*. Phytochemistry 29:3831–3837.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondria DNA restriction data. Genetics 131:479–491.

Issell BF, Muggia FM, Carter SK. 1984. Etoposide (VP-16)—Current Status and New Developments. Academic Press, Orlando, Florida, USA.

Jackson DE, Dewick PM. 1984. Biosynthesis of *Podophyllum* lignans. II: Interconversion of aryltetralin lignan in *Podophyllum hexandrum*. Phytochemistry 23:1037–1042.

Nadeem M, Palni LMS, Purohit AN, Pandey H, Nandi SK. 2000. Propagation and conservation of *Podophyllum hexandrum* Royle: an important medicinal herb. Biological Conservation 92:121–129.

Nautiyal MC, Rawat AS, Bhadula SK, Purohit AN. 1987. Seed germination in *Podophyllum hexandrum*. Seed Research 15:206–209.

Nayar MP, Sastry APK. 1990. Red Data Book of Indian Plants. Botanical Survey of India, Calcutta, India.

Nei M. 1972. Genetic distance between populations. American Naturalist 106:283–392.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Science (USA) 76:5269–5273.

Purohit AN, Lata H, Nautiyal S, Purohit MC. 1998. Some characteristics of four morphological variants of *Podophyllum hexandrum* Royle. Plant Genetic Resources Newsletter 114:51–52.

Purohit MC, Bahuguna R, Maithani UC, Purohit AN, Rawat MSM. 1999. Variation in podophylloresin and podophyllotoxin contents in different populations of *Podophyllum hexandrum.* Current Science 77:1078–1080.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences (USA) 81:8014–8018.

Weaver W, Shannon CE. 1949. The Mathematical Theory of Communication. University of Illinois, Urbana, Illinois, USA.

Sharma KD, Singh BM, Sharma TR, Meenu Katoch, Guleria S. 2000. Molecular analysis of variability in *Podophyllum hexandrum* Royle—an endangered medicinal herb of north-western Himalaya. Plant Genetic Resources Newsletter 124:57–61.

Tanksley SD, McCouch SR. 1997. Seed bank and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066.

Tyler VE, Brady LR, Robbers JE. 1988. Pharmacology (9th ed.). Lea and Febiger, Philadelphia, USA.

Young AG, Clarke GM. 2000. Genetics, Demography and Viability of Fragmented Populations. Cambridge University Press, Cambridge, UK.

Morpho-agronomic diversity of hyacinth bean (*Lablab purpureus* (L.) Sweet) accessions from Bangladesh

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Summary
Morpho-agronomic diversity of hyacinth bean (*Lablab purpureus* (L.) Sweet) accessions from Bangladesh
A study was conducted of the morpho-agronomic diversity among 88 accessions of hyacinth bean (*Lablab purpureus* (L.) Sweet) from 14 districts of Bangladesh. Considerable variation was observed in all 15 qualitative and 15 quantitative characters assessed. Among the quantitative traits the highest coefficient of variation was found in peduncle length, followed by rachis length, number of flowering nodes per peduncle and yield per plant; the lowest coefficient of variation was in days to first pod maturity. D2 analysis grouped the accessions into seven morpho-agronomic clusters. No relationship was found between geographic origin and clustering. These findings were confirmed by results of canonical analysis. Further investigation using molecular techniques is recommended to assist in establishing a core collection for the crop.

Key words: Morpho-agronomic characterization, diversity, cluster analysis, canonical, *Lablab purpureus*, Bangladesh.

Résumé
Diversité morpho-agronomique d'accessions de dolique pourpre (Lablab purpureus (L.) Sweet) du Bangladesh
Une étude de la diversité morpho-agronomique a été réalisée sur 88 accessions de dolique pourpre (*Lablab purpureus* (L.) Sweet) provenant de 14 districts du Bangladesh. Une variation considérable a été observée pour l'ensemble des 15 caractères qualitatifs et 15 caractères quantitatifs évalués. Les caractères quantitatifs présentant les coefficients de variation les plus élevés sont la longueur du pédoncule, suivi par la longueur du rachis, le nombre de nœuds florifères par pédoncule et le rendement par plante ; le coefficient de variation le plus faible est observé pour le nombre de jours avant la maturation de la première gousse. L'analyse D^2 répartit les accessions en sept groupes morpho-agronomiques. Aucune relation n'est observée entre l'origine géographique et le groupement. Ces observations sont confirmées par les résultats d'une analyse canonique. D'autres investigations faisant appel à des techniques moléculaires devraient permettre d'établir une collection de référence.

Resumen
Diversidad morfoagronómica de accesiones de zarandaja (Lablab purpureus (L.) Sweet) en Bangladesh
Se llevó a cabo el examen de la diversidad morfoagronómica de 88 accesiones de zarandaja (frijol de Egipto) (*Lablab purpureus* (L.) Sweet) provenientes de 14 distritos de Bangladesh. Se observó una considerable variación entre las 15 características cualitativas y las 15 cuantitativas evaluadas. Entre los rasgos cuantitativos el coeficiente de variación más alto estaba en el largo del panículo, seguido por el largo del raquis, el número de nódulos de floración por pedúnculo y el rendimiento por planta. El coeficiente de variación más bajo se daba en los días necesarios para la maduración de la primera yema. El análisis d^2 agrupó las accesiones en siete conglomerados morfoagronómicos. No se halló relación entre el origen geográfico y el agrupamiento. Estos resultados fueron confirmados por los de los análisis ortodoxos. Se recomiendan ulteriores investigaciones empleando técnicas moleculares que permita establecer una colección testigo del cultivo.

Introduction

Bangladesh is situated between 20º35′ and 26º75′ N latitude and 88º03′ and 92º75′ E longitude in South Asia. The elevation ranges from 3 to 45 m above sea level (m asl). The annual minimum temperature is 8 ºC and the annual maximum temperature is 36 ºC. Hyacinth bean (*Lablab purpureus* (L.) Sweet) is an important vegetable crop in Bangladesh. It is grown on approximately 11 000 ha across the country during the winter season, yielding an average of 4.53 t of fresh pods per ha for a total yield of about 50 000 t (BBS 2004). Hyacinth bean is also grown for fodder and as a cover crop. The leaves and seeds contain 20–28% protein, with a well-balanced amino acid composition (Schaaffhausen 1963). The fresh pods and green seeds are eaten boiled or used in curries. Mature seeds are used as pulses, often in soup. Sprouted seeds are occasionally sun-dried and stored for use as a vegetable.

The species is extremely diverse (Verdcourt 1970; Duke et al. 1981) and three subspecies are recognized. These are the wild, ancestral subspecies, subsp. *uncinatus* Verdc., and two cultivated subspecies, subsp. *purpureus* Sweet and subsp. *bengalensis* (Jacq.) Verdc. Subspecies *uncinatus*, which is found mainly in East Africa, has pods of about 40 × 15 mm. Subspecies *purpureus* includes a cultivated form with larger pods measuring 100 × 40 mm. Subspecies *bengalensis* has longer pods than other subspecies, up to 140 mm long but only 10–25 mm wide.

Shivashankar and Kulkarni (1992) described three groups among the cultivated plants based on relative position of seeds in the pods:

1. Cultivar group Lablab: The mature seeds have their long axis at right angles to the suture. Pods are dehiscent or indehiscent. Seeds are no more than one-third to three-quarters of the width of the mature pods. Widely distributed.

2. Cultivar group Ensiformis: The mature seeds lie with their long axis more or less oblique to the suture, nearly filling the mature pods. The pods are indehiscent. This group is difficult to distinguish from cultivar group Lablab when young. Found in South-East Asia and East Africa.
3. Cultivar group Bengalensis: The mature seeds lie with their long axis parallel to the suture, more or less filling the mature pod. The seeds are gibbous dorsally and at their base. The pods are indehiscent. Found in South Asia and East Africa.

Knowledge of genetic diversity within a crop is essential for the long-term success of a breeding programme and maximizes the exploitation of germplasm resources. Knowledge of the structure of genetic diversity within a large germplasm collection may be of great help in making decisions on germplasm management, as well as in developing breeding strategies. Recently, attempts have been made to use molecular markers to study genetic diversity in hyacinth bean. For example, Liu (1996) studied genetic variation among 40 accessions of hyacinth bean using random amplified polymorphic DNA. A high level of genetic variation was detected but mainly between cultivated and wild forms and not within cultivated forms. Genetic variation was significantly greater among Asian accessions of the cultivated genotypes than among African accessions. Pengelly and Maass (2001), using morphological and agronomic characters, found greater variation in wild forms from eastern and southern Africa than within cultivated landraces collected from Africa and Asia. They also found that the wild and cultivated forms from the East African highlands, particularly Ethiopia, belonged exclusively to subsp. *uncinatus* and were distinct from the remainder of the collection studied. In contrast, Maass et al. (2005), using amplified fragment length polymorphisms to assess genetic relationships between cultivated and wild forms of lablab, found that landraces from Africa and Asia belonged predominantly to subsp. *purpureus*, and showed moderate genetic diversity. They also found that accessions of the wild subsp. *uncinatus* from Africa had a higher level of genetic diversity than the cultivated forms.

Unfortunately, these and other similar studies (Wang et al. 2007; Venkatesha et al. 2007; Zhong-cheng et al. 2005; Wang et al. 2004; Joshi et al. 1995) included little or no germplasm from Bangladesh. Bangladesh is rich in hyacinth bean diversity; scientists of the Plant Genetic Resources Centre (PGRC) of the Bangladesh Agricultural Research Institute (BARI) have collected 551 accessions of hyacinth bean from different districts of Bangladesh and these have been characterized in the field (Islam et al. 2002; Islam 2004).

The current study used agromorphological characters to assess the nature and magnitude of diversity available in 88 of these accessions, with a view to contributing to the establishment of a core collection for this crop.

Materials and methods

The experiment was conducted at PGRC of BARI, Joydebpur, Gazipur at 8.4 m asl in 2002/03. The 88 accessions selected for the study were collected from 14 districts in Bangladesh, largely from Sirajganj and Kushtia, following the methods described by Arora (1991). The accessions were selected on the basis of coverage of different growing areas of the country, variation in fresh pods, seeds etc. Seedlings were established in polythene bags on 28 September 2002 and transplanted into the field after 12 days. Plants were established 4 m apart within rows

Table 1. Variation in 15 qualitative characters in 88 hyacinth bean accessions from Bangladesh.

Character	State 1	No.	State 2	No.	State 3	No.	State 4	No.	State 5	No.
Hypocotyl colour	Green	72	Purple	16	–	0	–	0	–	0
Epicotyl colour	Green	66	Purple	22	–	0	–	0	–	0
Stem colour	Green	51	Purple	37	–	0	–	0	–	0
Petiole colour	Green	53	Purple	35	–	0	–	0	–	0
Leaf vein pigmentation	Green	76	Purple	12	–	0	–	0	–	0
Flower colour	Purple	48	White	36	Greyish–white	4	–	0	–	0
Raceme position	Emerging from leaf canopy	42	Intermediate	34	Within foliage	12	–	0	–	0
Growth habit	Indeterminate climber	55	Indeterminate semi-climber	33	–	0	–	0	–	0
Fresh pod colour	Green	71	Very light green	12	Purple	3	Purple margin	2	–	0
Fresh pod shape	Flat	53	Elongated	31	Elongated–wavy	4	–	0	–	0
Fresh pod curvature	Straight	57	Slightly curved	24	Curved	7	–	0	–	0
Fresh pod beak shape	Thick	38	Long	23	Short	19	Medium	8	–	0
Seed colour	Black	42	Brown	30	Gray–orange	11	Black dot and brown	3	Bicolour	2
Seed shape	Round	37	Flat	34	Elongated	17	–	0	–	0
Seed texture	Medium ridged	68	Highly ridged	20	–	0	–	0	–	0

and with 4 m between rows to allow space for normal growth and development of the vine. Plants were grown on bamboo trails. The experiment was laid out in a randomized complete block design with three replicates. Each replicate consisted of a single row of five plants per accession. BARI-recommended cultural practices were followed (Islam et al. 2004). Data were recorded on all five plants in each replicate. Observations were recorded on 15 qualitative characters (Table 1) and 15 quantitative characters (Table 2). Range, mean and coefficient of variation were determined (Table 2). Genetic divergence, cluster, principal component analysis and canonical variance analysis were performed using Genstat 5 (1998).

Table 2. Variation in 15 quantitative characters in 88 hyacinth bean accessions from Bangladesh.

Character	Range	Mean	CV (%)
Days to first flowering	54–100	77	12.3
Days to first fresh pod harvest	77–137	111	11.9
Days to first pod maturity	116–153	140	4.5
Terminal leaflet length (cm)	7.4–10.6	9.0	7.8
Terminal leaflet width (cm)	6.9–10.7	8.6	9.1
Rachis length (cm)	1.4–15.0	7.6	50.5
Peduncle length (cm)	2.4–29.7	15.4	51.0
No. of flowering nodes per peduncle	2.2–8.2	4.5	35.7
Fresh pod length (cm)	5.3–17.0	9.8	23.1
Fresh pod width (cm)	1.6–4.3	2.4	23.2
Fresh pod weight (g)	3.4–15.9	8.6	30.7
Number of pods per cluster	2.6–9.5	5.4	24.8
Number of seeds per pod	2–5	4.0	12.2
100-seed weight (g)	17.3–48.0	28.9	23.8
Fresh pod yield per plant (g)	338.6–1573.1	847.7	30.8

Table 3. Distribution of hyacinth bean accessions in different clusters by district of origin in Bangladesh.

Cluster number	Number of accessions	Districts in Bangladesh							
		Sirajganj	Faridpur	Rajbari	Chittagong	Meherpur	Chuadanga	Kushtia	Various
I	17	BD-7964 (1), BD-7966 (3), BD-7979 (16), BD-7981 (18)	BD-7989 (26)	BD-7992 (29)	BD-7999 (36)		BD-8040 (77)	BD-8007 (44), BD-8009 (46), BD-8010 (47), BD-8022 (59), BD-8025 (62), BD-8026 (63), BD-8028 (65), BD-8045 (82)	BD-8005 (42) – Jessore
II	23	BD-7971 (8), BD-7972 (9), BD-7978 (15), BD-7980 (17), BD-7984 (21), BD-8043 (80)			BD-7996 (33), BD-7998 (35)	BD-8029 (66), -8030 (67), -8031 (68), -8032 (69)	BD-7976 (13), BD-8035 (72), BD-8036 (73), BD-8037 (74), BD-8038 (75)	BD-8014 (51), BD-8015 (52), BD-8021 (58), BD-8024 (61), BD-8047 (84)	BD-8004 (41) – Jamalpur
III	8	BD-7982 (19), BD-8041 (78)	BD-7990 (27)					BD-8018 (55), BD-8020 (57), BD-8023 (60)	BD-7987 (24), BD-8044 (81) – Feni
IV	9	BD-7965 (2), -7974 (11), BD-7977 (14)		BD-7994 (31)	BD-8001 (38)	BD-8027 (64), -8050 (87)	BD-8034 (71)	BD-8008 (45)	
V	17	BD-7967 (4), -7968 (5), BD-7969 (6), BD-7970 (7), BD-7973 (10), BD-7983 (20), BD-8042 (79)			BD-8002 (39)	BD-8033 (70), -8051 (88)		BD-8011 (48), BD-8016 (53), BD-8019 (56), BD-8046 (83), BD-8049 (86)	BD-7986 (23) – Lakhmipur; BD-8006 (43) – Narail
VI	13	BD-7975 (12)	BD-7988 (25), BD-7991 (28)	BD-7993 (30)	BD-7995 (32), BD-7997 (34), BD-8003 (40)			BD-8012 (49), BD-8013 (50), BD-8017 (54), BD-8048 (85)	BD-8000 (37) – Bandarban; BD-7985 (22) – Mymensingh
VII	1						BD-8039 (76)		
Total no. of accessions	88	23	4	3	8	8	8	26	8

Results and discussion

The accessions showed considerable variation in all qualitative (Table 1) and quantitative (Table 2) characters assessed. Among the quantitative traits the highest coefficient of variation was found in peduncle length, followed by rachis length, number of flowering nodes per peduncle and yield per plant and the lowest was in days to first pod maturity (Table 2). These results are similar to those of Pengelly and Maass (2001) in a study of 249 accessions of lablab including both *L. purpureus* subsp. *purpureus* and subsp. *uncinatus* from Asia and Africa.

On the basis of D^2 values, the 88 accessions were grouped into seven different clusters (Table 3). Most accessions (23) were in cluster II, followed by cluster I and V (17 accessions each). Cluster VII consisted of only one accession. Accessions collected from the same geographic origin (districts) were distributed among the different clusters. This is similar to findings in previous studies, e.g. Sultana et al. (2001). The absence of a relationship between geographic origin and genetic diversity suggests that forces other than agro-ecological conditions, such as exchange of breeding material, genetic drift, variation, natural and artificial selection are responsible for diversity, as reported earlier (Murty and Arunachhalam 1966). Adaptive gene complexes evolve in plant populations restricted to small geographic areas or subjected to identical environmental pressures. These gene complexes are conserved by genetic linkages or stringent natural or human selections. The clustering of accessions from different ecogeographic locations into a single cluster could be attributed to the free exchange of breeding materials between regions (Verma and Mehta 1976).

Mean intra- and intercluster distances in the hyacinth bean accessions are presented in Table 4. The intracluster distance ranged from 0 (cluster VII with only one accession) to 1.45 (cluster III). Maximum intercluster distance was estimated between clusters III and VII (25.04), followed by clusters II and III (18.75), suggesting wide diversity between these groups. The lowest intercluster distance was between clusters IV and VI (1.93). The magnitude of heterosis and potential for transgressive segregation largely depends on the degree of genetic diversity in the parental lines (Bhadra and Akhtar 1991). This suggests that parents for hybridization should be selected from two clusters with

Table 4. Mean intra- (bold) and intercluster distances in 88 hyacinth bean accessions from Bangladesh.

Cluster number	I	II	III	IV	V	VI	VII
I	1.21	2.92	16.47	3.04	8.55	4.84	11.02
II		1.41	18.75	5.17	11.02	7.10	8.14
III			1.45	13.61	7.97	11.69	25.04
IV				1.21	5.86	1.93	12.49
V					1.18	3.95	18.09
VI						1.14	14.22
VII							0

Table 5. Cluster means for 15 quantitative characters in 88 hyacinth bean accessions from Bangladesh.

	Cluster						
Character	I	II	III	IV	V	VI	VII
Days to first flowering	77.5	78.5	77.4	74.3*	76.1	77.9	90.0**
Days to first fresh pod harvest	109.4	114.8	111.1	105.1*	111.5	110.5	133.0**
Days to first pod maturity	138.4	140.4	139.9	135.7*	139.9	141.9**	141.0
Terminal leaflet length (cm)	9.0	8.9	9.3**	9.0	9.3**	9.1	7.9*
Terminal leaflet width (cm)	8.8	8.3	8.7	8.6	8.9**	8.6	7.3*
Rachis length (cm)	9.8**	6.0	5.5	9.5	7.2	8.7	4.4*
Peduncle length (cm)	17.30	12.3	10.8	22.2**	13.6	19.6	8.4*
No. of flowering nodes per peduncle	5.2	4.1	3.6	5.4**	4.0	5.0	3.5*
Fresh pod length (cm)	9.4	8.4	12.9**	10.1	10.7	10.1	5.3*
Fresh pod width (cm)	2.3	2.1	2.5	2.2	2.9**	2.3	1.6*
Fresh pod weight (g)	7.2	6.0	14.5**	8.4	10.8	9.2	3.4*
Number of pods per cluster	5.2	5.7	4.5*	6.1	4.6	5.7	7.6**
Number of seeds per pod	4.0	4.0	4.7**	3.8	4.2	4.0	2.1*
100-seed weight (g)	27.2	25.7	28.1	30.8	32.5**	32.1	17.3*
Fresh pod yield per plant (g)	709.3	591.3	1434.5**	828.4	1069.0	907.8	338.6*

Within rows, * and ** indicate minimum and maximum cluster mean value, respectively.

wide intercluster distance to get more variability among the segregants. However, yield potential should not be ignored in selecting parental lines.

Accessions in cluster III showed greatest terminal leaflet length, fresh pod length, pod weight, number of seeds per pod and yield per plant (Table 5) and thus must be considered useful parental material.

PC1 was heavily weighted by terminal leaflet length, terminal leaflet width and fresh pod weight while PC2 was dominated by terminal leaflet width, fresh pod width and number of seeds per pod (Table 6).

The cluster constellations obtained by D^2 analysis were confirmed by canonical analysis. Cluster constellations were also independently derived using principal component analysis (PCA) to verify grouping obtained through D^2 statistic in a two-dimensional chart (PC1–PC2). In PCA the first two components were observed to explain 57.25% of variation and the first three components accounted for 75% of the total variation. A similar observation was reported by Chowdhury and Mian (1996) in field pea. Accession scores obtained for the first two components were plotted on two main axes and then superimposed on the clustering found from the D^2 analysis (Figure 1), showing similar results. D^2 and PCA are thus alternative methods for deriving clusters. The scatter diagram suggests that accessions in cluster VI were most similar and those in cluster III were the most heterogeneous. This is confirmed by the clustering pattern obtained from D^2 analysis.

Conclusion

This study found considerable variability in hyacinth bean accessions in Bangladesh. The 88 accessions were grouped into seven clusters. Crosses between accessions belonging to widely divergent clusters would be expected to generate maximum heterosis and variation in subsequent generations.

Table 6. Relative contributions of different characters to the total divergence in 88 hyacinth bean accessions from Bangladesh.

Character	PC1	PC2
Days to first flowering	-0.061	-0.054
Days to first fresh pod harvest	0.033	0.079
Days to first pod maturity	0.031	0.011
Terminal leaflet length (cm)	-0.782	0.878
Terminal leaflet width (cm)	0.523	-1.016
Rachis length (cm)	-0.073	-0.125
Peduncle length (cm)	0.032	0.140
No. of flowering nodes per peduncle	-0.154	-0.826
Fresh pod length (cm)	0.119	-0.104
Fresh pod width (cm)	0.278	-1.364
Fresh pod weight (g)	1.073	0.203
Number of pods per cluster	-0.073	0.645
Number of seeds per pod	-0.172	-1.249
100-seed weight (g)	0.028	-0.106
Fresh pod yield per plant (g)	0.011	0.002

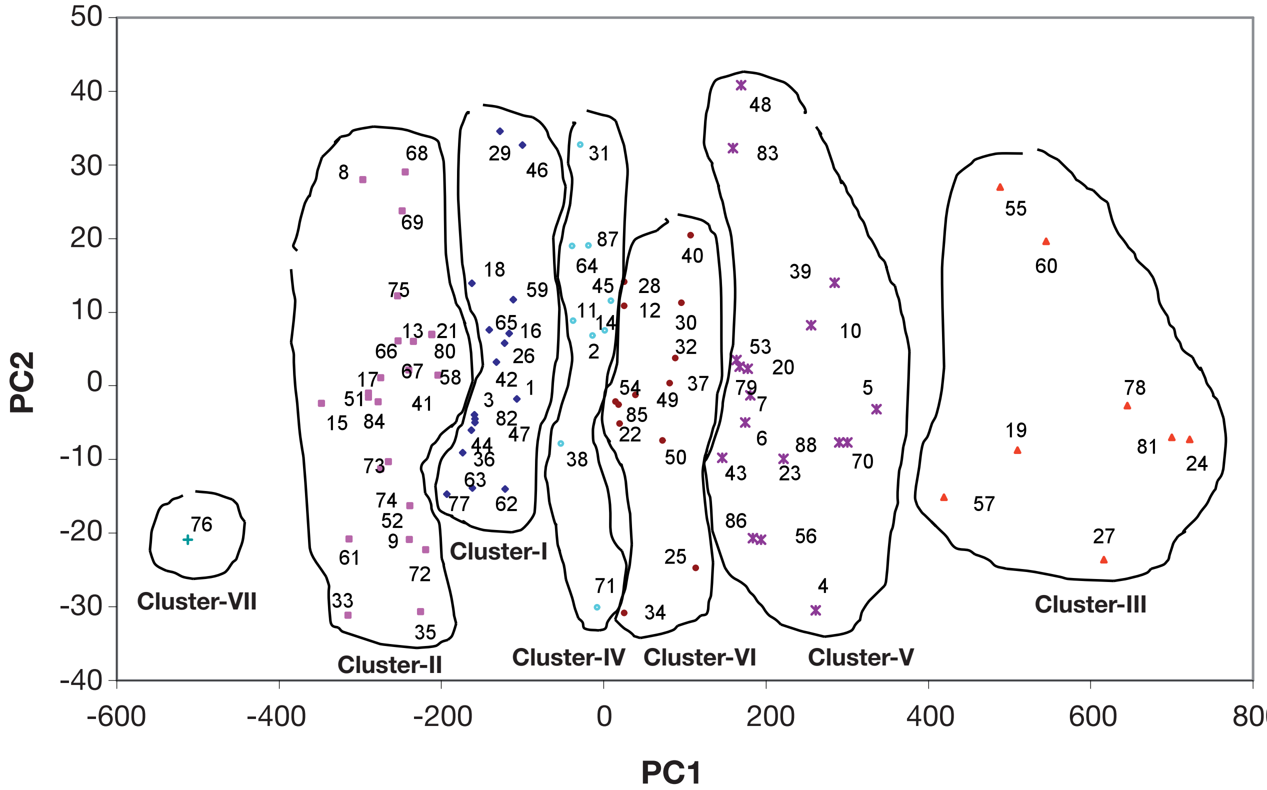

Figure 1. Canonical diagram of the first two canonical vectors showing the relative positions of 88 hyacinth bean accessions from Bangladesh.

This suggests that crosses should be made between the accessions in cluster III with accessions in cluster VII and cluster II.

Lablab is considered a neglected or underutilized crop and the ultimate goal is to develop a core collection of this species to enhance its utilization. This study has demonstrated considerable morpho-agronomic diversity in the lablab collection at the PGRC of BARI. However, this needs further investigation using molecular techniques in order to identify material that could be added the core collection proposed by Pengelly and Maass (2001). This effort would benefit from an international collaborative research project on germplasm collection, evaluation and exchange.

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References

Arora RK. 1991. Plant exploration and germplasm collection. In: Paroda RS and Arora RK, editors. Plant Genetic Resources Conservation and Management. IBPGR, Regional Office, New Delhi, India, pp. 55–88.

BBS. 2004. Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh, p. 106.

Bhadra SK, Akhtar MI. 1991. Genetic divergence for yield and its morphological components of mungbean (*Vigna radiata* (L.) Wilczek). SABRAO Journal of Breeding and Genetics 23(2):127–136.

Chowdhury MAZ, Mian MAK.1996. Genetic divergence in field pea (*Pisum sativum* L.). Annals of Bangladesh Agriculture 6:1–6.

Duke JA, Kretschmer AE Jr, Reed CF, Weder JKP. 1981. *Lablab purpureus* (L.) Sweet. In: Duke JA, editor. Handbook of Legumes of World Economic Importance. Plenum Press, New York, USA and London, UK, pp. 102–106.

Genstat 5. 1998. Fifth Edition (Beta) Procedure Library Release PL11 (Release 4.1). Laws Agricultural Trust, IACR-Rothamsted, UK.

Islam MT. 2004. Characterization of hyacinth bean (*Lablab purpureus* (L.) Sweet). In: Annual Research Report—2003–04. Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh, pp. 41–59.

Islam MT, Haque MM, Rahman MM. 2002. Catalogue on Hyacinth bean (*Dolichus lablab* (L.)). Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.

Islam MS, Satter MA, Rahman MM, Quyum MA, Alam SMN, Alam MS, Mustafi RA. 2004. Handbook of Agro-technology (3rd ed.). Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.

Joshi S, Sreekantaradhya R, Shambulingappa KBG, Shivashankar G, Jagannath DP. 1995. Genetic variation in pod characteristics in fieldbean (*Lablab purpureus* (L.) Sweet) and its relation to classification as subspecies. Crop Research (India) 9(1):78–80.

Liu CJ. 1996. Genetic diversity and relationship among *Lablab purpureus* genotypes evaluated using RAPD as markers. Euphytica 90:115–119.

Maass BL, Jamnadass RH, Hanson J, Pengelly BC. 2005. Determining sources of diversity in cultivated and wild *Lablab purpureus* related to provenance of germplasm by using amplified fragment length polymorphism. Genetic Resources and Crop Evolution 52:683–695.

Murty BR, Arunachalam V.1966. The nature of divergence in relation to breeding system in some crop plants. Indian Journal of Genetics 26:188–198.

Pengelly BC, Maass BL. 2001. *Lablab purpureus* – diversity, potential use and determination of a core collection of this multi-purpose tropical legume. Genetic Resources and Crop Evolution 48:261–272.

Schaaffhausen RV. 1963. *Dolichos lablab* or hyacinth bean: its uses for feed, food and soil improvement. Economic Botany 17:146–153.

Shivashankar G, Kulkarni RS. 1992. *Lablab purpureus*. In: van der Maesen LJG, Sadikin Somaatmadja, editors. Plant Resources of South-East Asia (PROSEA). No. 1. Pulses. Pudoc, Wageningen, The Netherlands, pp. 48–50.

Sultana N, Ozaki Y, Okubo H. 2001. Morphological and physiological variation in lablab bean (*Lablab purpureus* (L.) Sweet). Journal of the Faculty of Agriculture, Kyushu University 45 (2):465–472.

Venkatesha SC, Gowda MB, Mahadevu P, Rao AM, Kim D-J, Ellis THN, Knox MR. 2007. Genetic diversity within *Lablab purpureus* and the application of gene-specific markers from a range of legume species. Plant Genetic Resources: Characterization and Utilization 5(3):154–171.

Verdcourt B. 1970. *Lablab* Adans. In: Studies in the Leguminosae–Papilionoideae III. Kew Bulletin 24:409–411.

Verma VS, Mehta RK. 1976. Genetic divergence in lucerne. Journal of the Maharashtra Agricultural University 1:23–28.

Wang ML, Gillaspie AG, Newman ML, Dean RE, Pittman RN, Morris JB, Pederson GA. 2004. Transfer of simple sequence repeat (SSR) markers across the legume family for germplasm characterization and evaluation. Plant Genetic Resources: Characterization and Utilization 2(2):107–119.

Wang ML, Morris JB, Barkley NA, Dean RE, Jenkins TM, Pederson GA. 2007. Evaluation of genetic diversity of the USDA *Lablab purpureus* germplasm collection using simple sequence repeat (SSR) markers. Journal of Horticultural Science and Biotechnology 82(4):571–578.

Zhong-cheng T, Shu-yao W, Wen-long W, Liu L. 2005. Study of the diversity of germplasm resources of *Dolichos lablab* L. Natural Science Journal of Hainan University 23:53–60.