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Cover:
A close-up of *Phaseolus vulgaris* var. *aborigineus* flowers, discussed by Drewes on pp. 9-14.
Photo: Susana Drewes.

Couverture :
Fleurs de *Phaseolus vulgaris* var. *aborigineus*, article de Drewes pp. 9-14.
Photo: Susana Drewes.

Portada:
Primer plano de flores de *Phaseolus vulgaris* var. *aborigineus*, tema del artículo de Drewes, pp. 9-14.
Fotografía: Susana Drewes

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

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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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Geographical distribution of the Andean lupin (*Lupinus mutabilis* Sweet)

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Summary

Geographical distribution of the Andean lupin (*Lupinus mutabilis* Sweet)

Lupin (*Lupinus* spp.) is a globally important crop. The Andean lupin or tarwi (*Lupinus mutabilis* Sweet) originated in the Andean area of South America. It is the only American type of the genus *Lupinus* domesticated and cultivated, distributed from Colombia to the north of Argentina, although currently it is only of importance in Ecuador, Peru and Bolivia. Recently, interest has increased in Europe due to its high nutritional quality, as a valuable source of protein and oil, with an oil content of 14–24% and a protein content of 41–51%. It has great market potential for human use, and as an animal feed. However, some unfavourable characteristics have hindered the propagation of the crop, such as an indeterminate growth habit and a high alkaloid content. Scientists working with lupins are organized in the International Lupin Association (ILA), which among its objectives has the promotion of international cooperation. Only few scientists work with *Lupinus mutabilis*, but interest seems to be increasing. The main potential of the Andean lupin is in the Andean region of South America, where it should be possible to increase production and develop markets. Ecuador has done most, but the crop could be promoted also in Peru and Bolivia. Recently a workshop was held in Bolivia, with participants from universities and research institutions of Bolivia and neighbouring Peru and Argentina, in order to define minor crops of major potential. Andean lupin was selected as one of the crops on which to focus.

Key words: Andean crop, lupin, pearl lupin, small crop, tarwi, underutilized species.

Résumé

Distribution géographique du lupin andin (*Lupinus mutabilis* Sweet)

Le lupin andin ou tarwi (*Lupinus mutabilis* Sweet) est originaire de la zone andine de l'Amérique du sud. C'est le seul type américain du genre *Lupinus* domestiqué et cultivé. Son aire de répartition s'étend de la Colombie au nord de l'Argentine, mais il est surtout cultivé en Équateur, au Pérou et en Bolivie. Le lupin (*Lupinus* spp.) est une ressource agricole importante à l'échelle mondiale. L'Europe s'y intéresse pour ses qualités nutritionnelles. Avec une teneur en huile de 14–24 % et en protéines de 41–51 %, il présente un potentiel important en alimentation humaine et animale. Cependant, son mode de croissance indéterminé et une teneur élevée en alcaloïdes freinent son développement. L'Association internationale du lupin (ILA) s'efforce de promouvoir la coopération internationale de la recherche sur cette espèce. Le potentiel principal de *L. mutabilis* se situe dans la zone andine, où il est possible d'accroître la production et de développer les marchés. L'Équateur s'est le plus investi, mais la culture pourrait également être encouragée au Pérou et en Bolivie. Un atelier s'est tenu en Bolivie avec des participants péruviens et argentins, afin de définir les cultures mineures présentant le meilleur potentiel. Le lupin andin a été sélectionné comme l'une des cultures sur lesquelles les efforts doivent se concentrer.

Resumen

Distribución geográfica de lupino de los Andes (*Lupinus mutabilis* Sweet)

El lupino (*Lupinus* spp.) es un cultivo importante en todo el mundo. El lupino de los Andes o "tarwi" (*Lupinus mutabilis* Sweet), originario de la zona andina de América del Sur, es el único tipo americano del género *Lupinus* domesticado y cultivado desde Colombia hasta el norte de Argentina, aunque en la actualidad solo es importante en Ecuador, Perú y Bolivia. En Europa aumenta el interés por su cualidad nutricional como fuente de proteína y aceite para uso humano y como alimento para animales: 41-51% de proteína y 14-24% de aceite. Algunas características desfavorables obstaculizan la propagación del cultivo: hábitos inciertos de crecimiento y un alto contenido alcalino. Entre los objetivos de la Asociación Internacional del Lupino figura la promoción de la cooperación internacional. Pocos científicos trabajan con *Lupinus mutabilis*, pero el interés está aumentando. El mayor potencial del lupino de los Andes está en la región andina de América del Sur, donde es posible aumentar la producción y desarrollar mercados. Ecuador es el que más ha hecho, pero el cultivo también puede ser promovido en Perú y Bolivia. Recientemente se celebró un taller con participantes de universidades e instituciones de investigación de Bolivia, Perú y Argentina para definir cuáles son los cultivos menores con mayor potencial: el lupino de los Andes fue seleccionado entre ellos.

Distribution

Tarwi, tauri, chocho or pearl lupin (*Lupinus mutabilis* Sweet) is a legume cultivated in the Andean area of South America, and is of great importance in Ecuador, Peru and Bolivia. The countries where the species is regarded as of first priority are Peru, Bolivia, Ecuador and Chile, while in Argentina and Colombia it is of medium priority (FAO 1986). We estimate that the total area of tarwi is about 10 000 ha (Jacobsen and Mujica 2004).

The Andean lupin, described in Jacobsen and Mujica (2007), has less importance in South America than other Andean grain crops of increasing potential for production and commercialization on the national and international markets, compared with quinoa (Jacobsen and Mujica 2002) and amaranth (Jacobsen and Mujica 2003).

Lupin (*Lupinus* spp.) is a globally important crop. Tarwi (*Lupinus mutabilis* Sweet) originated in the Andean area of South America (Dávila 1987). It is the only American member of the genus *Lupinus* that has been domesticated and cultivated (Blanco 1982). It is distributed from Colombia to the north of Argentina, although currently it is only of agricultural importance in Ecuador, Peru and Bolivia. Recently, interest has increased in Europe due to its high nutritional quality, as a valuable source of protein and oil, with an oil content of 14–24% and a protein content of 41–51% (Gross et al. 1988).

Research has been carried out for 40 years on the Andean lupin in Peru and other Andean countries (Mujica et al. 2001, 2002). The germplasm collection in Peru consists of 1200 accessions, with vegetative periods of 140–230 days, seed yields of 800–2700 kg/ha, protein contents of 35–45% and oil contents of 15–23%. Tarwi fixes 160–220 kg N/ha, for a water consumption of 656 mm, according to Penman (1948). The main pests affecting the crop are *Copitarsia turbata* H.S., *Agromyza* sp., *Frankliniella tuberosi* Moulton and *Myzus* sp., while major diseases are *Colletotrichum gloeosporioides*, *Uromyces lupini* and *Fusarium oxysporum* (Mujica et al. 2002).

Although institutions in several countries maintain *Lupinus* accessions, cultivated material, and especially wild types from many areas, remain uncollected (Table 1).

In Peru, tarwi is mainly grown around Lake Titicaca, from Ilave to the border with Bolivia in Desaguadero, and in small areas in Cajamarca in northern Peru and Cusco in the south. The main production centres in Ecuador are Cotopaxi, with 2150 ha and 484 t (225 kg/ha); Chimborazo with 1013 ha and 230 t (227 kg/ha); and Pichincha with 585 ha and 190 t (325 kg/ha) as annual averages. In addition, in Ecuador, *chocho*, as tarwi is known locally, is produced in the provinces of Carchi, Imbabura, Tungurahua and Bolivar. The yields are low, only about 250 kg/ha (INIAP 1997).

It is estimated that Ecuador has almost 32 000 ha suitable for production of chocho, 17 000 ha have certain limitations, and a further 55 600 ha have major limitations (Table 2). The limitations refer in general to the presence of biotic and abiotic adverse factors, including pests, frost and steep slopes. (INIAP 1997).

Studies have been initiated to evaluate the potential of *L. mutabilis* in Europe. Cultivation of the Andean lupin under European conditions has often been characterized by a low seed yield and a long vegetation period (Sawicka-Sienkiewicz and Augiewicz 2002). Interspecific hybrids were studied in order to evaluate the chromosome number, which is 2n=48 for *L. mutabilis* and 2n=50 for *L. albus*. Hybrids mostly had 48 chromosomes, independent of the mother, and they were usually morphologically similar to the mother. The first hybrids between *L. polyphyllus* and *L. mutabilis* have been developed, with dominant characters of pod shatter, bitterness and colour (von Baer 2002). The challenge is to achieve early varieties that are sweet, with high protein content (up to 50%) and high oil content (up to 25%), but there is a negative

Table 1. Main genebanks conserving Andean lupin (*Lupinus mutabilis* Sweet) in the Andes.

Country	Genebank	No. of accessions	Approx. no. of acc. still to be collected
Peru	1. Centro de Investigación en Cultivos Andinos (CICA), Cusco. E-mail. aalvarezcaceres@yahoo.es	1800	150
	2. Centro de Investigación y Producción, Camacani-UNA, Puno. E-mail: amhmujica@yahoo.com	260	200
	3. E.E. Santa Ana, INIA-Huancayo	347	250
Bolivia	1. Foundation PROINPA (Promocion de Investigaciones en Productos Andinos), Calle Conchitas 790, piso 3, La Paz. Email: w.rojas@proinpalp.org	20	400
	2. Centro Investigaciones Fitoecogenéticas de Pairumani–CIFP, Cochabamba.	114	
	3. Universidad Mayor de San Andrés (UMSA), La Paz.	340	
Ecuador[†]	Instituto de Investigaciones Agropecuarias (INIAP), EE. Santa Catalina, Quito. E-mail: denaref@ecnet.ec	381 (257 from Ecuador)	200
Chile	Universidad Temuco.	180	80
Argentina	Instituto Nacional de Tecnologia Agropecuaria (INTA), Instituto de Recursos Biológicos, Las Cabañas y los Regeros s/n, 1712 Castelar, Buenos Aires. E-mail: ysidro@cirn.inta.gov.ar	180 (4 from Argentina)	20–100
Colombia	1. Universidad Nacional de Colombia (UNC), Facultad de Agronomía, A.A. 14490, Bogotá. E-mail: guiart7@LatinMail.com	20	50
	2. Instituto Colombiano Agropecuario (ICA), Tibaitatá, AA 151123, Bogotá.	40	

Sources: Thanks to personal comments and estimations from Angel Mujica, UNAP; Wilfredo Rojas, PROINPA; Eduardo Peralta, INIAP; Guillermo Corredor, UNC; and Daniel Bertero, UBA. [†] Data from INIAP 1998.

Table 2. Potential area (hectares) for the production of *chocho* (*L. mutabilis* Sweet) in Ecuador.

Production areas	No limitations	Minor limitations	Major limitations
1. Ibarra-Otavalo	938	911	2 576
2. Otavalo	0	1 324	990
3. Cayambe-Cangahua	4 011	230	4 933
4. Sigchos-Mulaló	5 704	7 323	7 556
5. Latacunga-Salcedo	12 223	3 054	6 993
6. Quero-Guano	4 946	2 988	16 526
7. Riobamba-Guamote	3 431	1 180	11 362
8. Tixán-Palmira	452	153	4 836
Total	31 704	17 162	55 771

Source: INIAP 1997.

correlation between protein and oil content. Determinate types should be used in crossings to secure earliness.

In Spain, work has focused on the species *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis* (López-Bellido et al. 1982; Guerrero 1982). Andean lupin did not function as a winter crop under the conditions in Córdoba (López-Bellido and Fuentes 1990).

Adaptation

While seed quality of *L. mutabilis* is well documented, little information exists on morphological characters, growth duration, dry matter (DM) production and seed yield. *L. mutabilis* has an indeterminate growth habit, where the plants produce a principal stem, ending in a terminal inflorescence, with branches appearing after flowering (Blanco 1982; Neves-Martins et al. 1992). Great variation has been observed in the number of branches (0 to 52; Blanco 1982) and in plant height (0.23–2.25 m; Neves-Martins et al. 1992). These morphological characters are influenced by genotype and climatic conditions. In years of drought or in dry regions, the plants are smaller with fewer branches, and mature earlier. The architecture of the plant seems to be closely related to earliness (Blanco 1982; Lenoble 1982; Huyghe 1992).

L. mutabilis is adapted to a temperate, neither too dry nor hot, climate, and is relatively susceptible to frost. Temperatures below -2°C cause plant death, especially if occurring during the first growth stages (Blanco 1982). This susceptibility limits its use as a winter crop. With spring sowing, the problem is slow initial growth, so that flowering often coincides with the summer drought, as for instance in southern Europe and in Australia. At the same time, dry conditions reduce the problem of indeterminate growth, but also reduce yield potential considerably.

In Europe, seed yield of Andean lupin is limited by competition between plants for nutrients, and the low DM yield. Despite the indeterminate growth of *L. mutabilis*, the leaf area is small. Leaf development is very slow during the early growth stages, and the crop does not cover the soil until by the end of the vegetative growth period (Hardy et al. 1997). The duration of the remaining period with a complete crop cover was too short to produce a satisfactory DM yield (Hardy et al. 1997).

Despite the high oil and protein content, the potential for increased production of tarwi is limited due to its low yield and its indeterminate growth. Tarwi yielded less in Europe (ca 1 t/ha) compared with other legumes (Table 3). However, the results are based on two very dry years. Other studies have shown higher yields, for example in England, where Masefield (1976) found yields of 1.8 to 6.0 t/ha when sowing in March, while the same genotypes produced 0.5–3.5 t/ha when sown in April. In Germany, tarwi yielded 0.68–3.0 t/ha (Weißmann and Weißmann 1992; Romer and Jahn-Deesbach 1992). Rubenschuh (1997) reported yields of 1.8–6.5 t/ha in 1991 and 0.2–2.4 t/ha in 1992. In Spain, the yield was 0.2–0.5 t/ha (López-Bellido 1992), and in France 1.0–2.5 t/ha (Lenoble 1982). In the Andes, the yield varies from 0.5 to 3.5 t/ha, with an average of 0.9–1.3 t/ha (González 1986). These results show that the productivity of *L. mutabilis* is unstable and highly influenced by environmental conditions (Caligari et al. 2000). The relatively low yields are due to the low number of pods and the low number of seeds per pod (Lenoble 1982; López-Bellido and Fuentes 1990; Romer and Jahn-Deesbach 1992). Flower loss also causes yield loss, reaching 59–73% (Neves-Martins and Silva 1994). The flower loss can be related to development of lateral branches, as also seen in *L. angustifolius*, *L. luteus* and *L. mutabilis* (Porter 1982).

Another problem with tarwi is the harvest index (HI), which was only 0.09–0.33 in several cultivars studied. In addition, 41–56% of pod dry weight was located in the pod walls (Hardy and Huyghe 1997).

Table 3. Average yield of seed (t/ha) for 16 genotypes of tarwi in Europe. Data from project Final Report (EU 1997).

	1994	1995	Average
France	1.8	1.2	1.5
Germany	0.8	0.5	0.7
Poland	0.4	0.9	0.7
Portugal	2.1	0.7	1.4
United Kingdom	0.9	1.4	1.2
Average			1.1
Average best cv.			1.2
Average worst cv.			0.7

However, a number of characteristics make tarwi a promising crop. Among these are the high nitrogen fixation capacity—from 120 to 160 kg N/ha per year—and a grain of high biological value, with 40% protein and 20% oil. Moreover, the plant can be incorporated into the soil as organic matter.

The DM and seed yield was studied in two genotypes of early (LM34) and late (LM268) maturity in 1994–95, at two sowing densities (35 and 55 seeds/m^2) in Lusignan, France (Hardy et al. 1997). DM yield was on average 6800 kg/ha. The main stem and the first order branches contributed most to the biomass. Pods were only produced on the main stem. The maximum leaf area index (LAI) was 2.8. LAI did not correlate with DM production, which varied with genotype. LM34 showed better growth of pods, its harvest index was 0.32 and seed yield on average was 1.28 t/ha. LM268 had more vegetative growth, and both HI and seed yield were lower (0.16 and 1.13 t/ha, respectively). The yields were similar at the two plant densities. Neither genotype showed translocation of assimilates from stem to pods (Hardy et al. 1997). LM34 gave a relatively stable seed yield, and its HI was equivalent to that of other indeterminate lupins. LM268 had better growth of pods and seeds, but vegetative growth stopped, so the plant did not mature. In hot and dry conditions, growth and formation of pods were reduced due to abortion of flowers and seeds (Hardy et al. 1997).

In Europe and Western Australia, *L. mutabilis* cannot compete in yield with other crops unless the seed quality has an additional value sufficient to compensate for the lower yield. In order to increase the potential for DM production of tarwi, an increase in LAI should be considered, for instance by increasing sowing density, which will cause an accumulation of green matter in stem and first-order branches, and little development of upper branches. Another possibility would be to modify the sowing date. It is not possible to sow before spring. A delayed sowing implies the development of more first-order branches; however, as flowering may also be delayed, it may coincide with a period of drought, so that maturity is delayed. A third solution would be to screen the available variability for intermediate genotypes, which develop sufficient, but not too much biomass. Such a genotype would require a vegetative development sufficient for capturing light over a long period, and thus optimizing biomass yield. Greater modifications of plant structure have little probability of increasing the yield of *L. mutabilis,* despite ensuring seed harvest, because it will also reduce the leaf area and limit the potential for biomass production. Modifications, such as a shorter phyllochron to reduce time to anthesis, could have an effect. This means that mechanisms that define plant structure, and the relationship between structure of the crop and the efficiency of light interception, should be analysed in order to decide whether *L. mutabilis* could be an alternative for areas outside the Andes (Hardy et al. 1997).

It was demonstrated that seed yield of *L. mutabilis* was limited in Europe due to low DM production. Twelve genotypes were sown on eight dates in five locations (France, United Kingdom, Germany, Poland and Portugal) in 1994 and 1995 (Hardy et al. 1998). The widest variation was observed for phenological and morphological characters. The variation in number of principal leaves was partly affected by temperature. Height and date of flowering were related to the number of leaves, but the time of flowering was also affected by environmental conditions. The number of branches was determined by the environmental conditions during growth. The number of leaves in the second branch of the first order was less susceptible to the environmental conditions than the number of leaves on the main stem. The heritabilities were high for all characters except for the number of leaves on the second branch of first order, and the interactions between genotype and environmental condition. It was concluded that it is possible to improve *L. mutabilis* by modifying its morphology (Hardy et al. 1998).

However, unstable yield and indeterminate maturity are the principal factors that limit the introduction of *L. mutabilis* to Europe. Before beginning a breeding programme, it is important to study carbon partitioning and to analyse its consequences for maturity and seed yield (Hardy et al. 1997).

A more fundamental analysis of the physiological mechanisms that define the structure of the plant and the function of the phyllochron, and a comprehension of the relationship between the main stem and branches, would lead to a better understanding of the optimal phenology of the plant for each environmental condition (Hardy et al. 1998).

Breeding

Andean lupin has a very long growth period, the reason for which is its indeterminate growth habit, with a continuous production of lateral branches. Selection of early maturing material of *L. mutabilis* began in Germany in 1983, and through conventional breeding a new variety, cv. Inti, was developed with 51% of protein and 16% oil (Gross et al. 1988). The most surprising result was the reduction of the alkaloid content to 0.0075%, which is even lower than the sweet lupin (0.02%) (Pearson and Carr 1977).

For the purpose of obtaining early maturing material, variability was created through induced chemical mutations (Romer et al. 1996). The two populations in M$_3$ showed genetic variability, with promising characteristics such as earliness and short plant stature. Plant height is related to earliness, so that short plants tend to be earlier. The selected plants had smaller seeds (Silva et al. 1996). Interspecific crossings were performed with a perennial lupin (*L. polyphyllus*), which is early, but no pods contained seeds. It seemed to be possible to use frozen pollen of *L. polyphyllus* (Romer 1995).

If the Andean lupin were to be introduced to Europe, it would be necessary to select the most adapted genotypes, with resistance to diseases, and begin breeding programmes using interspecific hybridization. *L. mutabilis* has been crossed with *L. termis, L. graecus* and *L. vavilovii.* Twenty seeds were obtained from 198 crosses (11%). The species differ in the number of chromosomes (*L. mutabilis* 2n=48, the others 2n=50). The same genotypes of *L. mutabilis* were crossed with several wild types from the USA (*L. elegans, L. pubescens, L. hartwegii* and *L. nanus*), from which were obtained 11 seeds from 155 flowers (7%), even though the progenitors had the same number of chromosomes (2n=48). Interspecific offspring with the desired characters were obtained, such as hybrids between the line KW1, crossed with *L. pubescens* and *L. elegans.* Thus, interspecific hybridization in *L. mutabilis* seems to be possible, but with a low efficiency (Sawicka-Sienkiewicz and Brejdak 1996).

In vitro regeneration provides a powerful tool for creating disease-free genotypes, for application of genetic transformation, and for multiplying rare breeding material (Schafer-Menuhr 1985). Furthermore, *in vitro* techniques could rescue hybrid embryos that normally do not form roots (Schafer-Menuhr 1985; Gulati and Jaiwal 1990). This regeneration technique has been used in several lupin species (Sator 1985; Schafer-Menuhr 1985; Vuillaume et al. 1985; Sroga 1987; Podyma et al. 1988; Upadhyaya et al. 1992; Pigeare et al. 1994). However, the frequency of regeneration has always been low and with problems upon transferring the plants to natural condition.

Regeneration was achieved by organogenesis of immature seeds of *L. mutabilis*, using the modified method of Schenk and Hildebrandt (1972), complemented with tidiazuron (2 mg/L). There were only successful crossings between two of the five lines evaluated, with a multiplication factor of up to 12.4. Very few offspring produced roots (Rahim and Caligari 1996).

One possibility could be the use of *L. mutabilis* as a progenitor for interspecific crossings in order to improve seed quality of other cultivated lupin species (*L. albus*, *L. angustifolius* and *L. luteus*). However, such hybridizations have not succeeded so far (Hardy and Huyghe 1997).

The optimal plant type has a determinate growth, with one or two lateral branch orders, with, for instance, eight primary branches. This type was called semi-determinate (Romer et al. 1996). It must be early, with a low alkaloid content. A semi-determinate plant, that is one with a determinate habit with additional primary branches, may be the most appropriate form for the future (Caligari et al. 2000).

Incidence of pests and diseases

The principal factor limiting production of *Lupinus* spp. is the disease anthracnose, which is now common in *L. albus* in Europe (Gondran et al. 1996), in North and South America (von Baer and Hashagen 1996), and recently in Australia (Dept. of Agriculture 1996). *L. mutabilis* is also very susceptible to this disease. It was previously reported that anthracnose was caused by the fungus *Colletotrichum gloeosporioides* (Gondran et al. 1994), but there is now proof that the causal organism is the fungus *C. acutatum* (Gondran et al. 1996). The disease spreads rapidly in the field if moisture is present. There is a need for strategies to control this disease if lupin production shall succeed. Blanco (1982) demonstrated genetic differences with respect to anthracnose in *L. mutabilis* in Peru, but such differentiated behaviour was not seen in the European material.

In order to control the disease, it is recommended to use high-quality, disease-free seed, and to select for resistance. With no differences in behaviour against anthracnose, it could be necessary to treat the seeds with fungicides. Results from France showed that a mixture of Carbendazime and Iprodiona was effective (Gondran et al. 1990). A treatment with Landor C (Fludioxonil + Difenoconazole) was evaluated, but was only effective if the contamination of seeds did not exceed 2% (Romer 1997). Application of Landor C in combination with Harvesan (Carbendazime + Flusilazole) should begin in the four-leaf stage and be repeated every three weeks.

A preventive fungicide application followed by continuous applications, especially under humid conditions, is efficient. However, this strategy is too expensive in commercial fields, and obviously not sustainable, and not possible in organic agriculture. The key to control the disease is seed production of good quality, which will give farmers the possibility of producing Andean lupin without the use of pesticides. Simultaneously, plant breeding programmes should include disease resistance. This is obviously a long-term process, and its success uncertain.

In Japan, there were problems with blight and root rot in tarwi. Soil sterilization was recommended, and when sowing crops susceptible to this disease, it was done in colder seasons or areas (Sato et al. 1999). Root rot was also seen in the USA. Several pathogenic fungi were isolated from lupin grown in Minnesota. *Fusarium* sp. was associated with root rot symptoms; *Rhizoctonia* sp. attacked parts of the lower stem; and *Ascochyta* sp. caused a necrosis of the stem and pod lesions. Seed treatments were usually ineffective. The use of adequate cultural controls, such as crop rotation and clean seed, can reduce the risk of loss caused by diseases (Putnam 2001).

The area of the Peruvian-Bolivian altiplano, with its altitude of 3800 masl and its harsh climatic conditions, is regarded as disease-free for tarwi. Therefore it is an excellent area for seed production of disease-free seed (Lescano et al. 1991).

Photoperiod response

Photoperiod sensitivity to grain filling has an important function in the adaptation of plants to the Andean environment, which is a climate characterized by drought and by frost towards the end of the growing season. The sensitivity promotes accelerated grain filling when the daylength is short. However, this character can limit the adaptation of tarwi to higher latitudes. The adaptation to high latitudes should thus consist of a selection for less sensitivity to daylength effects on grain filling. Knowledge of the variation in the sensitivity to daylength and its genetic basis makes it possible to obtain genotypes for high latitudes with little or no sensitivity, and cultivars in the Andes with greater sensitivity.

Oil content of tarwi cultivated in Europe was less than in its region of origin, due to climatic factors (FAO 1982). Hackbarth (1961) mentioned that *L. mutabilis* was neutral to daylength, but it was observed that 7 of 12 cultivars presented a higher oil content in short days. Two cultivars did not show variation and three cultivars had a lower oil content. It has been shown in several species and varieties of lupin that the effect of vernalization is a reduction in the growth period and a higher yield (Krasulina 1937; Silvester-Bradley 1980). In tarwi, no reaction to vernalization was observed.

The environmental response should be quantified by a daily registry of maximum and minimum temperature and calculations of the daylength (Charles-Edwards et al. 1986). Data on the locality of origin, such as latitude, longitude and altitude, should be recorded, as well as historical data on precipitation, number of days without frost, intensity of frost, potential evapotranspiration, duration of growth season, and average temperature (FAO 1986). All these data will be useful to interpret specific responses of the genotypes (Bertero et al. 1999).

The Andean lupin is adapted to a temperate climate and is strongly influenced by daylength. In its region of origin, the Andes, *L. mutabilis* is cultivated at altitudes up to 3800 masl. *L. mutabilis* is resistant to frost during the period of grain filling, while earlier in its growth it is sensitive. *L. albus* resists temperatures below 0°C (FAO 1982). In its area of origin, plant development is affected by low temperatures, especially towards maturity. However, in Europe, low temperatures affect *L. mutabilis* during the first growth phases, causing plant death (López-Bellido 1992). This limits the adaptation of *L. mutabilis* to latitudes different from the area of origin (von Baer and von Baer 1988). The economic viability of *L. mutabilis* in regions with winter crops would require improved tolerance to low temperatures during the initial growth stages.

Uses

The slopes of the region of Cochabamba, Bolivia, between 2500 and 4000 masl, are characterized by a multitude of microclimates for crop production. The poor productivity of food crops in this region has been associated with a reduction in soil fertility. The time allowed for fallow has declined as more soil is demanded for crop production. The use of legumes as cover crops during the fallow period, in order to recover soil fertility, could be an option (Wheeler et al. 1999). For high elevations near 3800 m, only *Vicia villosa* subsp. *dasycarpa* and *V. faba* of Bolivia are potentially adapted. These two species, in addition to *L. mutabilis* and *V. faba* of Nepal, are crops of potential cover for use to around 3500 masl.

The biological value of lupin protein was determined. In bitter varieties of *L. albus*, *L. angustifolius*, *L. consentinii* and *L. mutabilis* the seeds have to be boiled and washed in order to eliminate the alkaloids (Savage et al. 1982). A digestibility test varied between 95% in *L. mutabilis* and 80% in *L. angustifolius*, and the biological value of *L. mutabilis* was also the highest, at 75%. The biological value of all species was improved by the addition to 0.5% methionine to the diet. In Ecuador, new products have been developed, such as processed and washed seeds, to be sold as a delicacy for salads, snacks, etc.

Conclusion

Lupinus mutabilis has been an important source of protein in human nutrition for more than 2000 years. Today its cultivation in the Andean region is restricted to small fields as a subsistence crop. The composition of the seeds, the almost neutral photoperiodic requirement, the white and large seeds, and the adaptation to harsh climatic conditions, makes an introduction to other parts of the world possible. A study of the adaptation of *Lupinus mutabilis* to Europe showed that it does not produce seed of a sufficiently high yield in order to become economically feasible. The reasons for the low yield are its low potential for accumulation of dry matter due to a low LAI, its indeterminate growth habit, and a high proportion of pod wall relative to seed.

The characters requiring modification in order to adapt *L. mutabilis* to European conditions are a dwarf gene in order to reduce plant height and a genotype with determinate growth and some additional compensatory branches. The discovery of a mutant with determinate growth opens up the potential for producing a crop with a new architecture that could be established in Europe.

The main potential of the Andean lupin is in the Andean region of South America, where it should be possible to increase production and develop markets. INIAP, Ecuador, has done most, and published a CD on the production, post-harvest and agro-industry of the crop (Peralta and Ayala 2001). The crop could also be promoted in Peru and Bolivia. Recently, a workshop was held in Bolivia, with participants from universities and research institutions from Bolivia and neighbouring Peru and Argentina, in order to define minor crops with a major potential for production, use and market sale. Andean lupin was selected as one of the crops on which to focus (Jacobsen et al. 2004).

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Prospección y colecta de germoplasma silvestre de *Phaseolus vulgaris* en la zona central de Argentina

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Resumen

Prospección y colecta de germoplasma silvestre de *Phaseolus vulgaris* en la zona central de Argentina

El origen americano del poroto común, *Phaseolus vulgaris* L., ha sido reconocido teniendo a *P. vulgaris* var. *aborigineus* (Burkart) Baudet como ancestro silvestre y considerando que el proceso de domesticación tuvo lugar en América Central y del Sur como dos acontecimientos separados. Los cultivares comerciales poseen una base genética bastante estrecha y la variabilidad genética presente en los bancos de germoplasma es limitada; por lo tanto, se considera de primordial importancia rascatar materiales silvestres, con el fin de preservar la diversidad. La inexistencia en bancos de germoplasma de material de la provincia de Córdoba (Argentina), motivó la búsqueda intensiva en las zonas serranas de los Departamentos: Ischilín, Cruz del Eje, Punilla, Totoral, Colón, Capital, Santa María, San Alberto y Calamuchita. Este trabajo se realizó con el objetivo de contribuir al rescate y disponibilidad de germoplasma silvestre y localizar poblaciones de *P. vulgaris* var. *aborigineus.* Como resultado de las expediciones, se encontraron dos poblaciones de poroto silvestre, ambas en el Departamento de Punilla. En estas poblaciones, el muestreo se realizó en micro sitios para cubrir las subpoblaciones locales y cada planta recolectada fue numerada; se colectaron 122 entradas de poroto silvestre y se hicieron 8 ejemplares de herbario. Los materiales colectados se caracterizaron utilizando descriptores de semilla. En el área explorada no se registraron cultivos de poroto ni otras especies de *Phaseolus*. Debido al aumento de la ganadería, al desmonte y los incendios de la zona, estas poblaciones se encontrarían en peligro de extinción.

Résumé

Prospection et collecte de matériel génétique sauvage de *Phaseolus vulgaris* dans la zone centrale de l'Argentine

Phaseolus vulgaris var. *aborigineus* (Burkart) Baudet, ancêtre du haricot commun, a été domestiqué en Amérique centrale et du sud, en deux étapes séparées. Les cultivars commerciaux ont une base génétique relativement étroite et la variabilité génétique dans les banques de gènes est limitée. Il est donc primordial de préserver le matériel sauvage et la diversité. Une recherche intensive a été réalisée dans les collines des départements de Ischilín, Cruz del Eje, Punilla, Totoral, Colón, Capital, Santa María, San Alberto et Calamuchita, dans la province de Córdoba (Argentine) afin de localiser les populations de *P. vulgaris* var. *aborigineus*, de sauvegarder et de rendre disponible le matériel génétique sauvage de cette région, jusque là non représenté dans les banques de gènes. Deux populations ont été détectées dans le département de Punilla. Les échantillons collectés représentent des sous-populations locales, et chaque plante a été numérotée ; 122 accessions ont été collectées et 8 ont été conservées sous forme de spécimens d'herbier. Le matériel collecté a été caractérisé au moyen de descripteurs de semences. Aucun cultivar de haricot ou d'autres espèces de *Phaseolus* n'a été observé dans la zone explorée. En raison de l'extension de l'élevage, du défrichage et des incendies, les populations observées sont menacées d'extinction.

Summary

Prospecting for and collection of wild germplasm of *Phaseolus vulgaris* in the central zone of Argentina

The American origin of the common kidney bean *Phaseolus vulgaris* L. has been recognised as belonging to *P. vulgaris* var. *aborigineus* (Burkart) Baudet as the wild ancestor and that domestication took place in Central and South America as two separate events. Commercial cultivars have a rather narrow genetic base and genetic variability in genebanks is limited; therefore it is considered to be of fundamental importance to preserve wild material and hence preserve diversity. The absence in genebanks of germplasm from Córdoba (Argentina) prompted an intensive search in the hill areas in the Departments: Ischilín, Cruz del Eje, Punilla, Totoral, Colón, Capital, Santa María, San Alberto and Calamuchita. The aim of the study was to contribute to the rescue and availability of wild germplasm and to locate populations of *P. vulgaris* var. *aborigineus.* During the expeditions two populations of wild kidney bean were found, both of them in the Department of Punilla. The populations were sampled in microsites to include local sub-populations and each plant numbered; 122 accession of wild kidney bean were collected and 8 made into herbarium specimens. Collected material was characterized by means of seed descriptors. No cultivars of kidney bean or other species of *Phaseolus* were observed in the exploration area. Because of increased cattle farming, land clearing and fires in the area, the populations that were found are in danger of extinction.

Key words: Argentina, germplasm, *Phaseolus vulgaris, Phaseolus vulgaris* var. aborigineus, wild bean, prospection.

Introducción

El origen americano del poroto o frijol común, *P. vulgaris* L. es conocido y aceptado (Berglund Brücher y Brücher 1976; Debouck et al. 1993). Burkart (1941 y 1943) publicó los primeros informes de la existencia de plantas silvestres de poroto común en Sudamérica, específicamente en el NO de Argentina. En 1952 creó *Phaseolus aborigineus* Burk. como la forma silvestre de *P. vulgaris* que difiere por su hábito grácil, floración tardía, legumbre dehiscente y semillas pequeñas. Jaffé y Brücher (1968) realizaron comparaciones del contenido de fitohemaglutinina entre *P. vulgaris* y *P. aborigineus* colectado en los Andes venezolanos, para establecer las relaciones taxonómicas entre estas dos especies. Estos trabajos han sido utilizados como parte de los argumentos actuales sobre el origen de *P. vulgaris*, teniendo a *P. aborigineus* como ancestro silvestre y considerando que el proceso de domesticación de esta especie tuvo lugar en América Central y del Sur en dos acontecimientos separados. Baudet (1977) en estudios sobre el origen de especies cultivadas del género *Phaseolus* establece la entidad *Phaseolus vulgaris* L. var. *aborigineus* (Burk.) Baudet.

Los *Phaseolus* silvestres están distribuidos desde el norte de México hasta el centro de Argentina (Debouck et al. 1993; Beebe et al. 1995; Drewes 2006) encontrándose marcadas diferencias en las características morfológicas (Kaplan 1965; Debouck y Tohme 1989) y moleculares (Gepts et al. 1986, 1988) entre los dos extremos de su distribución geográfica. Las poblaciones silvestres de *P. vulgaris* representan un típico elemento de la vegetación climax de los bosques montañosos que cubren el lado oriental de los Andes y presentan una diferenciación regional, encontrando *P. aborigineus* var. *hondurensis* en América Central y razas locales en Colombia, Venezuela y Argentina (Brücher, 1988). Singh et al. (1991a, b) identificaron seis razas en el germoplasma de poroto, tres de origen mesoamericano y tres de origen andino. El trabajo de Debouck et al. (1993) es una importante contribución al conocimiento sobre el origen del poroto cultivado. Buscando los acervos genéticos de *P. vulgaris* investigaron detalladamente las poblaciones silvestres y cultivadas del noroeste de América del Sur (Colombia, Ecuador y Perú); concluyen que la distribución geográfica del poroto silvestre está localizada en las montañas andinas y presente en tres regiones discontinuas.

Las legumbres son una de las fuentes más importantes de la dieta de proteína en zonas tropicales, subtropicales y templadas de todo el mundo. Actualmente, cinco especies de *Phaseolus* contribuyen a satisfacer los requisitos alimenticios de la población de América Latina y otras regiones. El poroto común, *Phaseolus vulgaris* L., es una de las legumbres comestibles más importantes del mundo, y en las Américas está en segundo lugar después de la soja (Stenglein et al. 2004). El principal obstáculo de la mejora genética de las legumbres para alimento es la estrecha base genética de las plantas cultivadas existentes (Mercado-Ruaro y Delgado Salinas, 1988). Asimismo, la variabilidad genética presente en los bancos de germoplasma es bastante limitada. En este sentido, se considera de primordial importancia rescatar de los materiales silvestres, con el fin de preservar la diversidad y hacer uso de estos acervos genéticos en la ampliación de la base genética de los cultivares comerciales y en la búsqueda de alternativas para los sistemas de producción (Valois, 1996). Por lo tanto, los porotos silvestres constituyen recursos para el mejoramiento genético de las plantas cultivadas. También la prospección y el estudio de los materiales nativos y silvestres es una importante contribución al conocimiento de la diversidad genética y de la evolución del género *Phaseolus* en América Latina (Gutiérrez et al. 2006).

Las actividades de recolección de germoplasma vegetal en Argentina comenzaron en el año 1986 con la recolección sistemática de semillas del género *Phaseolus* del NO de Argentina para su conservación. Existe una valiosa colección de materiales de la forma primitiva de *P. vulgaris* y de la variedad silvestre entre los que se cree se encuentran presuntos híbridos entre la forma silvestre y los cultivares primitivos (Menéndez Sevillano et al. 2003).

El presente trabajo se realizó con el objetivo de localizar poblaciones de poroto silvestre en el centro de Argentina (extremo de distribución sur de la especie). Permitió encontrar dos poblaciones de la var. *aborigineus,* incrementar el número de entradas de germoplasma silvestre y caracterizar el mismo.

Materiales y métodos

Viajes de prospección

Actividades previas a la prospección. Se realizó una búsqueda bibliográfica y se relevó toda la información presente en los ejemplares de herbario de *Phaseolus vulgaris* depositados en los herbarios de la Facultad de Ciencias Exactas y Naturales (BAFC), Instituto Darwinion (SI) y Universidad de Córdoba (COR). De todos los ejemplares examinados, sólo había uno procedente de la Provincia de Córdoba recolectado por Stuckert en el año 1903. Además, se analizaron cartografía e imágenes satelitales de la zona central de Argentina, para determinar el área de exploración y las posibles rutas de acceso.

Las expediciones de prospección se realizaron entre los meses de febrero y mayo de los años 2004, 2005 y 2006. Se utilizó un vehículo con doble tracción, que sirvió como base para emprender las caminatas por los ríos y arroyos en las quebradas. Estos recorridos presentaron gran dificultad, debido al terreno rocoso y la vegetación cerrada y espinosa que frecuentemente impedía el paso. Se recabó información de los lugareños, a quienes se les mostraron fotos de las plantas y de las legumbres.

En el área explorada (Fig.1) se visitaron las siguientes localidades y parajes:

Dpto. Punilla: Villa Carlos Paz, Icho Cruz, Copina, El Cóndor, Estancia Vieja, Santa Cruz del Lago, Empalme, Tanti, Cabalango, Tres Cascadas, Flor Serrana, Río Los Chorrillos, El Coyuyo, Mallín, Puente Subiría, Cosquín, Río Toro Muerto, El Durazno, Mataderos, Cuesta Blanca, El Alto, Los Gigantes, Rio Yuspe, Río Las Mojarras, Mirador del Lago, Parque Siquiman, Bialet Massé, Santa María, Villa Caeiro, La Falda, Cuchi Corral, La Cumbre, Huerta Grande, Villa Giardino, Capilla del Monte, Los Paredones, Río Dolores, Río Tramontana, Río Seco.

Dpto. Colón: Cerro Pan de Azúcar, Vaquerías, Arroyo Vaquerías.

Dpto. Ischilín: Río Pinto, Quebrada de los Terrones, Valle de la Luna.

Dpto. Cruz del Eje: San Marcos Sierras, Río Pintos, Río Quilpo.

Dpto. Totoral: Cerro Uritorco, Río Calabalumba, Río de La Plata, Río Los Alazanes y Arroyos Huertas Malas, Los Rocillos y Minas.

Dpto. Calamuchita: La Cumbrecita, Villa General Belgrano, Santa Rosa de Calamuchita, Villa del Dique, Embalse.

Dpto. Santa María: La Cantera, Río-Suquía, Laguna Azul, Villa San Roque, La Ochoa, Malagueño, Alta Gracia.

Dpto. Capital: La Calera, Río Suquía.

Dpto. San Alberto: Pampa de Achala, Mina Clavero.

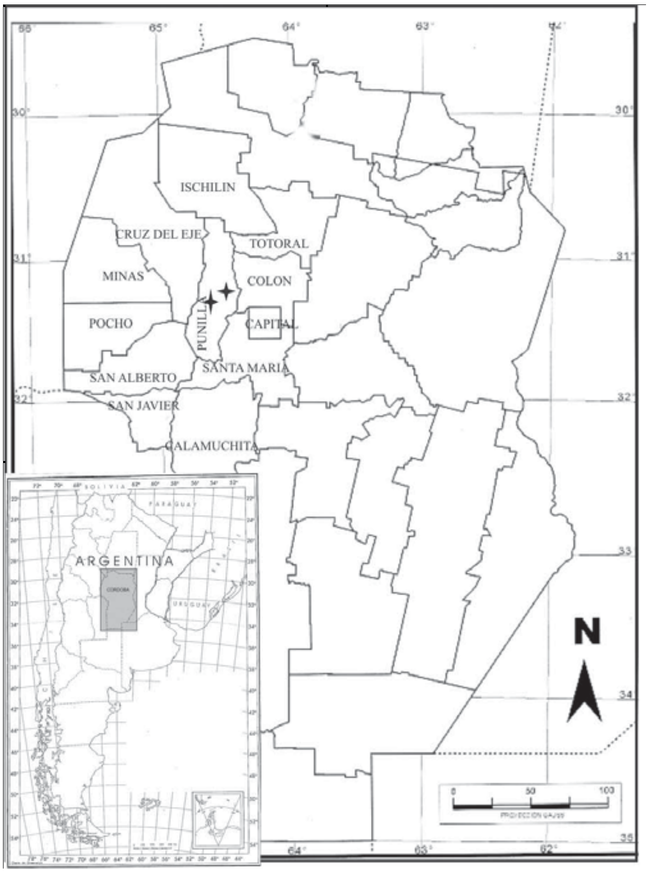

Figura 1. Ubicacion de las poblaciones de *Phaseolus vulgaris* var. *aborigineus* en la Provincia de Cordoba.

El muestreo en poblaciones naturales se realizó con una estratificación en micro sitios para cubrir la posible existencia de subpoblaciones locales. Cada planta recolectada fue numerada y las legumbres cosechadas se separaron en distintas bolsas de papel con igual número de referencia según su estado de madurez (legumbres maduras "M", legumbres verdes "V"). Los ejemplares recolectados fueron depositados en el Herbario BAFC. Se tomaron registros fotográficos de las poblaciones.

Caracterización del germoplasma

Se realizó la caracterización de la semilla, debido a la importancia de este órgano en la clasificación de *Phaseolus*. En la semilla se pueden considerar los criterios: tamaño, forma, color y la combinación de todos éstos (Voysest 1983, 2000). Siguiendo las recomendaciones de los descriptores de *P. vulgaris* (IBPGR, 1982) y *P. lunatus* (IPGRI, 2001) y el sistema de evaluación estándar para frijol del Centro Internacional de Agricultura Tropical (CIAT), se tomaron diez semillas de cada material al azar y se describieron según las características siguientes: color principal (negro, rojo morado, rojo ladrillo, marrón y blanco), color secundario (presencia y ausencia), patrón de distribución del color secundario (sin patrón, poco moteado, medianamente moteado y muy moteado), brillo de la semilla (opaca o mate, intermedia y brillante), aspecto de la testa (liso y rugoso), color del hilo (negro, rojo, marrón, amarillo y blanco), largo (mm), ancho (mm), grueso (mm), forma de la semilla (redonda, ovalada, cúbica, arriñonada y truncada) y peso de 100 semillas en gramos (pequeñas con menos de 25 g, medianas entre 25 g y 40 g y grandes con más de 40 g).

Resultados y discusión

Zonas de recolección

Las expediciones se realizaron en ambientes de distrito chaqueño serrano bosque húmedo (Cabrera 1971); se recorrieron zonas íntermontanas, situadas entre 400 y 1800 m de altura en las serranías caracterizadas por superficies de cierta amplitud, temperaturas promedio entre 18°C y 25°C y lluvias abundantes en época estival. En la región serrana la vegetación se organiza en pisos diferenciados sobre la base de la topografía, altitud, humedad, temperaturas, exposición a los vientos y asoleamiento. En el piedemonte de las sierras se mezclan especies de la llanura: "algarrobos" (*Prosopis nigra, Prosopis alba*), "espinillo" (*Prosopis torquata)*, "tala" *(Celtis spinosa)*, "chañar" (*Geoffrea decorticans*), "quebracho" *(Aspidosperma quebracho-blanco)* con otras propias del bosque serrano. Entre los 500 y 1300 msm se establece el bosque serrano, caracterizado por la presencia del "horco-quebracho" (*Schinopsis haenkeana)* y del "molle de beber o molle blanco" (*Lithraea ternifolia)* asociado con otras especies como el "coco o cochucho" (*Fagara coco)*, el "tala" *(Celtis spinosa)*, el "churqui" (*Acacia caven*), el "molle u horco molle" *(Schinus areira)*, el "sombra de toro" (*Jodina rhombifolia*), el "manzano del campo" (*Ruprechtia apetala*), el "visco" (*Acacia visco*), el "yuchán" (*Chorisia insignis*) y el "piquillin" (*Condalia microphylla*). En las Sierras Grandes aparece el "tabaquillo" *(Polylepis australis)*. Entre los 1350 y los 1700 msm se afirma un tipo de vegetación arbustiva dominada por el "romerillo" (*Baccharis coridifolia*), la "carqueja" (*Baccharis trimera*) y algunos elementos arbóreos. Por encima de los 1700 msm, desaparecen los árboles, salvo en las quebradas. En los faldeos, altiplanicies y cumbres dominan los pastizales de altura, con presencia de gramíneas como la *"stipa"* y la *"festuca"*.

El hallazgo de dos poblaciones de *P. vulgaris* var. *aborigineus* en el Departamento de Punilla fue el resultado de las expediciones. La primera población fue hallada en febrero de 2004 y tiene su hábitat natural a lo largo del Arroyo Tanti (afluente del Río Las Mojarras). Las plantas de poroto silvestre estaban distribuidas a lo largo de aproximadamente 3 km desde la naciente del arroyo. Se registraron 6 lotes en cuyas áreas el poroto silvestre era dominante (Fig. 2 y Fig. 3 a y b). En algunos lugares de suave pendiente la vegetación predominante en el margen del arroyo estaba compuesta por gramíneas y herbáceas que alcanzaban una altura de hasta 1 m y algunos árboles de escaso porte. Gran cantidad de plantas de poroto se enredaban con facilidad en las gramíneas y árboles como "molle" y "tala" alcanzando alturas de 3–4 m. En áreas de suelo rocoso y pendiente abrupta las plantas de poroto trepabana los árboles y arbustos. En todos los lotes las plantas se encontraban con abundante floración y con frutos verdes. Se recolectaron frutos verdes y maduros en mayo del 2005 y 2006, cosechando un total de 117 plantas.

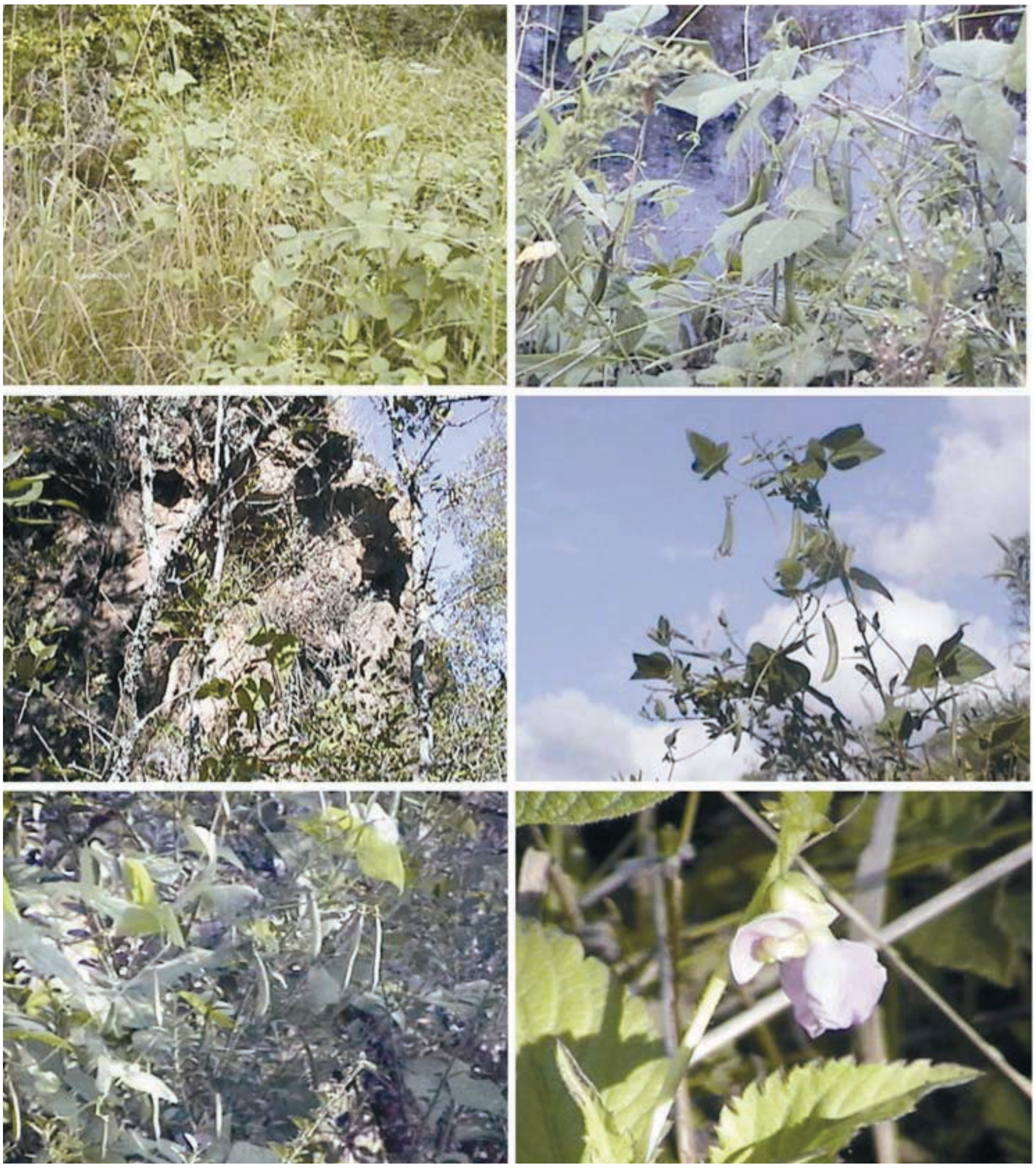

Figura 2. Plantas de *Phaseolus vulgaris* var. *aborigineus* en la población Arroyo Tanti a, b, c y d: aspecto de las plantas. e: legumbres. f: flor.

La segunda población, hallada en mayo del 2006, ocupaba un área estrecha de 10 m [mult] 3 m en la margen del Arroyo Los Chorrillos, en una profunda quebrada con pendientes abruptas de más de 100 m de altura. Se encontraron sólo 5 plantas ya secas con las legumbres maduras pendientes del árbol al que trepaban (Fig. 3 c y d).

Semillas

Las semillas colectadas son de tamaño pequeño (menos de 25 g por cada 100 semillas), color oscuro, color secundario negro, moteadas, brillantes, color de hilo blanco, las formas predominantes son ovaladas y cúbicas truncadas, y se relacionan con la posición dentro de la legumbre (Estévez et al. 2005). El 80 % con testa lisa corresponde a las legumbres maduras, mientras que el 20% con testa rugosa corresponde a las legumbres cosechadas verdes. No se encontraron diferencias significativas entre el tamaño de las semillas provenientes de las legumbres verdes (que fueron madurando ya embolsadas), con las semillas provenientes de legumbres recolectadas secas.

Conclusiones

Como resultado de las expediciones de prospección y colecta de materiales silvestres del género *Phaseolus*, encontramos dos poblaciones de la var. *aborigineus*, ambas en el Departamento Punilla (Córdoba). Las mismas se hallaron en márgenes de arroyos; estos sitios eran quebradas con pendientes muy pronunciadas ya que cuando los lugares eran más abiertos la presencia de ganado modificaba notoriamente la vegetación. Cabe destacar el alto grado de desmonte apreciado en los faldeos de los cerros para realizar plantaciones de *Pinus*.

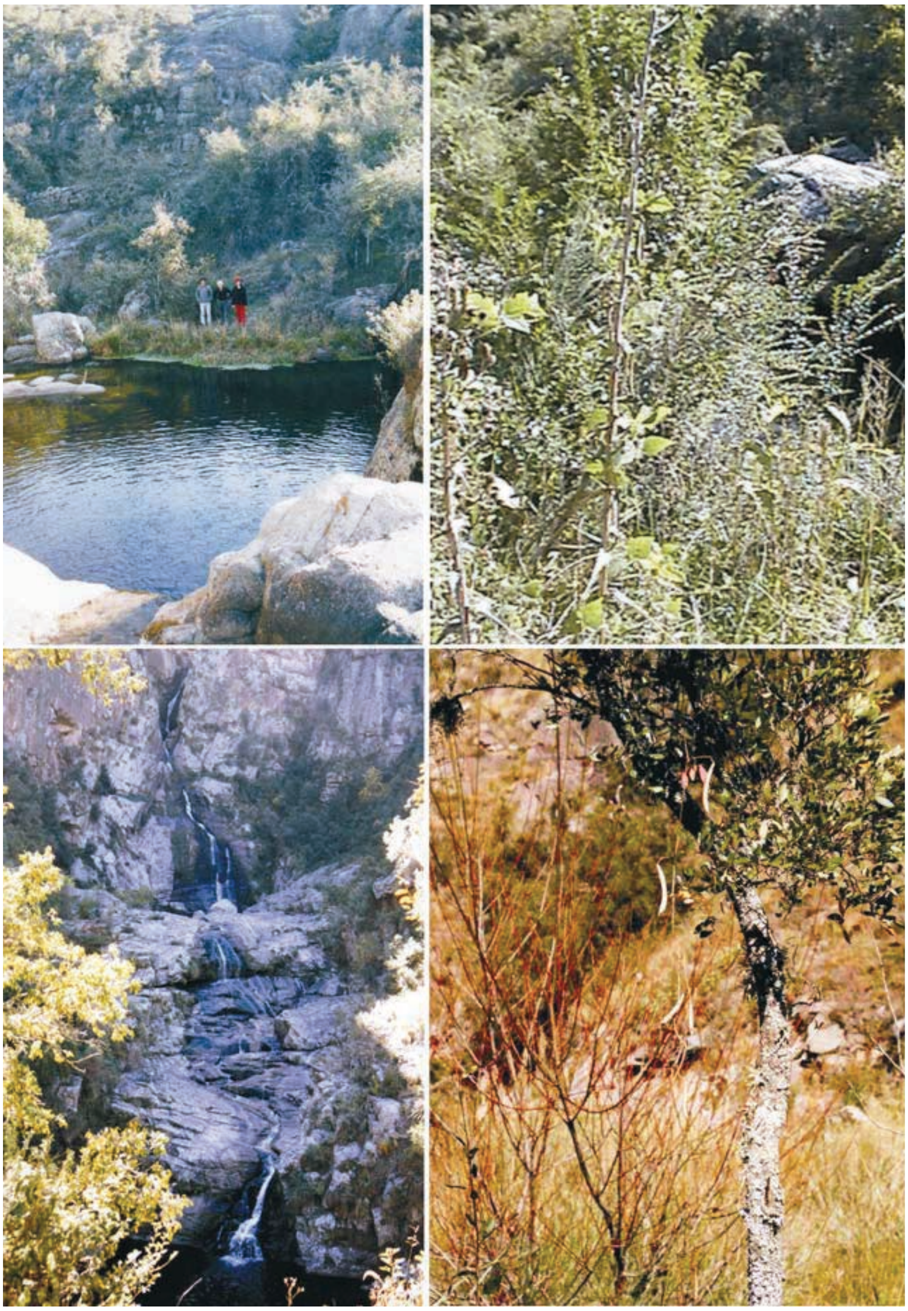

Figura 3. a: Arroyo Tanti; b: aspecto de las plantas en marzo 2004; c: Arroyo Los Chorrillos; d: aspecto de las plantas en mayo 2006.

Se destaca que en la región no se registraron cultivos comerciales ni cultivares primitivos o razas locales, normalmente encontradas en el norte del país en comunidades de pequeños agricultores. Distintos autores han observado hibridación de *Phaseolus vulgaris* var. *aborigineus* con la forma cultivada, dando como resultado ejemplares con características intermedias entre ambas entidades, formando los denominados complejos "wild-weedy-crop" mencionados para México, Colombia, Perú, y Bolivia (Vanderborght, 1983; Delgado Salinas et al. 1988; Debouck y Thome, 1989; Freyre et al. 1996; Beebe et al. 1997; Gepts et al. 2003; Zirrmbo-Villareal 2005). En Argentina se ha citado la existencia de poblaciones con presunto origen híbrido (Menéndez Sevillano et al. 1998; Hoc et al. 2003; Burkart y Brücher 1953). El estudio de la biología floral y el seguimiento de las poblaciones de la variedad silvestre en el NO de Argentina, ha demostrado la existencia de híbridos o introgresiones con los cultivos de *Phaseolus vulgaris*, *Phaseolus augustii* y cultivares primitivos que se desarrollan en la región (Hoc et al. 2006).

Las poblaciones halladas se encuentran aisladas y su acervo genético no ha recibido ningún tipo de flujo génico no deseado.

El incremento del número de entradas en las colecciones existentes y su mantenimiento ofrecerá a los fitomejoradores una amplia variabilidad genética, como base para la creación de cultivares de mayor adaptabilidad y, por lo tanto, serán favorecidos por menores exigencias de insumos con consecuentes beneficios medioambientales. La diversidad genética es una condición para alcanzar alta productividad y estabilidad del rendimiento mientras que por otra parte ha sido, a lo largo del tiempo, la materia prima utilizada por los fitomejoradores para desarrollar variedades mejoradas (Gepts y Papa, 2003).

La información suministrada por los pobladores sobre la utilización del poroto silvestre indica que en la región recorrida no hay antecedentes de su consumo como recurso alimenticio. Por estas razones, por la presencia de ganado vacuno y caprino, por el desmonte y por los incendios de la zona, es probable que estas poblaciones se encuentren en peligro de extinción.

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Collecting pasture legumes in Israel with a focus on species of importance to southern Australia.

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Summary

Collecting pasture legumes in Israel with a focus on species of importance to southern Australia

A seed collecting expedition was undertaken in Israel during May 2006 that focused on pasture legume species with potential for southern Australia. Israel was chosen because of high plant diversity, including species of *Trifolium* L. in particular. The relatively low latitudes dissecting Israel, coupled with dry Mediterranean conditions in the east and south increased the opportunity of finding plants well adapted to southern Australia. Locations known to be rich in pasture species were targeted. Between the upper Golan Heights in the north and Be'e'r-Sheva in the south, 54 collecting sites were identified. Seed of 558 accessions from 100 identified pasture and crop species was sampled. *Trifolium* was the most frequently collected genus, accounting for 318 accessions from 35 species. Species varied greatly in their distribution and abundance depending on seasonal conditions, and adaptation to the environment, including soil type, altitude and grazing pressure. Occurrence of four species of particular importance, *Trifolium glanduliferum* Boiss., *T. spumosum* L., *T. dasyurum* C. Presl and *Biserrula pelecinus* L., is discussed in relation to their adaptive strategies. Future seed collecting should focus in the south, where plants adapted to drought are likely to be found. Surveys of rare species should also be undertaken to fully assess their risk status and guide the Israeli Genebank in their conservation activities.

Key words: germplasm collection, *ex situ* collecting, pasture improvement, pasture plants, *Trifolium* species.

Résumé

Collecte de légumineuses pastorales en Israël ciblant des espèces importantes pour l'Australie méridionale

En mai 2006, des graines ont été collectées en Israël, en particulier celles d'espèces de légumineuses pastorales présentant un potentiel pour l'Australie méridionale. Israël a été choisi entre autres pour la diversité des espèces de *Trifolium* L. Du fait de sa latitude et des conditions méditerranéennes sèches dans l'est et le sud, la probabilité d'y trouver des plantes bien adaptées à l'Australie méridionale est élevée. 54 sites de collecte ont été déterminés entre les hauteurs du Golan au nord et Be'e'r-Sheva au sud connus pour leur richesse en espèces pastorales. Des échantillons de graines de 558 accessions de 100 espèces identifiées, pastorales et cultivées, ont été prélevés. *Trifolium* est le genre le plus fréquemment collecté : 318 accessions pour 35 espèces. La distribution et l'abondance des espèces varient considérablement suivant les conditions saisonnières et l'adaptation à l'environnement, incluant type de sol, altitude et pression de pâturage. L'occurrence de quatre espèces importantes, *Trifolium glanduliferum* Boiss., *T. spumosum* L., *T. dasyurum* C. Presl et *Biserrula pelecinus* L., est discutée en relation avec leurs modes d'adaptation. Les futures collectes de graines devraient se concentrer sur le sud du pays, où la probabilité de trouver des plantes adaptées à la sécheresse est la plus élevée. La prospection des espèces rares devrait permettre d'évaluer dans quelle mesure elles sont menacées et guider la banque de gènes d'Israël dans ses activités de conservation.

Resumen

Recolección de legumbres forrajeras en Israel, concentrada en especies importantes para Australia meridional

En mayo de 2006 se emprendió una expedición de recolección de semillas en Israel, concentrada en especies de legumbres forrajeras útiles para Australia meridional. Se escogió a Israel por su gran diversidad vegetal, en particular de especies de *Trifolum* L. Las latitudes relativamente bajas de Israel y las condiciones secas del este y sur del Mediterráneo aumentan la posibilidad de hallar plantas que se adapten bien a Australia meridional. Se buscaron ubicaciones ricas en especies forrajeras, identificando 54 sitios entre las alturas del Golan al norte y Be'e'r-Sheva al sur. Se muestrearon semillas de 558 accesiones de 100 especies forrajeras y de cultivos. El género *Trifolium* se recolectó con mayor frecuencia, presentando 318 accesiones de 38 especies. La distribución y abundancia de especies variaban mucho según las condiciones de la temporada y la adaptación al medio ambiente, el tipo de suelo, la altura y la presión del pastoreo. Se examinaron cuatro especies de particular importancia, *Trifolium glanduliferum* Boiss., *T. spumosum* L., *T. dasyurum* C. Presl y *Biserrula pelecinus* L. y sus estrategias de adaptación. Las futuras recolecciones de semillas deberían concentrarse en el sur, donde es probable encontrar plantas adaptadas a la sequía. También se deberían emprender búsquedas de especies raras a fin de evaluar a fondo el estado de riesgo y guiar las actividades de conservación del Banco de Genes Israelí.

Introduction

There have been significant developments in new pasture legumes for southern Australia over the past 15 years (Nichols et al. 2007). However, developing new cultivars for short-season areas of the grain zone has been difficult, partly due to the limited germplasm with early flowering times available in Australian genebanks. Most pasture germplasm originates from more northerly latitudes in the Mediterranean (Snowball et al. 2006). This shortage of pasture germplasm prompted a number of collecting missions to several Mediterranean countries between 2003 and 2006. Israel was selected as a target for collecting for several reasons.

Firstly, the country possesses a rich diversity of pasture legume species (Zohary 1972). The *Trifolium* genus in particular is well represented (Zohary and Heller 1984), with 50 species (Feinbrun-Dotan and Danin 1998; Danin 1998). Recent and imminent commercialization of little-known species of *Trifolium* in Western Australia highlights the importance of the genus for pasture plant improvement (Loi et al. 2005a, b; Nichols et al. 2007).

Secondly, Israel lies at relatively low latitudes (31 to 33°N), corresponding to short-season areas in the Mediterranean region of southern Australia. Flowering time in pasture species from Mediterranean environments is promoted by increased day length (Evans 1959; Aitken 1974), although temperature and vernalization requirements can also be important (Evans 1959). Longer days occur earlier in spring at lower latitudes.

Thirdly, Israel possesses a large diversity of ecogeographical, climatic and edaphic environments in a relatively small area (Feinbrun-Dotan and Danin 1998; Zohary 1966). An equally diverse range of plant genotypes is likely to arise given this diversity. Early flowering genotypes were considered likely to be found in shorter season environments, including low altitude areas near the coast and Jordan Valley, low rainfall areas in the east and south, and on shallow or coarse-textured soils. The transitional zone between the Mediterranean woodlands and shrublands and the semi-steppe shrublands were also likely to possess plant genotypes with useful adaptive features.

Fourthly, existing seed collections originating from Israel are species rich but genotype poor. For example, the collection of *Trifolium* held in the Australian genebank consists of 44 species with an average of 10 accessions per species. At least a modest genetic diversity in germplasm collections is desirable for effective plant selection and breeding.

Finally, the large diversity of species and environments present in Israel offered the opportunity to collect pasture legumes important for other priorities. These include annual species for longer seasons, salt-tolerant annual species, and perennial species for recharge control and extending the period of green feed on offer.

Most germplasm held in Australian genebanks originating from Israel was collected between 1951 and 1973. C.M. Donald and J.F. Miles collected in 1951 (Neal-Smith and Johns 1967), C.A. Neal-Smith in 1954 (Neal-Smith 1963), J.H. Leigh in 1965 (Neal-Smith and Johns 1967), J.A. Carpenter, E.J. Crawford and E.T. Bailey in 1967 (Crawford 1967), C.A. Neal-Smith and F.W. Hely in 1968 (Neal-Smith and Johns 1971) and B.J. Quinlivan in 1973 (Quinlivan 1974). Israeli researchers J.S. Katznelson and colleagues made significant collections in the 1950s and 1960s. In 1997, a collection of root nodules and seed was undertaken by J.H. Howieson (ATGRC Database). A seed and herbarium collection of pasture legumes resides with the National Herbarium at the Hebrew University of Jerusalem under the management of I. Herrnstadt and U. Plitmann (Bioversity International; BioGIS). Germplasm of Israeli origin held in the Australian Trifolium Genetic Resource Centre (ATGRC) has already proved its value with the commercial release of *Trifolium glanduliferum* originating from the Yehudiyya Forest, north of the Sea of Galilee (Nutt and Loi 2002). However, there is a need to increase the genetic diversity as well as the species diversity of the seed collections to enable more effective plant improvement.

The aim of this seed collecting mission was to collect from a broad diversity of environments for pasture legume species with potential for southern Australia. Important annual species include *Trifolium glanduliferum, T. spumosum, T. dasyurum* and *Biserrula pelecinus.* While short-season areas were targeted, species from longer-season areas were also collected to increase genetic diversity of collections and to satisfy other priorities. Collecting also provided an opportunity to comment on the conservation status of pasture legumes in Israel.

Materials and methods

The first step in planning the mission involved an analysis of Flora Palaestina and the BioGIS website. Areas were identified where high priority species were reported to occur. These included most areas north of Netanya and the West Bank, particularly in Upper Galilee and the Golan Heights, and the coastal strip south of Tel-Aviv. A number of specific sites east of the Gaza strip and south of the West Bank were also identified. The next stage of the mission involved planning the route and site selection. The area south of Tel-Aviv was visited first, followed by the area north of Netanya and the West Bank, to best correspond with the growing season and ripening of seeds. The area south of the West Bank was visited last because of reports of late rain that might have delayed plant development. Collecting sites were selected according to botanist's knowledge of specific localities where plant biodiversity was high or where target species were known to occur. This approach proved to be effective in finding a high diversity of target species, and efficient in terms of time and resources. Navigation to specific locations identified from the BioGIS database was attempted but quickly abandoned, primarily because of the difficulty in navigating through a heavily developed landscape. Nevertheless, navigation to some BioGIS sites south of the West Bank where *Biserrula pelecinus* had been reported proved successful.

A number of geographical and edaphic characteristics were assessed at each site. Site location was recorded using a Magellan 315 GPS meter while the collecting area at each site was estimated. Altitude was recorded using a Barigo altimeter. Slope angle and aspect were assessed using a Suunto tandem clinometer and compass. Annual rainfall at each site was estimated using data from the BioGIS database. Soil pH (water) was measured at each site using a CSIRO quick test kit (Raupach and Tucker 1959). Other site characteristics were assessed visually, including physiography, habitat, water relations, grazing pressure, associated species, parent rock, and soil characteristics including texture, colour, depth, hardness and stoniness.

Familiarity with the target species and published Floras aided the identification of samples during collecting. Species abundance and spatial distribution were estimated visually, and the numbers of plants and seed heads or pods sampled was counted. When a species was abundant, a small number of seeds were collected from a large number of locations in order to best capture the genetic diversity of the population. Conversely, when species were rare, seed quantity was the first priority. Seed head and pod samples were dried at 60°C for 24 hours prior to being hand rubbed to enable the separation, cleaning and conservation of seeds. Root nodules were collected from a limited number of species according to priorities set by the Centre for Rhizobial Studies, Murdoch University, Western Australia. When nodules could not be found, soil was collected to enable trapping of rhizobium in the glasshouse at a later date.

Results and discussion

Fifty-four collecting sites were identified between the upper Golan in the north and near Be'e'r-Sheva in the northern Negev in the south (Figure 1). The Mount Scopus Botanic Garden in Jerusalem (Site 13) also donated seed of plants collected from other Israeli locations. These accessions are not included in the discussion on site characteristics. Average annual rainfall of sites ranged from 1100 mm at the most northerly site in the upper Golan to 105 mm at Site 55, east of Be'e'r-Sheva (Table 1). Low rainfall and low altitude sites were found in the upper Jordan area and the coast south of Tel Aviv. Acid soils were encountered throughout, but were more common in the Golan and upper Galilee, and associated with granite. Coarse textured soils were less common than fine textured soils, and were more common in coastal sites.

Seed of 548 accessions was collected from 12 important pasture legume genera (Table 2). A total of 100 identified and 4 unidentified pasture and crop species were sampled (Table 3). *Trifolium* was the most frequently collected genus, accounting for 318 accessions from 35 species. Half of the species were found at one or two collecting sites, and 75% of species were found at between one and five sites. The most frequently occurring species (from 10 or more sites) were 13 *Trifolium* species, *Hymenocarpos circinnatus* (L.) Savi, *Medicago orbicularis* (L.) Bart, *Lotus peregrinus* L., *Astragalus hamosus* L. and *Biserrula pelecinus*. *Trifolium purpureum* Lois. and *T. tomentosum* L. were the most frequently encountered of all species. Of the 50 species of *Trifolium* recorded in Israel, 15 were not encountered (Feinbrun-Dotan and Danin 1998; Danin 1998).

Abundance of individual species at each collecting site (data not presented) varied from rare (<0.1% of surface area) to high (>25% of surface area). Two-thirds of collections were rare or very scarce (<1% of surface area) and 89% of collections were rare, very scarce or scarce (<5% of surface area). Of the more frequently encountered species, all sightings of *Trifolium spumosum*, *Astragalus hamosus* and *Biserrula pelecinus* were rare or very scarce. Very high levels of hard seed found in these species (Zoghlami and Zouaghi 2007; Loi et al. 1999, 2003) may result in low levels of abundance in some years, while also contributing to their long-term survival. Alternatively, their prostrate habit may result in being shaded out by more erect species under light grazing. *Trifolium palaestinum* Boiss. was the only species recorded as common (5–25% of surface area) or highly abundant at more than 50% of sites where it was encountered, while *Hymenocarpus circinnatus*, *Trifolium pilulare* Boiss. and *T. purpureum* were common or highly abundant at five or more sites. High levels of abundance might be attributed to superior adaptation to a difficult environment (such as infertile, coarse-textured soils in the case of *T. palaestinum*) or an ability to compete with other species from having large seeds (in the case of *H. circinatus*

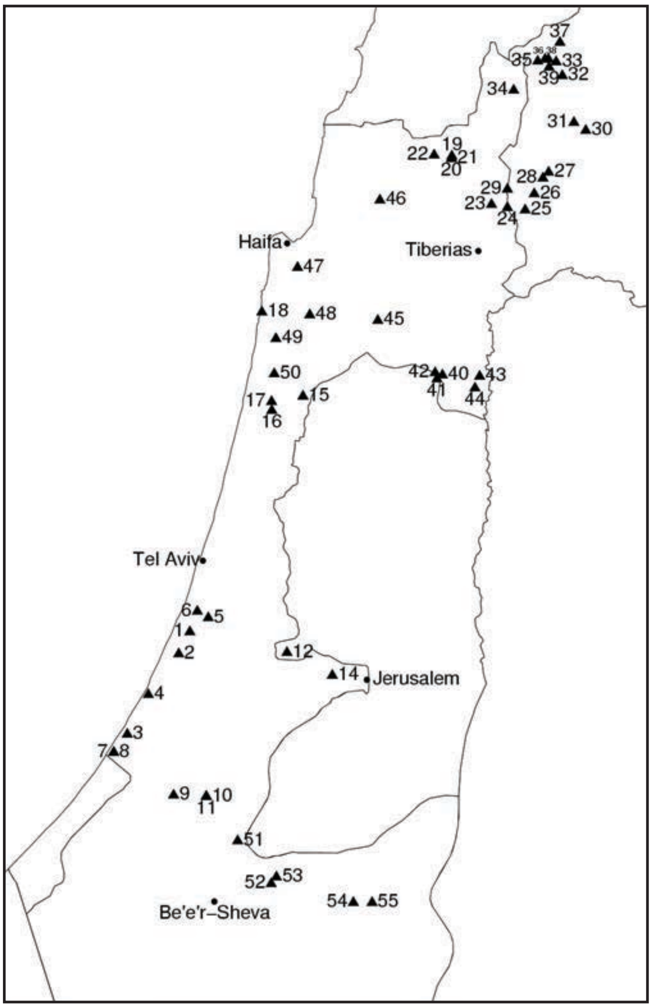

Figure 1. Collecting sites.

Table 1. Geographical and edaphic characteristics of 54 collecting sites recorded in 2006 in Israel.

Site number	Annual rainfall (mm)	Altitude (masl)	Parent rock	Soil pH	Soil texture†	Water relations‡
1	500	32	Dune	7.5	S	FD
2	490	35	Dune	7.5	S	FD
3	430	64	Dune	7.5	S	FD
4	470	7	Dune	7.5	S	FD
5	550	37	Alluvial	7.0	SL	ImpD
6	550	7	Dune	7.5	S	FD
7	430	2	Dune	7.5	S	FD
8	430	0	Alluvial	9.5	L	Poor
9	340	159	Limestone	8.5	SL	FD
10	300	182	Limestone	9.0	SL	FD
11	300	182	Alluvial	7.5	L	Poor
12	600	277	Limestone	6.0	L	FD
14	600	710	Limestone	7.0	L	FD
15	600	50	Limestone	8.0		FD
16	590	31	Alluvial/sandstone	6.0	S	FD
17	590	10	Alluvial	9.0	CL	Swamp
18	530	0	Dune	9.0	S	FD
19	650	840	Basalt	6.0	CL	ImpD
20	600	850	Basalt	5.5	CL	Poor
21	600	860	Basalt	6.0	L	FD
22	750	750	Limestone/alluvial	8.0	CL	ImpD
23	450	0	Basalt	6.5	CL	FD
24	430	-200	Alluvial	8.0	SL	Poor
25	420	-150	Basalt	8.5	S	Swamp
26	500	150	Basalt	6.5	CL	FD
27	750	470	Basalt	7.0	C	FD
28	700	360	Basalt	8.5	CL	FD
29	610	65	Basalt	8.5	CL	FD
30	840	1060	Basalt	6.0	L	FD
31	820	970	Basalt	5.5	L	FD
32	1070	1100	Basalt	5.5	SL	FD
33	1070	1100	Sandstone	5.0	L	FD
34	600	69	Alluvial	9.0	CL	FD
35	800	420		8.0	CL	FD
36	900	750	Limestone	8.5	CL	FD
37	1100	1500	Limestone	7.5	L	FD
38	900	850	Limestone	8.0	CL	FD
39	900	750	Limestone	8.5	CL	FD
40	430	90	Limestone	9.0	L	FD
41	450	420	Limestone	9.0	L	FD
42	450	480	Limestone	8.5	L	FD
43	350	-150	Alluvial	9.0	CL	FD
44	300	-140	Alluvial	8.5	SL	FD
45	500	130	Alluvial	8.0	L	Swamp
46	750	250	Limestone	7.5	C	Poor
47	700	490	Limestone	8.0	SL	FD
48	700	280	Limestone	8.0	L	FD
49	600	100	Porous stone	7.5	SL	FD
50	600	90	Alluvial	variable	S	ImpD
51	250	400	Limestone	9.0	CL	FD
52	225	400	Schist	5.5	SL	FD
53	225	415	Schist	variable		FD
54	130	590	Basalt/Schist	8.5	SL	FD
55	105	425		9.0	SL	FD

Notes: Site 13 is not included; †: S = sand, L = loam, C = clay, SL = sandy loam, CL = clay loam; ‡: FD = freely drained, ImpD = imperfectly drained, Poor = poorly drained.

Table 2. Number of accessions of important pasture legume genera collected in Israel in 2006.

Genus	No. of accessions
Astragalus	21
Biserrula	15
Bituminaria	8
Coronilla	5
Hymenocarpos	26
Lotus	24
Medicago	39
Melilotus	18
Ononis	20
Ornithopus	4
Trifolium	318
Trigonella	10

and *T. pilulare*) or being large, upright plants (in the case of *T. purpureum*). Alternatively, species abundance in any single year is likely to be strongly influenced by seasonal conditions and grazing pressure. For example, drought conditions at the southern sites (Sites 52–55) in 2006 are likely to have been a major cause of the very low abundance of species.

The distribution of species varied according to site characteristics, including altitude, soil texture, soil pH, annual rainfall, water relations, latitude and proximity to the ocean (Table 1, Figure 1, and Table 3). *Trifolium palaestinum* and *T. philistaeum* Zoh. were found in coastal areas on sandy soils that receive 400 to 600 mm average annual rainfall. *Trifolium physodes* Stev. ex M.B. was found only at high altitudes. *Trifolium glanduliferum, T. pauciflorum* D'Urv., *T. scutatum* Boiss. and *Ornithopus compressus* L. were found in higher-rainfall areas with between 500 and 1100 mm average annual rainfall. Species that occurred more frequently on acid soils included *Biserrula pelecinus* and *Trifolium glanduliferum,* while those more frequent on alkaline soils included *Astragalus hamosum, Lotus peregrinus, Trifolium cherleri* L., *T. purpureum* and *T. tomentosum.*

The distributions of four important species, *Trifolium glanduliferum, T. spumosum, T. dasyurum* and *Biserrula pelecinus,* are illustrated in Figures 2a to 2d. Sites are also illustrated for seed collections held in the ATGRC made prior to 2006, and observations from the BioGIS database. Sites of prior seed collections are those visited by both Israeli and Australian collectors and for which there is accurate location data. Observations from the BioGIS database include seed collections held at the Hebrew University of Jerusalem.

New seed collections of *Trifolium glanduliferum* made during this mission have added significantly to existing collections (Figure 2a). However, collecting sites do not cover the full distribution range described by BioGIS. A number of plant or site factors may be implicated. *Trifolium glanduliferum* is a relatively small plant that produces very small (0.5 mg), hard seeds that soften mostly in autumn in southern Australia (Loi et al. 2003). A high proportion of these seeds will spread in animal dung due to their small size (Thomson et al. 1990; Squella and Carter 1996). An early autumn commencement to the growing season coupled with a history of low intensity grazing throughout the year would disadvantage its growth. Grazing intensity was low at most collecting sites and autumn rainfall was above average. These may account for the limited occurrence of this species during the collecting mission. The first cultivar of *Trifolium glanduliferum* (cv. Prima) was released into commerce in 2001 by the Department of Agriculture and Food, Western Australia, and originates from the Yehudiyya Forest (Nutt and Loi 2002). An important characteristic of the species is its resistance to red-legged earthmite (*Halotydeus destructor* Tucker), a serious pest of pastures at the seedling stage. New germplasm collected in 2006 from the upper Golan could improve pasture production in high-rainfall areas in

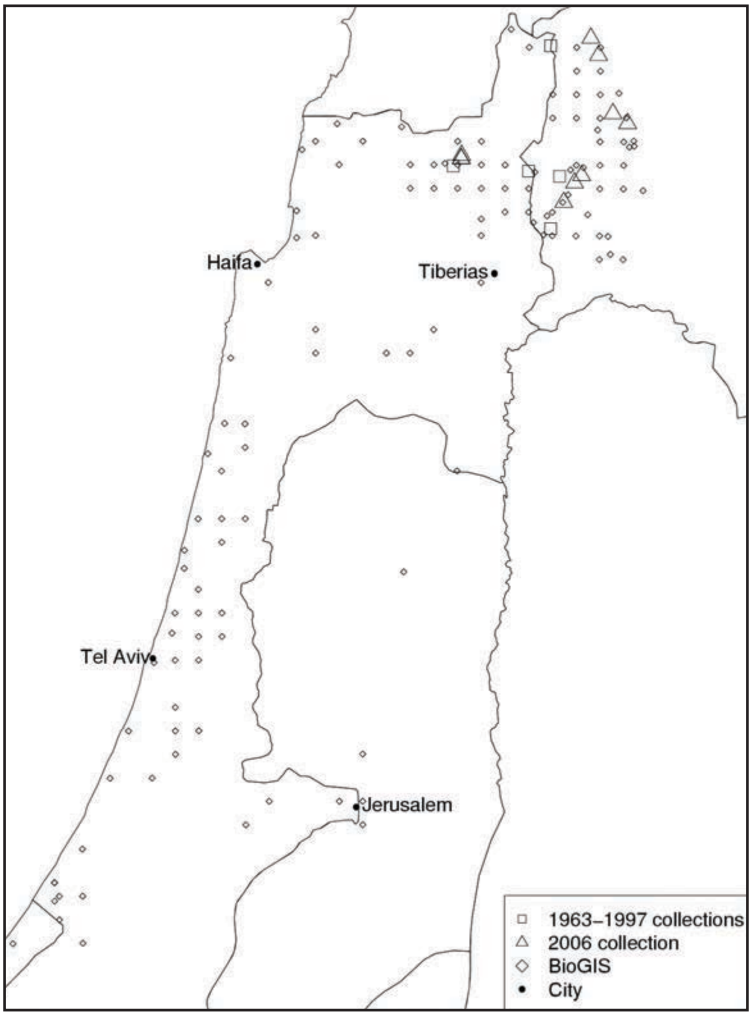

Figure 2a. Map of Israel showing seed collecting sites in 2006, previous seed collecting sites and observation sites from BioGIS for *Trifolium glanduliferum*.

Table 3. Site numbers of species collected in Israel in 2006.

Species and Site numbers
Anthyllis tetraphylla 9, 29
Argyrolobium uniflorum 9
Astragalus asteria 37, 55
Astragalus boeticus 3, 5, 6
Astragalus hamosus 6, 8, 22, 24, 27, 29, 30, 36, 38, 41, 48, 49, 52, 54, 55
Astragalus sp. 7
Biserrula pelecinus 5, 12, 15, 16, 19, 20, 26, 29, 30, 31, 32, 33, 52, 54, 55
Bituminaria bituminosa 13, 14, 23, 25, 34, 39, 41, 42
Cicer pinnatifidum 12, 48
Coronilla repanda 7
Coronilla rostrata 12, 36, 47
Coronilla scorpioides 48
Glycyrrhiza glabra 43, 44
Hippocrepis unisiliquosa 52, 55
Hymenocarpos circinatus 9, 10, 12, 14, 15, 16, 18, 19, 21, 23, 26, 29, 31, 33, 35, 36, 38, 40, 42, 47, 48, 49, 51, 52, 53, 55
Lathyrus aphaca 27
Lathyrus blesspharicarpus 5, 6, 37
Lathyrus hierosolymitanus 17
Lathyrus nissolia 37
Lathyrus ochrus 5, 18, 38, 45
Lens orientalis 47
Lotus conimbricensis 20
Lotus creticus 7
Lotus cytisoides 13
Lotus edulis 47
Lotus glaber 44
Lotus halophilus 3
Lotus palustris 24, 25
Lotus peregrinus 1, 4, 5, 6, 10, 14, 15, 16, 18, 25, 40, 47, 49, 51, 52
Lupinus luteus 16
Lupinus micranthus 2, 3
Lupinus palaestinus 7, 8, 16
Lupinus varius 21, 23, 31, 48
Medicago doliatus 6, 10, 24, 50
Medicago laciniata 55
Medicago orbicularis 5, 6, 8, 9, 12, 14, 15, 16, 19, 27, 29, 32, 33, 35, 36, 38, 47, 48, 51, 52, 53
Medicago polymorpha 14, 30
Medicago rigidum 42, 48
Medicago rugosa 15, 19, 28
Medicago scutellata 15, 28, 40, 47, 51
Medicago tuberculata 47
Melilotus albus 24, 25
Melilotus indicus 1, 2, 8, 18, 24, 45, 46, 49
Melilotus siculus 17, 45, 50
Melilotus sulcatus 17, 24, 27, 45, 50
Ononis alopecuroides 24, 28, 45
Ononis mitissima 17, 45, 47
Ononis natrix 1, 3, 5, 7
Ononis ornithopodioides 12, 41
Ononis pubescens 25, 36
Ononis serrata 47
Ononis sicula 1, 10, 47
Ononis sp. 10, 12
Ornithopus compressus 16, 17, 32, 33
Pisum sativum 50
Pisum syriacum 12
Sanguisorba minor 9
Securigera securidaca 35
Tetragonolobus palaestinus 15, 22, 23, 28, 29
Tetragonolobus requinii 28
Trifolium alexandrinum 17, 18, 50
Trifolium argutum 12, 15, 16, 17, 19, 20, 25, 26, 27, 28, 29, 31, 32, 33, 35, 36, 38, 46, 48, 49
Trifolium arvense 33
Trifolium berytheum 24, 28, 45
Trifolium boissieri 22, 23, 36, 38
Trifolium campestre 51
Trifolium cherleri 9, 12, 14, 15, 18, 21, 22, 29, 33, 35, 36, 38, 40, 41, 42, 49
Trifolium clypeatum 12, 14, 15, 22, 27, 33, 35, 36, 38, 40, 41, 42, 47, 48, 49
Trifolium dasyurum 9, 12, 13, 14, 15, 20, 27, 30, 31, 32, 36, 37, 38, 41, 51, 53
Trifolium dichroanthum 6, 13, 17
Trifolium echinatum 15, 22, 28, 33, 49
Trifolium erubescens 22, 37
Trifolium fragiferum 50
Trifolium glanduliferum 19, 20, 21, 26, 27, 28, 30, 31, 32, 33
Trifolium globosum 33
Trifolium grandiflorum 32, 37
Trifolium hirtum 25, 29
Trifolium israeliticum 21, 22
Trifolium lappaceum 12, 15, 38, 41, 46, 47, 50
Trifolium lucanicum 33, 36
Trifolium nigrescens 17, 20, 22, 23, 24, 27, 29, 35, 46, 48
Trifolium palaestinum 1, 2, 3, 4, 5, 6, 7, 16, 18
Trifolium pauciflorum 19, 20, 21, 26, 27, 30, 31, 32, 33, 35, 36, 38
Trifolium philistaeum 2, 4, 7, 16
Trifolium physodes 13, 21, 32, 33, 36, 37, 38
Trifolium pilulare 14, 15, 16, 19, 20, 21, 22, 23, 26, 27, 28, 29, 30, 31, 32, 33, 35, 36, 37, 38, 40, 41, 42, 48
Trifolium purpureum 5, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 22, 23, 25, 26, 27, 28, 30, 31, 35, 36, 38, 40, 41, 42, 44, 45, 46, 47, 49, 50, 51
Trifolium repens 27
Trifolium resupinatum 12, 18, 22, 24, 27, 46, 50
Trifolium scutatum 19, 20, 21, 22, 27, 30, 31, 33, 37, 48
Trifolium spumosum 10, 11, 12, 15, 16, 19, 20, 21, 22, 23, 25, 27, 28, 29, 30, 31, 32, 35, 36, 37, 38, 40, 46, 48, 49
Trifolium stellatum 14, 15, 19, 20, 21, 22, 25, 29, 30, 31, 32, 33, 35, 36, 37, 38, 40, 41, 42, 47, 49
Trifolium subterraneum 23, 32
Trifolium tomentosum 1, 5, 6, 10, 11, 12, 14, 15, 16, 18, 19, 20, 22, 25, 27, 29, 30, 31, 32, 33, 35, 36, 37, 38, 41, 42, 46, 47, 48, 49, 53
Trifolium vavilovii 23, 27, 40, 41
Trigonella arabica 10
Trigonella foenum-graecum 14
Trigonella monspeliaca 37, 47
Trigonella sp. 27
Trigonella spinosa 10, 12, 15, 41, 47
Vicia galilea 21
Vicia narbonensis 5
Vicia sativa 4, 5, 6, 8, 14, 16, 22, 25, 30, 38, 45, 50
Vicia sp. 14, 24, 25, 27, 30

southern parts of Australia where cold tolerance is required. Germplasm of *T. glanduliferum* held in Australian genebanks originates from Israel, Turkey and Syria (Snowball et al. 2006). However, seed collected in Turkey in 1996 by Bennett et al. (1998) was later re-identified as *Trigonella spicata* Sibth. & Sm.

Trifolium spumosum was found to be more widespread than *T. glanduliferum* (Figure 2b), ranging from the Golan Heights south to Site 10, located between the Gaza strip and the West Bank. Site 10 receives on average approximately 300 mm of rainfall per annum and may represent the limit of its distribution. In addition, this is the most southerly location in the northern hemisphere where seed has been collected. It was, however, recorded on Gran Canaria at latitude 28°N by Hansen and Sunding (1985) and in the Arava Valley, 70km south of the Dead Sea, at latitude 30°36′N (National Herbarium, Hebrew University of Jerusalem), although no seed collections are reported. Ghamkhar et al (2007) has also identified Wadi Al Jayb in the Jordan Valley, south of the Dead Sea, as an area where *T. spumosum* may potentially occur. Clearly, searching Wadi Al Jayb and surrounding areas will be important to better define the distribution of *T. spumosum*. Characteristics of this species that contribute to survival include high seed producing capacity, high level of hard seed dormancy, late autumn seed softening, and a very thick and strong seed coat (Loi et al. 2003, 2005a, b). The low abundance and frequent occurrence of *T. spumosum* at a broad range of collecting sites is likely to be the result of a sound survival strategy of producing large quantities of persistent hard seeds. It also is a prostrate species and may be out-competed by more erect species in the absence of grazing. The first cultivar will be released to commerce in 2008 in Australia (A. Loi, pers. comm.). Future cultivar development will benefit from a core collection of the species created using ecogeographical and molecular data (Ghamkhar and Snowball 2006).

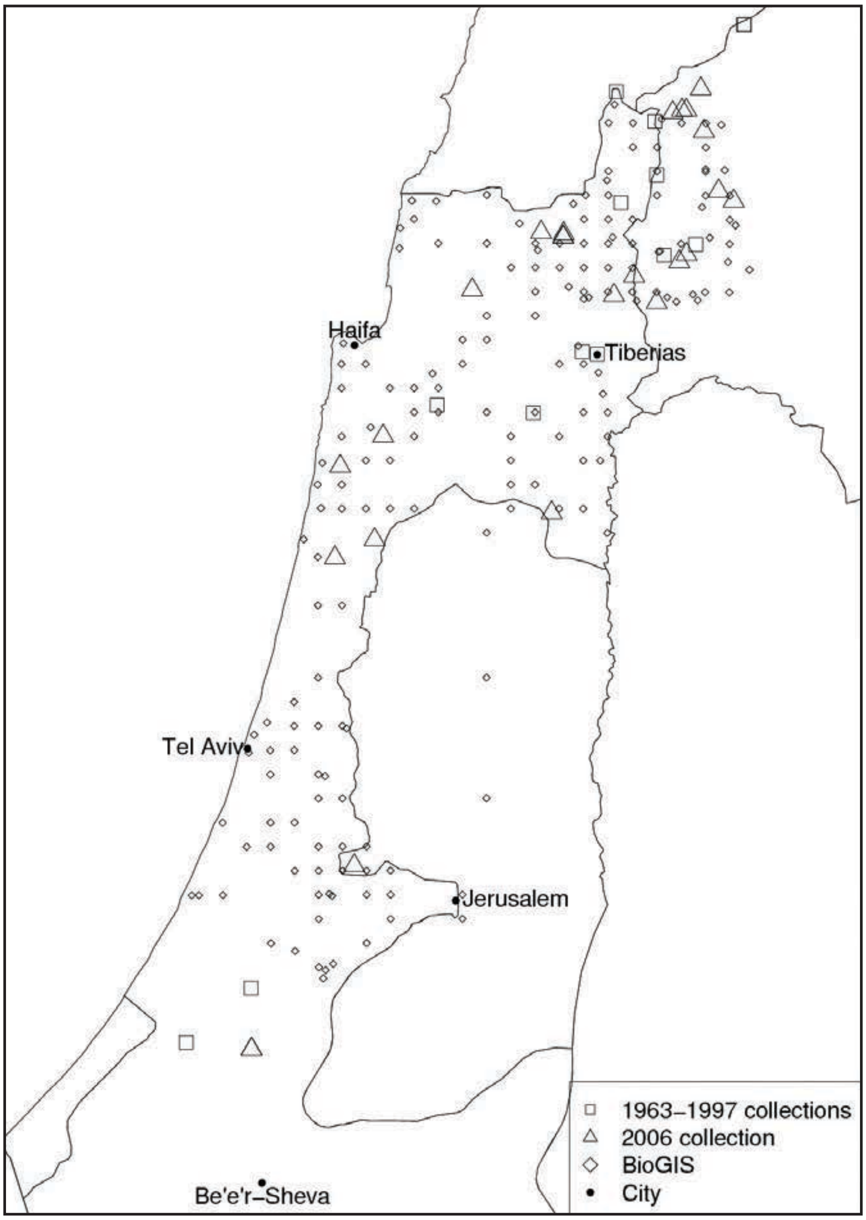

Figure 2b. Map of Israel showing seed collecting sites in 2006, previous seed collecting sites and observation sites from BioGIS for *Trifolium spumosum*.

Biserrula pelecinus is another prostrate species with a survival strategy similar to *T. spumosum*. It produces a large number of hard seeds, but they are much smaller (0.7–2.3 mg; Bazihizina 2005), and have a higher level of hard seed dormancy and a slower rate of softening (Loi et al. 2005a, b). This may explain the low abundance and high frequency at a broad range of collecting sites (Figure 2c). Its occurrence at Sites 52, 54 and 55, which received approximately 50 mm of rainfall during the growing season of 2006, demonstrates its ability to survive in very harsh environments. It is worth noting that these three sites were located using GPS records on the BioGIS website, made earlier by other botanists. Two commercial cultivars of *B. pelecinus,* cvs. Casbah and Mauro, are successfully grown in a long rotation with cereal crops in the wheatbelt of southern Australia in areas receiving between 325 and 700 mm average annual rainfall (Loi et al. 2005a, b). Regeneration of the pasture depends on a small but significant degree of seed softening during the cropping phase. Collections from the southern sites may be well adapted to the low-rainfall areas of the southern Australian wheatbelt, where growing seasons are very short and less reliable. Collections from high-rainfall sites in the northern districts of Israel may produce seeds that soften

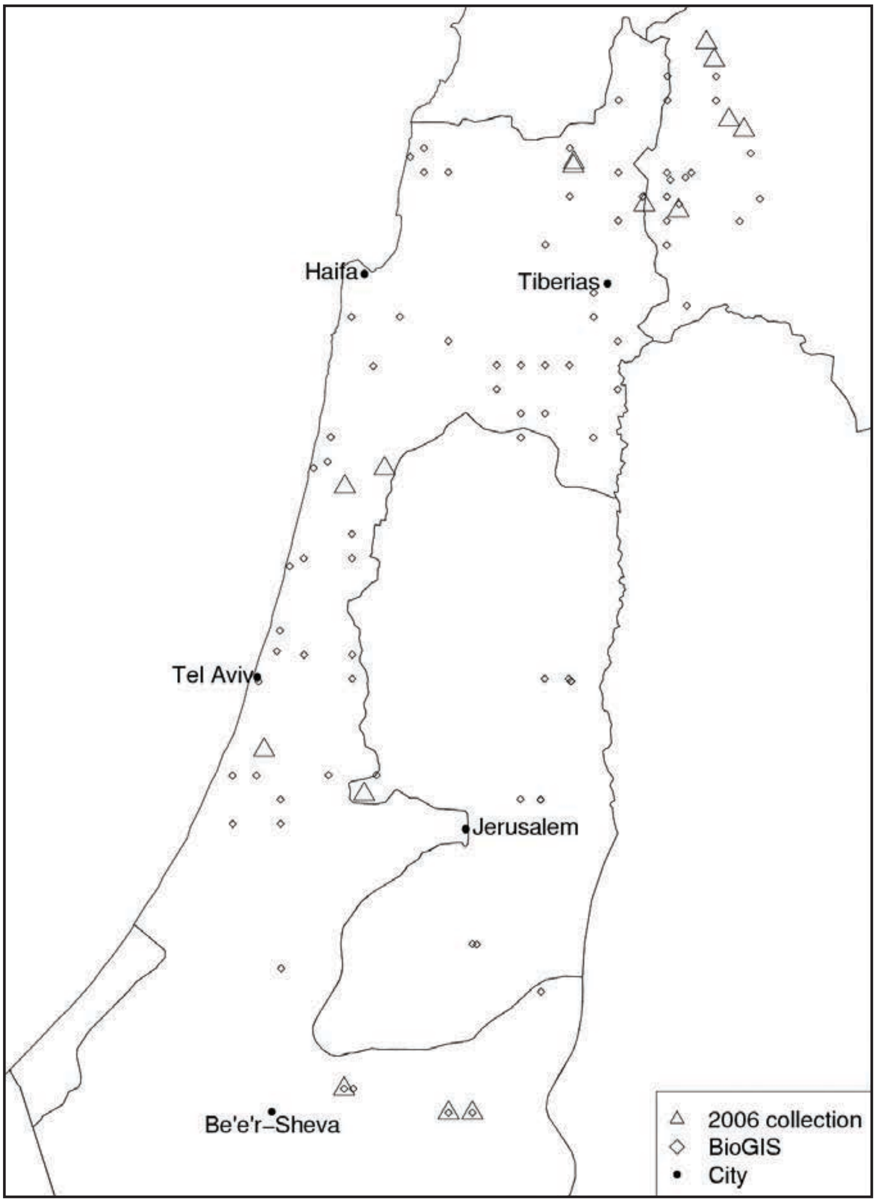

Figure 2c. Map of Israel showing seed collecting sites in 2006 and observation sites from BioGIS for *Biserrula pelecinus*.

more readily, thereby ensuring adequate regeneration in short crop rotations or continuous pasture systems. Collecting Sites 31–34 possess a combination of high rainfall, high altitude and acid soil not found in existing collections of *B. pelecinus* from other countries (ATGRC database).

The distribution of *Trifolium dasyurum* is similar to that of *B. pelecinus*, but it is far more frequent according to data from BioGIS (Figure 2d). Success of this species can be attributed to several survival strategies. Like many other wild pasture species, *T. dasyurum* produces seeds with high levels of hard seed dormancy and low rates of seed softening (Snowball, unpublished data). However, most seed softening occurs in winter in cv. Sothis compared with summer or autumn in many other pasture species (Loi et al. 2007). This is clearly a safe strategy to deal with a lack of critical autumn rainfall.

This character has also been utilized in the control of crop weeds during the pasture phase. Seeds of *T. dasyurum* are relatively large (2–6 mg) with less than 25% expected to be able to survive passage through the grazing animal (Thomson et al. 1990). However, flowering is determinate, seed ripening is rapid, and seed heads and calyces dehisce soon after ripening, whence they are effectively spread by wind and escape grazing animals. It has been suggested that rapid seed ripening can explain differences in the nutritive value between plant parts of *T. dasyurum*, and that it maximizes seed production and therefore ecological fitness (Norman et al. 2005a, b). Future collecting south and east of Be'e'r-Sheva should be attempted in years of average or above average rainfall to obtain more germplasm of *T. dasyurum*, *B. pelecinus* and *T. spumosum*. Existing seed collections held in the Hebrew University of Jerusalem also need to be examined.

Studies of annual forage species in Mediterranean environments have shown a variety of adaptive characteristics that provide strong protection from genetic erosion. Norman et al. (2005a, b) found annual species of *Trifolium* collected from the same sites possess different reproductive strategies to achieve persistence. These include large seeds that give competitive advantage, large number of small seeds, and high levels of long-term hardseededness. Four species of *Trifolium* (including *T. glanduliferum* and *T. purpureum*) originating from Israel displayed a breakdown in hardseededness delayed until mid- to late autumn, and *T. clypeatum* L. displayed slow germination (Norman et al. 1998), both important strategies that avoid seedling death when insufficient rain falls at the break of the season. Studies undertaken in Israel across an aridity gradient showed that annual plant communities at the arid end benefit from a close association with adjacent shrub species, probably through amelioration of drought stress from shade and improved soil structure and infiltration (Holzapfel et al. 2006). In similar studies, size characteristics in plants were important in determining species composition. Small species with tiny seeds, like *T. campestre* Schreb., *Trigonella monspeliaca* L. and *Hippocrepis unisiliquosa* L., were at an advantage at low productive sites when grazed, compared to larger plants with very large seeds, like *Hymenocarpos circinnatus*, which benefited from no grazing (Osem et al. 2004). The relative importance of plant size versus seed size in the response to grazing was thought to change with productivity level (Osem et al. 2006). Grazing intensity has a direct impact on the composition and diversity of natural pastures. In Israel, heavy, continuous grazing resulted in a more species rich plant community (Sternberg et al 2000). High grazing pressure has been blamed for the degradation of many Mediterranean ecosystems. In Israel, however, heavy grazing can be employed while imposing limited damage (Zaady et al. 2001). It is clear that heavy grazing can generally increase the diversity of natural pastures, but severe grazing over an extended period of time on ever decreasing areas of habitat is likely to have the reverse effect. Natural rangelands in Israel are well managed and their species diversity is not likely to be at risk from overgrazing. However, it is not clear how much at risk they are from under-grazing or from loss of their habitats from competing land use. While Israel has established many nature reserves that are well managed, genetic diversity is almost certainly being lost when habitats disappear to urban, industrial and agricultural development. A desirable course of action would be to assess the loss in genetic diversity of a small number of key species. This should be undertaken by re-visiting sites recorded on BioGIS to assess species abundance over a number of years. *Trifolium glanduliferum* was found at relatively few sites compared with observations from BioGIS, is rare in the Mediterranean, with few *ex situ* seed collections, and thus should be one of the first species for such a proposal. There are many other species that were infrequent or not encountered during the mission that could also be evaluated. Gaining a better appreciation of the threats to existing natural pastures will assist the Israeli Genebank in priority setting.

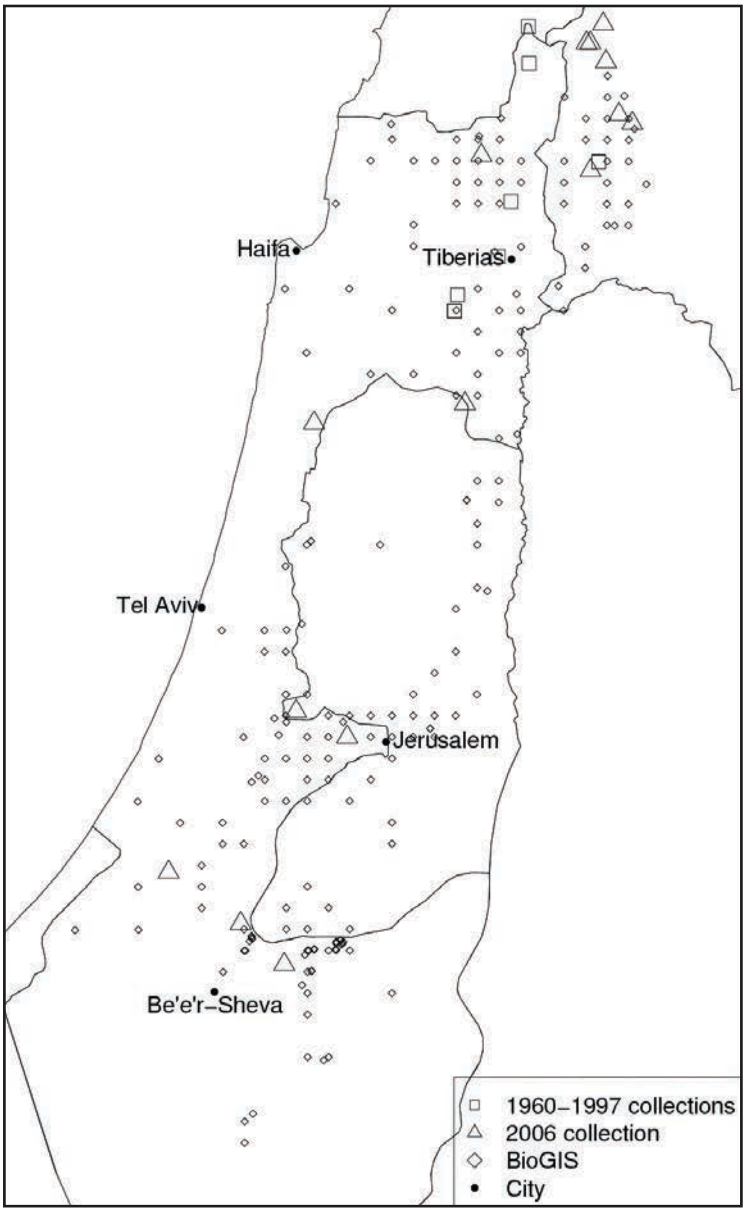

Figure 2d. Map of Israel showing seed collecting sites in 2006, previous seed collecting sites and observation sites from BioGIS for *Trifolium dasyurum*.

Conclusion

Israel possesses a large diversity of native pasture legumes, including 50 species of *Trifolium*. This collecting mission revealed that while some species were found to be widespread, many were far less frequent. Some species have characteristics that enable their spread and persistence, including hard seed dormancy, small seed size, wind-borne seed pods and tolerance to grazing. Other species were less frequent than expected from existing records. These, as well as the more rare species, should be surveyed to better assess their distribution and abundance, and, if necessary, seed collecting be undertaken for the purpose of *ex situ* conservation in the Israeli Genebank. Future collecting of species important for southern Australia should focus on the northern Negev and Wadi Al Jayb, where drought tolerance is likely to be found.

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Figures in this article were constructed using the R statistical system (R Development Core Team 2007) based on the R library called maps and a soon to be released library called GPSoR (D'Antuono and Snowball – see http://www.agric.wa.gov.au/biometrics).

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Prospections et collectes des écotypes de baobab (*Adansonia digitata* L.) au Mali

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Résumé

Prospections et collectes des écotypes de baobab (*Adansonia digitata* L.) au Mali

Le baobab africain (*Adansonia digitata* L.) est une espèce ligneuse alimentaire appartenant à la famille des Bombacacée. Son aire de distribution se situe du Sénégal à Madagascar. L'espèce présente des fonctions socio-économiques très importantes dans les pays du Sahel en Afrique. Sa production est limitée cependant par le vieillissement des parcs, la longue phase d'adolescence de l'espèce, l'absence de régénération et la méconnaissance des ressources génétiques et des techniques sylvicoles. L'objectif de ce travail est d'enquêter et d'identifier les variétés de baobab selon les critères des paysans puis de collecter les fruits sur ces variétés, de même que sur des populations dans le but de les caractériser et d'identifier des 'arbres plus'. La connaissance de la variabilité agro-morphologique et biochimique devrait permettre de proposer une ou des stratégie de gestion de l'espèce et de mettre en place des méthodes de multiplication végétative des 'arbres plus'. Des enquêtes et prospections effectuées au Mali en 2005 et 2006 ont montré que certaines populations disposent de critères de sélection du baobab qui leur ont permis d'identifier des morphotypes. Ces critères varient selon les régions et selon les groupes ethniques. La distinction entre morphotypes est effectuée à partir de la forme des fruits par les groupes ethniques Malinkés et Kassonkés en zone soudanienne sud et sahélienne sud, elle est faite à partir de la coloration du tronc au niveau du groupe ethnique Bambara en zone soudanienne nord. La sélection humaine semblerait donc exercer une influence sur la distribution des morphotypes à travers le pays. Dix fruits ont été collectés sur 399 morphotypes identifiés de même que sur 28 populations distribuées dans quatre zones agro-climatiques du Mali (guinéen nord, soudanien sud, soudanien nord et sahélien sud). Ces morphotypes et ces populations seront caractérisés du point de vue agro-morphologique et biochimique.

Resumen

Prospección y recolección de ecotipos de baobab (*Adansonia digitata* L.) en Malí

El baobab africano (*Adansonia digitata* L.) es una especie leñosa alimenticia de la familia Bombaceae distribuida de Senegal a Madagascar. Tiene funciones socioeconómicas muy importantes para los países del Sahel, pero su producción es limitada debido al envejecimiento de los parques, el largo tiempo de maduración, la falta de regeneración y el desconocimiento de los recursos genéticos y técnicas silvícolas. Este trabajo pretende examinar e identificar las variedades de baobab según los criterios de los productores, y recolectar frutos para caracterizarlos e identificar "árboles súper". Las investigaciones y prospecciones efectuadas en Malí en 2005 y 2006 mostraron que los criterios de selección empleados por ciertas poblaciones permiten identificar morfotipos. Estos criterios varían según las regiones y los grupos étnicos. Los grupos étnicos Malinkés y Kassonkés de la zona sudanesa del sur y saheliana del sur distinguen entre morfotipos a partir de la forma de los frutos; el grupo étnico Bambara de la zona sudanesa norte lo hace según la coloración del tronco. La selección humana parecería ejercer una influencia sobre la distribución de los morfotipos en el país. Se recogieron diez frutos sobre 399 morfotipos identificados entre 28 poblaciones distribuidas en cuatro zonas agroclimáticas de Malí (guineana norte, sudanesa sur, sudanesa norte y saheliana sur). Estos morfotipos y poblaciones serán caracterízadas desde el punto de vista agromorfológico y bioquímico.

Summary

Prospecting and collection of ecotypes of baobab (*Adansonia digitata* L.) from Mali

African baobab (*Adansonia digitata* L.), a species of woody crop belonging to the Bombaceae family, is distributed from Senegal to Madagascar. The species is of high socio-economic importance in the Sahel. Production is limited by the aging of the parks, the length of time needed to reach maturity, a lack of regeneration and a lack of information on genetic resources and sylvicultural management. The objective of the study is to investigate and identify varieties of baobab according to criteria of the producers, then to collect fruits with the aim of characterizing and identifying 'super trees'. A knowledge of the agro-morphological and biochemical variability should result in one or more strategies for production of the species and the introduction of methods for the vegetative multiplication of 'super trees'. The investigation and plant hunting was carried out in Mali in 2005 and 2006 and demonstrated that certain populations displayed selection criteria that could be used to identify baobab morphotypes. The criteria varied according to region and cultural origin. Morphotypes were differentiated by fruit shape in the Malinkés and Kassonkés cultural groups from the south Sudan and south Sahel zones, and from trunk colour at the level of the Bambara cultural group in the north Sudan zone. Selection by man would seem to influence morphotype distribution throughout the country. Ten fruits each were collected from 399 morphotypes identified from 28 populations distributed in four agro-climatic zones of Mali (north Guinea, south Sudan, north Sudan and south Sahel). The morphotypes and populations were characterized on the basis of their agro-morphology and biochemistry.

Key words: baobab, collecting, morphotypes, pedoclimatic gradiant, populations, prospection.

Introduction

Les espèces ligneuses alimentaires constituent la base du développement socio-économique des populations soudano-sahéliennes d'Afrique. Elles ont été utilisées et conservées par les populations rurales pendant des millénaires. Cependant, leurs ressources génétiques sont en péril en raison de la pression anthropique et des changements climatiques. Parmi elles, le baobab tient une place de choix. Son aire de distribution se situe en Afrique tropicale au niveau des isohyètes variant entre 400 et 1000 mm de pluie. On le retrouve du Sénégal à Madagascar en passant par les forêts de l'Afrique centrale où il est rare, puis près des côtes et des îles du Kenya et de la Tanzanie (Savart 2003). L'espèce vit de façon préférentielle sur les sols calcaires, mais on la retrouve aussi sur les sols latéritiques, limono-sableux à sableux, et même sur les sols gravillonnaires. Le baobab appartient à la famille des Bombacacées et au genre *Adansonia*. Le genre *Adansonia* renferme plusieurs espèces, la plus commune *Adansonia digitata* est celle que l'on retrouve en Afrique de l'Ouest, du Centre et de l'Est. Il existe à Madagascar et en Australie d'autres espèces telles qu'*Adansonia madagascariensis* et *A. gregorii* (Aubreville 1950 ; Wickens 1982).

Cet arbre, de 10 à 20 mètres de hauteur, peut vivre pendant 3000 ans. Le tronc peut mesurer jusqu'à 10 mètres de circonférence, il est brillant, lisse, de couleur gris clair à foncé ou rougeâtre avec toute une gamme de couleur intermédiaire (Terrible 1991 ; Arbonnier 2000). Les fleurs sont solitaires d'un diamètre d'environ 20 cm avec des pédoncules de 25 cm de longueur. Elles sont blanc jaunâtre et portent de nombreuses étamines. Le fruit est une capsule indéhiscente de couleur vert clair à vert jaunâtre renfermant un péricarpe à l'intérieur duquel se trouve la pulpe entourant de nombreuses graines (Cunny et al. 1997).

L'espèce joue des fonctions socioéconomiques importantes en Afrique sahélienne. Les feuilles et la pulpe des fruits sont utilisées dans l'alimentation. Les graines servent à fabriquer un condiment pour assaisonner les sauces. La pulpe renferme des quantités importantes de sels minéraux (Fe, Ca, Mg, Zn, etc.) ainsi que de la vitamine C et du sucre. Les feuilles sont riches en provitamine A et en oligoéléments. L'écorce est utilisée dans l'artisanat et sert à fabriquer des cordes, des paniers. Elle possède également des utilisations médicinales et culturelles (Maydell 1990).

L'espèce fait l'objet d'un commerce important entre le Mali et la sous-région en particulier le Sénégal et constitue une source importante de revenu pour les populations rurales.

La production du baobab connaît des contraintes liées à l'irrégularité de la production fruitière due au vieillissement des arbres dans les parcs, la sécheresse, la dégradation des sols et la longue phase d'adolescence de l'espèce. Il faut également citer l'absence de régénération dans les parcs, l'exploitation sévère pour la production de feuilles qui réduit considérablement la fructification, la méconnaissance des techniques sylvicoles et des ressources génétiques. En dépit de la méconnaissance des techniques sylvicoles, le baobab reste fortement sollicité par les populations du Sahel, et là où les plantations n'existent pas, le désir de réintroduction est vif. C'est ainsi qu'au Mali, les paysans des plaines de Ségou et du Seno cultivent de jeunes plants de baobab aux abords des cases, qu'elles entretiennent et replantent par la suite au pourtour de leurs champs (Sidibé et al. 1996).

Les pratiques humaines (sélection pour des caractères avantageux, récolte systématique des fruits, échanges des fruits) associées aux changements climatiques ont probablement eu un impact sur la structuration de la diversité génétique de ces caractères.

Dans le souci de connaître la diversité génétique du baobab et sa distribution au Mali, puis de proposer une ou des stratégies de gestion de l'espèce, il est opportun de prospecter et de collecter les ressources génétiques du baobab. Les prospections et collectes permettront de caractériser les morphotypes selon les critères paysans puis les populations en fonction du gradient pédo-climatique. Elles serviront également à identifier les « arbres plus » qui pourront être multipliés par voie végétative. Le présent article traite des prospections et des collectes des ressources génétiques du baobab dans les différentes zones agro-climatiques du Mali.

Matériels et méthodes

Sites d'étude

Les prospections et collectes ont eu lieu dans quatre zones agro-climatiques du Mali : le sahélien sud avec une pluviométrie variant entre 400 à 600 mm par an, le soudanien nord où la pluviométrie varie entre 610 et 850 mm, le soudanien sud (860 à 1100 mm) et le guinéen nord (1110-1500 mm). Les sols varient de latéritique à calcaire, puis sableux, sablo-limoneux ou gravillonnaire. Le vent est de type mousson en hivernage (juin à octobre) tandis que l'harmattan (vent sec et chaud) souffle en saison sèche (de novembre à février).

Prospections

Missions exploratoires et d'enquêtes

Une première mission exploratoire a eu lieu en 2004 afin de visiter les sites à baobab et d'identifier les pieds à récolter. Dans chaque zone, les responsables de la conservation de la nature et les autorités coutumières nous ont guidé dans le choix des sites. Puis, au niveau des villages, les élus communaux tels que les chefs de village nous ont accompagnés sur le terrain. D'autres missions ont eu lieu en 2005, dans le but de discuter avec les populations. Il s'agissait d'enquêtes rapides semi structurées au cours desquelles les femmes, les hommes et les enfants ont été interrogés. Les questionnaires ont porté sur l'importance socioéconomique du baobab, les usages des différentes parties du baobab, les critères de sélection des morphotypes, les noms des morphotypes et l'état de la ressource (pression humaine, pastorale, régénération).

Les collectes

La collecte a concerné les fruits puisque chez le baobab la reproduction est essentiellement sexuée bien que la multiplication végétative par greffage soit possible. Les collectes ont eu lieu dans les champs (champs de case et champs de brousse) et les jachères en compagnie des populations et après entretien. Il est à signaler que les champs de case et les jachères appartiennent en général aux paysans tandis que les champs de brousse appartiennent à la communauté villageoise. Les collectes ont été réalisées dans toutes les zones agro-écologiques comportant le baobab avec tous les types de sol et de climat possibles dans le but de capturer le maximum de variabilité. Bien que les sites soient surtout localisés dans les soudanien sud, soudanien nord et sahélien sud, des récoltes ont eu lieu également dans le guinéen nord plus humide qui constitue une zone marginale pour le baobab afin d'avoir une large gamme de conditions climatiques.

Missions de récolte des fruits

La récolte des fruits du baobab a lieu entre janvier et mars, période de maturité des fruits. Les récoltes ont débuté en janvier 2005 pour s'achever en 2007. Elles ont porté à la fois sur les morphotypes identifiés par les paysans et sur les populations.

Récolte des morphotypes

La première catégorie de récolte a eu lieu sur les morphotypes identifiés selon les critères paysans. Sur ces arbres, 10 fruits mûrs ont été récoltés de manière aléatoire sur l'arbre, mis dans des sacs en coton et identifiés par les données de passeport à savoir, le nom vernaculaire, le nom du propriétaire du champ ou de la jachère, le caractère organoleptique, l'âge de l'arbre selon le paysan. Chaque arbre récolté a été géoréférencé à l'aide d'un GPS de type Garmin 2000. Les arbres ont également été immatriculés à l'aide de peinture blanche, la numérotation consiste à écrire l'initiale du baobab en plus du chiffre donné en fonction de l'ordre de la collecte.

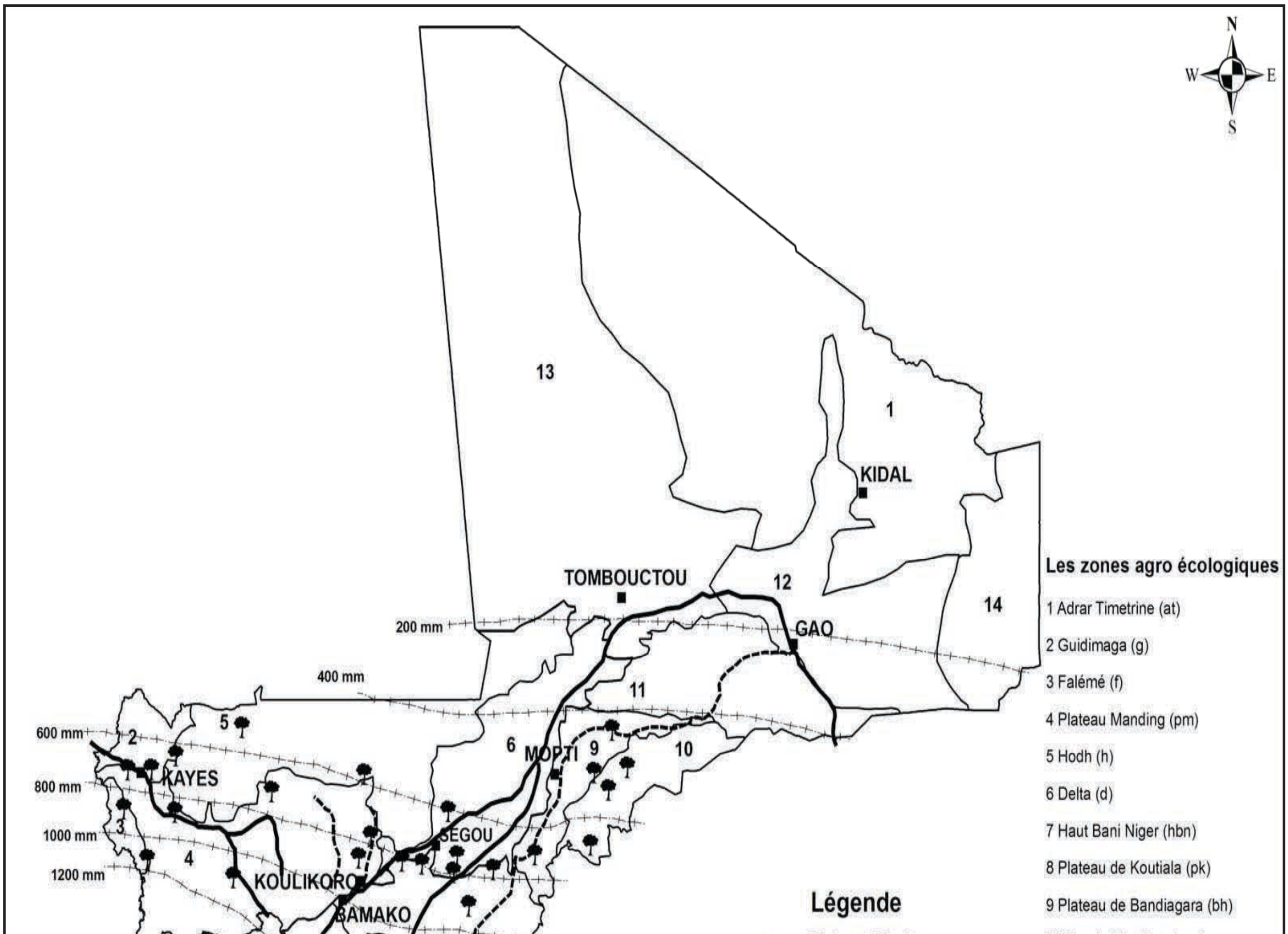

Figure 1. Carte de l'aire de collecte du baobab au Mali.

Collecte des fruits dans les populations

La collecte a été effectuée de manière aléatoire sur 40 arbres dans les champs ou les jachères des terroirs villageois. La valeur généralement élevée de la variance intra population pour les caractères de la croissance et des fruits (Kremer 1994 ; Maley & Parker 1992 ; Bani Aameur & Ferradous 2001 ; Ouédraogo 1995 ; Sanou et al. 2006), a contraint de récolter un minimum de 30 arbres par population pour les études de la variabilité. Une distance minimale de 100 mètres a été laissée entre les arbres afin d'éviter la consanguinité. Les arbres ont été également géoréférencés à l'aide du GPS et immatriculés. 10 fruits ont été également collectés sur chaque arbre et placés dans des sacs en coton.

Méthodes d'analyse des données

Pour l'établissement de la carte de distribution du baobab dans le pays, les coordonnées géographiques des sites prospectés ont été introduites et analysées par le SIG (système d'information géographique) de l'Institut d'économie rurale (IER) à l'aide du logiciel Map Info 6.0. Le test de Khi2 pour comparer les effectifs des morphotypes dans les différentes zones agro-climatiques a été réalisé à l'aide du logiciel XL-stat.

Résultats et discussion

Description des sites de collecte

Les collectes des ressources génétiques du baobab ont eu lieu dans 28 sites, répartis entre neuf zones agro-écologiques du Mali qui sont: le Guidimaga, le Hodh, la Falémé, le Plateau Mandingue, le Haut Bani Niger, le Plateau de Koutiala, le Plateau de Bandiagara, le Delta, et le Gondo Mondoro. Les récoltes ont eu lieu essentiellement dans les champs, mais aussi dans les jachères pour quelques rares cas. La carte des sites de collecte de même que les zones agro-écologiques correspondantes sont présentées dans la figure 1. Les listes des morphotypes et des populations récoltées sont fournies respectivement par les tableaux 1 et 2.

Le Guidimaga : est limité au nord par la Mauritanie, au sud par le Plateau Mandingue, à l'ouest par la république du Sénégal et à l'est par le Hodh. Il s'étend sur la rive droite du fleuve Sénégal à l'ouest du massif Sarakollé. Il se trouve également à cheval sur les zones agro-climatiques du sahélien sud et du soudanien nord avec une pluviométrie variant entre 500 et 850 mm par an. C'est une vaste plaine dont l'altitude moyenne est d'environ 100 m avec la présence de quelques collines gréseuses culminant à 200 m d'altitude. Le sol est sableux à sablo-limoneux. La région est traversée par les prolongements du mont Tambaoura, et par le Kolimbiné qui est un affluent du fleuve Sénégal. La zone est située à environ 550 km de Bamako la capitale. Trois villages ont été prospectés dans cette zone agro-écologique : Samé, Kabaté et Tringa-Marena. A Kabaté et Tringa-Marena, l'ethnie dominante est Sarakollé, tandis qu'à Samé on trouve à la fois les ethnies Khassonké et Ouolof, cette dernière étant originaire du Sénégal.

Le Hodh : Cette zone est essentiellement sahélienne avec une pluviométrie variant entre 350 et 700 mm par an. Située à 500-600 km de Bamako, elle est limitée à l'est et au sud-est par le Delta central nigérien, au sud par le Plateau Mandingue. Le sol est sableux à sablo-limoneux. On y distingue à l'ouest la présence du massif Sarakollé, la partie centrale est une vaste dépression localement ensablée tandis que les marges orientales sont des parties renfermant des sédiments du Continental Terminal (sable, grès argileux). Deux sites y ont été prospectés : Gadiabakadjel et Mourdiah. Le premier site est habité essentiellement par des Peulhs, le second est peuplé de Maures et de Sarakollés.

La Falémé : cette zone agro-écologique se situe au dessous de celle du Plateau Mandingue et s'étend à l'ouest de la falaise de la Tambaoura. Elle est traversée par la rivière Falémé (un affluent du fleuve Sénégal) et est bordée par le plateau du Fantofa. Le relief est une plaine comportant des collines, des buttes et des glacis de faible pente. Elle se situe entre les zones agro-climatiques du soudanien nord et du soudanien sud. Les deux villages prospectés sont Djinguilou et Kegnety habités essentiellement par le groupe Mandé composé de Khassonkés et de Malinkés.

Le Plateau Mandingue : se situe également dans les zone agro-climatiques du soudanien sud et du soudanien nord avec une pluviométrie variant entre 700 et 1100 mm. C'est un ensemble de surfaces d'aplanissement de buttes, de collines, de grès et de dolérite. Les villages prospectés sont les suivants : Kourougué, Sékokoto, Banamba, Nambolia et Sirado. Le village de Banamba est peuplé par les Bambaras, Peulhs et Maures, ceux de Sékokoto et Kourougué sont constitués essentiellement de Malinkés et celui de Sirado est surtout dominé par les Bambaras.

Le Haut Bani Niger : Cette zone est limitée au nord-ouest par le Plateau Mandingue, au nord-est et à l'est par le le Plateau de Koutiala. Le climat est du type nord guinéen avec environ 1200-1500mm de pluie par an, la végétation est la savane de type arboré. Les sols sont d'origine alluviale à colluvio-alluviale. On y trouve les sites de Koumadiobo et de Zegoua. Ces villages sont habités par les Bambaras et les Sénoufos. Le baobab n'est pas très abondant dans cette zone à cause de l'humidité plus importante.

Le Plateau de Koutiala : renferme les sites de N'Tarla, Kolokoba, Zambougou, Boidié, et N'Golosso. La zone est limitée au nord par le Delta du Niger, à l'est par le Plateau de Banfora, à l'ouest et au sud-ouest par le Haut Bani Niger. Ce plateau est constitué essentiellement de grès siliceux très friables qui s'altèrent facilement. Le relief est constitué par une succession de surfaces sommitales plates séparées par des versants glacis, des versants d'accumulation et des vallées alluviales. La pluviométrie est de l'ordre de 850 à 1000 mm. La population est composée majoritairement de Miniankas, Sénoufos et Bambaras.

Le Plateau de Bandiagara : est limité au nord par le Gourma et le Delta, au sud par le Plateau de Koutiala, à l'ouest et au nord-ouest par le Delta, à l'est par le Gondo Mondoro. La région est constituée par les grès supérieurs

Tableau 1. Liste des morphotypes collectés et coordonnées géographiques des sites correspondants.

Site	Zones agro ecologiques	Zone agro-climatique	Longitude	Latitude	Morphotype collecté	Nombre d'accessions	Lieu de collecte
Kabaté	Guidimaga	Soudanien nord	11,237	14,510	-		Champ
Samé	Guidimaga	Soudanien nord	11,662	14,501	Sitoburumba Sitodindjan Kalakaladensito Sitodenmissin Tamadensito Sitodima, Nonosito Sitodima	3 3 4 3 2 3 3 2 3	Champ, jachère
Tringa Maréna	Guidimaga	Sahélien sud	10,814	14,701	Pas de critères de différenciation	-	Champ
Gadiabakadjel	Hodh	Sahélien sud	9,667	15,102	Bokidanedjo Bokibaledjo Bokibodedjo	5 4 6	Champ, jachère
Djinguilou	Falémé	Soudanien sud	11,722	13,952	Sitaburumba Sitadindjan Kalakaladensita Sitadenmissin Tamadensita Sitadima, Tamadensita Folosita	3 3 4 2 3 3 3 3	Champ
Kegnety	Falémé	Soudanien sud			Sitaburumba Sitadindjan Kalakaladensita Sitadenmissin Tamadensita Sitadima, Tamadensita Folosita	4 2 3 4 3 5 4 3 5	Champ
Sekokoto	Plateau Mandingue	Soudanien sud	9,803	12,983	Sitaburumba Sitadindjan Kalakaladensita Sitadenmissin Tamadensita Sitadima Sitodima	3 4 3 3 2 2 5 4	Champ
Sirado	Plateau Mandingue	Soudanien sud	13,259	7,629	Sirablé, Siradjè Sirafing, Sirakoloso Siramoloni	3 4 4 3 4	Champ
Nambolia	Plateau Mandingue	Soudanien nord	10,831	13,907	Sitoburumba Sitodindja Kalakaladensito Sitodenmissin Tamadensito Sitodima Sitodima	3 2 4 3 2 2 1 4	Champ
Kourougué	Plateau Mandingue	Sahélien sud	9,147	14,193	Sitaburumba Sitadindjan Kalakaladensita Sitadenmissin Tamadensita Sitadima, Nonosita Sitodima	3 2 4 3 4 5 3 2 3	champ

Tableau 1 (cont.). Liste des morphotypes collectés et coordonnées géographiques des sites correspondants.

Site	Zones agro ecologiques	Zone agro-climatique	Longitude	Latitude	Morphotype collecté	Nombre d'accessions	Lieu de collecte
Banamba	Plateau Mandingue	Soudanien nord	13,565	7,416	Sirablé, Siradjè Sirafing, Sirakoloso Siramoloni	4 4 4 4 5	champ
Koumadiobo	Haut Bani Niger	Guinéen nord	6,943	11,524	Pas de critères de différenciation	-	champ
Zegoua	Haut Bani Niger	Guinéen nord	5,658	10,504	Pas de critères de différenciation	-	champ, jachère
N'Tarla	Plateau de Koutiala	Soudanien sud	5,707	12,585	Pas de critères de différenciation	-	champ, jachère
Kolokoba	Plateau de Koutiala	Soudanien sud	5,990	11,193	Pas de critères de différenciation	-	champ
Zambougou	Plateau de Koutiala	Soudanien nord	6,524	13,177	Sirablé, Siradjè Sirafing, Sirakoloso Siramoloni	3 4 3 4 2	champ
Boidié	Plateau de Koutiala	Soudanien nord	6,871	13,215	Sirablé, Siradjè Sirafing, Sirakoloso Siramoloni	2 3 4 4 2	champ
N'Golosso	Plateau de Koutiala	Soudanien sud	5,284	13,092	Pas de critères de différenciation	-	champ
Koïrabery	Plateau de Bandiagara	Sahélien sud	3,208	15,064	Pas de critères de différenciation	-	champ
Lougouroukoumbo	Plateau de Bandiagara	Sahélien sud			Pas de critères de différenciation	-	champ
Komodiguili	Plateau de Bandiagara	Sahélien sud	3,371	14,429	Pas de critères de différenciation	-	champ
Zoura	Plateau de Bandiagara	Sahélien nord			Pas de critères de différenciation	-	champ
Fouan	Delta	Soudanien nord	5,976	13,055	Sirablé, Siradjè , Sirafing, Sirakoloso Siramoloni	3 4 3 2 4	champ
Nabougou	Delta	Soudanien nord	5,911	13,290	Sirablé, Siradjè Sirafing, Sirakoloso Siramoloni	4 4 3 3 3	champ
Bewani	Delta	Soudanien nord	6,068	13,916	Sirablé, Siradjè Sirafing, Sirakoloso Siramoloni	4 5 3 5 5	champ
Koporo Pen	Gondo Mondoro	Sahélien sud	3,275	14,221	Pas de critères de différenciation	-	champ
Dah	Gondo Mondoro	Sahélien sud	3,595	13,431	Pas de critères de différenciation	-	champ

de la bordure sud-ouest du bassin de Taoudénit avec des placages de quartz et de dolérite. La pluviométrie est de l'ordre de 400 à 700 mm. Les collectes ont eu lieu ans les villages de Koïrabery, Komodiguili, et Zoura. Les ethnies rencontrées dans ces villages sont majoritairement les Dogons et les Bobos.

Le Delta : est limité au nord par le l'Aklé Azaouad et le Gourma, à l'ouest par le Hodh, au sud par le Plateau de Koutiala et à l'est par les contreforts du Plateau de Bandiagara. La pluviométrie varie entre 250 à 800 mm. La zone est très arrosée par le fleuve Niger et son affluent le Bani qui font qu'on y distingue :

Tableau 2. Liste des populations collectées et coordonnées géographiques des sites correspondants.

Sites	Zones agro ecologiques	Zone agro-climatique	Longitude	Latitude	Nombre d'arbres collectés par population	Lieu de récolte
Kabaté	Guidimaga	Soudanien nord	11,237	14,510	40	champ
Samé	Guidimaga	Soudanien nord	11,662	14,501	40	champ
Tringa Maréna	Guidimaga	Sahélien sud	10,814	14,701	40	champ
Gadiabakadjel	Hodh	Sahélien sud	9,667	15,102	40	champ
Mourdiah	Hodh	Sahélien sud			30	champ
Djinguilou	Falémé	Soudanien sud	11,722	13,952	40	champ
Kegnety	Falémé	Soudanien sud			40	champ
Sekokoto	Plateau Mandingue	Soudanien sud	9,803	12,983	40	champ
Sirado	Plateau Mandingue	Soudanien sud	13,259	7,629	40	champ
Nambolia	Plateau Mandingue	Soudanien nord	10,831	13,907	40	champ
Kourougué	Plateau Mandingue	Sahélien sud	9,147	14,193	40	champ
Banamba	Plateau Mandingue	Soudanien nord	13,565	7,416	40	champ
Koumadiobo	Haut Bani Niger	Guinéen nord	6,943	11,524	40	champ
Zegoua	Haut Bani Niger	Guinéen nord	5,658	10,504	15	champ
N'Tarla	Plateau de Koutiala	Soudanien sud	5,707	12,585	30	champ
Kolokoba	Plateau de Koutiala	Soudanien sud	5,990	11,193	30	champ
Zambougou	Plateau de Koutiala	Soudanien nord	6,524	13,177	40	champ
Boidié	Plateau de Koutiala	Soudanien nord	6,871	13,215	40	champ
N'Golosso	Plateau de Koutiala	Soudanien sud	5,284	13,092	40	champ
Koïrabery	Plateau de Bandiagara	Sahélien sud	3,208	15,064	40	champ
Lougouroukoumbo	Plateau de Bandiagara	Sahélien sud			40	champ
Komodiguili	Plateau de Bandiagara	Sahélien sud	3,371	14,429	40	champ
Zoura	Plateau de Bandiagara	Sahélien nord			40	champ
Fouan	Delta	Soudanien nord	5,976	13,055	40	champ
Nabougou	Delta	Soudanien nord	5,911	13,290	40	champ
Bewani	Delta	Soudanien nord	6,068	13,916	40	champ
Koporo Pen	Gondo Mondoro	Sahélien sud	3,275	14,221	40	champ
Dah	Gondo Mondoro	Sahélien sud	3,595	13,431	40	champ

- des plaines d'innondation (delta vif) où les sols sont franchement innondés de façon saisonnière à certains endroits par les crues du fleuve Niger,
- des plaines fossiles constituées surtout de sols limono argilo sableux abritant de nombreuses mares saisonnières,
- le Moyen Bani Niger drainé par les eaux du Niger et du Bani avec des sols légèrement sableux et profonds,
- une zone lacustre avec une faible proportion de terre sèche (5%) et environ 95% de zone humide constituée par un ensemble de lacs dont certains ont été asséchés depuis des décennies. La population est composée de Bambaras, de Peulhs et de Bozos, les villages prospectés sont Fouan, Nabougou et Bewani.

Le Gondo Mondoro: est situé entre le Plateau Mossi (Burkina Faso) et celui de Bandiagara. La zone est située entièrement dans le sahélien sud avec une pluviométrie de l'ordre de 400 à 600 mm par an, la végétation est steppique à épineuse. Cette région comporte par endroit une épaise dalle cuirassée sous des cordons dunaires, mais aussi des formations sédimentaires du Continental Terminal (sable, grès argileux). Les sols sont d'origine alluviale ou éolienne. Les ethnies rencontrées ici sont les Dogons, les Dafings et les Peulhs ; les villages prospectés sont Koporo-Pen et Dah.

Classification paysanne du baobab dans les différentes régions et collecte des fruits:

Les méthodes de classification varient en fonction des sites et des ethnies. Les enquêtes ont montré que certains groupes ethniques sélectionnent le baobab en fonction de la coloration du tronc et des caractéristiques des fruits (précocité, succulence, forme des fruits, caractère organoleptique des feuilles), et de la forme de l'arbre. D'autres n'ont pas de critères de distinction. Les résultats sont consignés dans le tableau 1. Au total, 15 morphotypes différents ont été recensés et 399 accessions ont été collectées pour ces morphotypes. Ainsi, si on fait une analyse en fonction des groupes ethniques, on constate que les Sarakollés n'ont pas de critères de différenciation du baobab.

Par contre, les Khassonkés font la distinction entre les types de baobab en fonction des caractéristiques des fruits. On a pu recenser selon ce groupe ethnique les morphotypes suivants:

- Sitoburumba : baobab à gros fruits
- Sitodindjan : baobab à fruits allongés
- Kalakaladensito : fruits en forme de meule
- Sitodenmissin : arbre à petits fruits
- Tamadensito : fruits en forme de tambour
- Sitodima : arbre à fruits succulents
- Nonosito : fruits à pulpe blanchâtre (laiteux)
- Sitodima : baobab au fruit sucré

Pour ce groupe ethnique (villages de Samé et Nambolia), huit variétés ont été recensées avec 47 accessions collectées.

En milieu malinké, la classification du baobab est faite en fonction de la forme des fruits, du goût du fruit, de la coloration de la pulpe tout comme chez les Khassonkés, cependant les appellations diffèrent légèrement. On a pu noter ici les morphotypes suivants:

- Sitaburumba : baobab à gros fruits
- Sitadindjan : baobab à fruits allongés
- Kalakaladensita : fruits en forme de meule
- Folosita: selon eux, la consommation des fruits de cet arbre donne le goître.
- Sitadenmissin : arbre à petits fruits
- Tamadensita : fruits en forme de tambour
- Sitadima : arbre à fruits succulents
- Nonosita : fruits à pulpe blanchâtre (laiteux)
- Gonkoungolo : fruits ayant la forme de la tête du singe (circulaire).

Pour ce groupe ethnique vivant à Djinguilou, Kegnety, Sekokoto et Kourougué, sept variétés correspondant à un total de 112 accessions ont été recensées.

En milieu bambara, la distinction est faite selon la couleur du tronc et selon le port de l'arbre. Les morphotypes suivants ont été notés :

- Sirablé : baobab à tronc de couleur rouge
- Siradiè : baobab à tronc de couleur gris clair
- Sirafing : baobab à tronc gris foncé
- Sirakolosso : tronc de coloration constituée par un mélange de gris, de blanc et de rouge
- Siramoloni : baobab de petite taille

125 morphotypes ont été récoltés chez ce groupe ethnique se trouvant dans les villages de Sirado, Banamba, Zambougou, Boidié, Nabougou, Fouan et Bewani.

Les peulhs interrogés ont affirmé ne pas avoir de critères de classification mais, s'ils devaient faire comme les Bambaras, ils auraient distingué les morphotypes suivants :

- Bokidanedjo : baobab à tronc gris clair
- Bokibaledjo : baobab à tronc gris foncé
- Bokibodedjo : baobab à tronc rougeâtre

Le nombre d'accessions récoltées est de 15 (village de Gadiabakadjel).

Les autres ethnies (Sénoufo, Minianka et Dogon) ont affirmé ne pas avoir de critères de classification du baobab bien que les feuilles soient très consommées par ces populations. Cependant, elles reconnaissent les arbres dont les feuilles possèdent des caractères culinaires intéressants. Par exemple, dans le Guidimaga le nom « Ba M'Piè » a été donné à un baobab dont les fruits et les feuilles sont très succulents, ce nom est celui du propriétaire de l'arbre.

Outre les observations effectuées sur les paramètres dendrométriques de l'arbre et des fruits, une analyse biochimique de la pulpe des fruits et des feuilles sera effectuée. Ces résultats permettront de comprendre comment se distribuent ces morphotypes dans les populations anthropisées de baobab au Mali, quelle est la relation entre ces morphotypes, et aussi de mettre en place un descripteur agro-morphologique pour le baobab. Par ailleurs, les morphotypes performants seront sélectionnés et multipliés par greffage.

Collecte des fruits dans les populations

Les sites prospectés lors de la collecte des morphotypes ont été visités pour la collecte dans les populations. Il s'agit des 28 villages mentionnés dans le tableau 2. En général, dix fruits ont été récoltés par arbre sur 40 arbres sauf à Zegoua où le nombre d'arbres a été de 15, et à Mordiah, N'Tarla et Kolokoba où le nombre d'arbres a été de 30. Au total, 1065 arbres ont été caractérisés, le nombre de fruits collectés a été de 10650. Les paramètres de l'arbre ont été observés sur place, les fruits et les graines ont été caractérisés une fois arrivés en station. La caractérisation des feuilles a été realisée pendant l'hivernage. Les résultats qui sont en cours d'analyse seront présentés par la suite. La connaissance de la variabilité en fonction du gradient pédo-climatique permettra de mettre en place une ou des stratégies de gestion de l'espèce. Un tel travail avait été effectué par Ouédraogo (1998) sur *Parkia biglobosa* ; Lovett et *al.* (2000) sur *Vitellaria paradoxa* au Ghana ; Sanou et al. (2006) sur *Vitellaria paradoxa* au Mali.

Impact de la sélection humaine sur la distribution des morphotypes de baobab

La sélection humaine semble avoir un effet sur la distribution des morphotypes de baobab et cette sélection serait dirigée par les connaissances locales sur l'espèce. En effet, les paysans ont reconnu l'existence de morphotypes de baobab seulement dans les zones soudaniennes et sahéliennes. En zone guinéenne, ils ont affimé ne pas avoir de critères de distinction au sein de l'espèce. La zone guinéenne est la zone la plus humide du pays, (pluviométrie autour de 1110-1500 mm par an), la densité en baobab y est faible, et les connaissances sur l'espèce y sont limitées. Pour les zones agro-climatiques où des critères de distinction des morphotypes existent, le test d'indépendance du khi2 (Tableau 3) a montré qu'il n'y a pas de différence significative pour la présence ou l'absence des morphotypes de baobab (p=0,199, α=0,05) entre ces zones.

Par ailleurs, 54,88% des morphotypes recensés en général se trouvaient dans le soudanien nord, 31,57% se trouvaient dans le soudanien sud et 13,54% dans le sahelien sud. Parmi les morphotypes identifiés sur la base des critères des fruits, 51,56% se trouvaient dans le soudanien sud, 32,03% se trouvaient dans le soudanien nord et 16,40% dans le sahelien sud (Tableau 4). Parmi les morphotypes identifiés sur la base des critères des fruits, Sitadiman (20%) possèdait la fréquence la plus élevée et était surtout représenté dans le soudanien sud, il est le moins représenté dans le sahélien sud. Sitaburumba, Kalakaladensita, Sitadenmissen, Tamadensita et Sitadendjan représentent respectivement 14,85%, 18,18%, 14,85%, 13,3% et 12,5%. Ces morphotypes ont été surtout rencontrés dans le soudanien sud. Les morphotypes les moins représentés étaient Nonosita et Folosita avec 3,15% chacun ; le premier a été rencontré dans les soudanien sud et nord tandis que le deuxième a été rencontré seulement dans le soudanien sud.

Parmi les morphotypes classés sur la base des critères de coloration du tronc, 12,38% se trouvaient dans le soudanien sud, 74,33% se trouvaient dans le soudanien nord, et 13,27% dans le sahelien sud (Tableau 4). Les morphotypes Sirablé (22,12%), Siradjè (30%), Sirafing (25,73%), Sirakolosso (22,12%) ont été rencontrés surtout dans le soudanien nord.

Le morphotype Siramoloni est representé seulement en zone soudanienne nord (84%) et soudanienne sud (16%). Ce morphotype est un baobab de petite taille, donc qui n'est pas âgé. En zone soudanienne, les paysans ont pris l'habitude de planter et de conserver les régénérations de baobab dans les cours des maisons afin de récolter et d'utiliser les feuilles pour l'alimentation. Ces plants sont tellement exploités pour les feuilles qu'ils ne rentrent pas en fructification. De telles pratiques existent au sein des groupes ethniques bambara et dogon.

La distinction des morphotypes basée sur les caractéristiques des fruits a été signalée seulement par les groupes ethniques malinké et khassonké, tandis que celle basée sur la coloration du tronc a été signalée par le groupe ethnique bambara. La sélection humaine a donc sans doute influencé la distribution des morphotypes de baobab et la régénération même si les semis des graines sont presque inexistantes dans les différentes zones. L'impact de la sélection humaine sur la distribution de la diversité avait été signalée chez d'autres espèces de la zone soudano-sahélienne d'Afrique. Maranz et Wiesman (2003) ont montré au cours d'une étude chez des populations africaines de karité, que pour les paramètres des fruits, les valeurs les plus élevées et les plus homogènes se rencontraient au niveau du Plateau Central Mossi (Burkina Faso). Ils en ont conclu que cela pourrait être dû à l'action de l'homme qui aurait intensement sélectionné et conservé des karités à gros fruits au niveau de cette zone. Sanou et *al.* (2006) ont également montré au niveau des populations de karité du Mali, que les fruits à quantité de pulpe importante se localisaient essentiellement dans le nord-est de l'aire du karité au Mali et que cela serait dû à la sélection humaine qui aurait favorisé la conservation et la multiplication de ce type de karité dans le nord-est du pays. Selon Seignobos (1982), Boffa (1999), Lovett & Haq (2000a), la sélection humaine serait liée à l'importance socio-culturelle de l'espèce et aux groupes ethniques et pourrait par conséquent influencer sur la densité de l'espèce dans les parcs agroforestiers.

Tableau 3. Comparaison des fréquences de distribution des morphotypes de baobab en fonction des différentes zones agro-climatiques.

Test d'indépendance du Khi2	
Khi2 observé	6,000
Khi2 valeur critique	9,488
ddl	4
p-value unilatérale	0,199
Alpha	0,05

Tableau 4. Pourcentages des morphotypes rencontrés par zone agro-climatique selon les deux critères (fruits et coloration du tronc des arbres).

Zones agro-climatique	Pourcentage des morphotypes identifiés sur la base des critères de fruit	Pourcentage des morphotypes identifiés sur la base de la coloration du tronc
Sahélien sud	16,40	13,27
Soudanien nord	32,03	74,33
Soudanien sud	51,56	12,38

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Método rápido y económico para caracterizar clones de yuca a través de marcadores bioquímicos

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Resumen

Método rápido y económico para caracterizar clones de yuca a través de marcadores bioquímicos

Los bancos de germoplasma son caracterizados mediante descriptores morfológicos, marcadores bioquímicos y moleculares. Los descriptores bioquímicos ofrecen ventajas tales como mayor información que los métodos tradicionales, relativo bajo costo, fácil ejecución y se recomiendan para bancos de germoplasma con gran número de entradas. En esta investigación se evaluaron algunos marcadores bioquímicos y se ajustó la metodología para caracterizar clones de yuca provenientes del Banco de Germoplasma de Yuca del INIA-CENIAP, Maracay, Venezuela. El ajuste del protocolo se elaboró mediante las respuestas electroforéticas de extractos derivados de tejidos de hojas apicales y puntas de raíces, de un grupo de 65 clones de yuca.

Résumé

Méthode rapide et peu coûteuse de caractérisation de clones de yuca par des marqueurs biochimiques

Le matériel génétique dans des banques de gènes est caractérisé au moyen de descripteurs morphologiques ainsi que de marqueurs biochimiques et moléculaires. Les descripteurs biochimiques sont plus avantageux, fournissant notamment une meilleure information que les méthodes traditionnelles, ils sont relativement peu coûteux, faciles à mettre en œuvre et recommandés pour les banques de gènes présentant un grand nombre d'accessions. Cette étude évalue divers marqueurs biochimiques et modifie la méthodologie de la caractérisation des clones de yuca provenant de la banque de gènes de yuca, INIA-CENIAP, Maracay, Venezuela. Le protocole a été modifié à partir de la réponse électrophorétique d'extraits de tissus foliaires apicaux et d'extrémités racinaires d'un groupe de 65 clones de yuca.

Summary

Rapid low cost method for characterizing yuca clones by bochemical markers

Germplasm in genebanks is characterized by means of morphological descriptors, and biochemical and molecular markers. Biochemical descriptors have advantages such as better information than traditional methods, relatively low cost and easy implementation, and are recommended for genebanks with large numbers of accessions. This investigation evaluated various biochemical markers, and modified the methodology for the characterization of yuca clones originating from the the Yuca Genebank, INIA-CENIAP, Maracay, Venezuela. The protocol was modified by means of the electrophoretic response of extracts from apical leaf tissue and root tips from a group of 65 yuca clones.

Key words: Genebank, yuca, ***Manihot esculenta***, **biochemical markers.**

Introducción

La mayor importancia de la colección y conservación de germoplasma en el mundo consiste en resolver estatus taxonómicos y relaciones evolutivas entre y dentro de las especies. Sin embargo, la principal justificación es su utilidad para el mejoramiento de los cultivos, aprovechando la variabilidad del banco y determinando los atributos genéticos del germoplasma, mediante los caracteres deseables disponibles en el mismo.

La caracterización y evaluación sistemática es la principal herramienta que permite medir estos atributos. Usualmente los caracteres morfológicos, que son altamente heredables, se emplean para la caracterización realizada por los curadores de los bancos, cuya función se basa en que los caracteres de cada accesión tienen un valor y ayudan al curador del banco a mantener los clones y a comprobar su integridad genética a lo largo de los años. La segunda función está relacionada con el uso en sí mismo de estos materiales.

Como complemento de estas herramientas se han desarrollado e incorporado a la base de datos de los bancos los resultados del uso de las técnicas de marcadores bioquímicos. Estas ofrecen diversas ventajas como proporcionar mayor información respecto de los métodos tradicionales, ser de relativo bajo costo y fácil ejecución en comparación con las técnicas de análisis molecular que requieren de reactivos y equipos de altos costos; además permiten caracterizar, en relativamente poco tiempo, los bancos de germoplasma con gran cantidad de entradas, debido a que se pueden analizar varios locus isoenzimáticos rápida y simultáneamente, y la visualización de las bandas es rápida (Ferreira y Grattapaglia 1996; González et al. 2002).

Entre los marcadores bioquímicos más empleados está la electroforesis de proteínas e isoenzimas, que básicamente involucra los tres siguientes pasos: la extracción de las proteínas del tejido vegetal, la separación de las proteínas a través de la electroforesis y por último la tinción histoquímica del gel, lo que permite visualizar el producto en forma de bandas.

Las proteínas e isoenzimas pueden producirse o acumularse en tejidos específicos; por lo tanto la selección del tejido a partir del cual se extraen éstas es determinante para el éxito de su caracterización. Pueden estar a concentraciones diferentes y variar según el estado de diferenciación celular. Todas estas características determinan el significado biológico que poseen los sistemas enzimáticos (Markert 1977).

La mayoría de los tejidos vivos de las plantas pueden ser usados para el análisis por electroforesis de proteínas e isoenzimas; sin embargo se prefieren tejidos tales como hojas, polen, raíces y semillas por su facilidad de obtención y extracción. Se debe tener en cuenta que al utilizarse material colectado en el campo, es necesario que esté libre de fitopatógenos y organismos epifíticos (Wendel y Weeden 1989).

Una vez identificado el tejido más adecuado, este es macerado en presencia de una solución que permite la extracción de las proteínas, la conservación de su actividad catalítica y la preservación de su oxidación por compuestos fenólicos asociados (Alfenas et al. 1998).

La difusión del uso de la electroforesis de isoenzimas ocurrió gracias al desarrollo de métodos eficientes para su visualización y aplicabilidad inmediata en biología, siendo más significativo su uso en estudios clásicos de genética poblacional (Gottlieb 1971; Pierce y Brewbaker 1973) de un gran número de organismos (Quiroz 1991; Ferreira y Grattapaglia 1996).

Específicamente en el cultivo de la yuca (*Manihot esculenta* Crantz) existen trabajos al respecto. En América Latina, Ramírez et al. (1987) y Hussain et al. (1987) ambos del Centro Internacional de Agricultura Tropical (CIAT) – Colombia, y De Oliveira et al. (1992) de EMBRAPA – Brasil. Estos lograron caracterizar enzimáticamente cultivares de yuca mediante el sistema αβ-esterasas. Montarroyos et al. (2003) determinaron que los sistemas más polimórficos fueron el Glutamato Oxalacético Trasaminasa (GOT) y la Peroxidasa (PRX) en una colección de yuca del Instituto Pernambucano de Agricultura – Brasil.

Resultados similares reportaron Lefèvre y Charrier (1993 a y b), los cuales fueron desarrollados en el Institut Français de Recherche Scientifique pour le Développement en Coopération (INRA) en Francia.

A nivel nacional, Polanco (1998) caracterizó la colección de yuca de la Facultad de Agronomía de la Universidad Central de Venezuela con αβ-esterasas y MDH.

La caracterización agronómica es muchas veces ineficiente para detectar diferencias que permitan discriminar individuos genéticamente cercanos, o que se acentúan cuando se consideran clones diferenciados por mutación en uno o pocos genes que codifican características no cualitativas. Es por esto que muchos curadores de bancos de germoplasma hacen uso de estos marcadores moleculares indirectos como herramienta para detectar más diferencias entre los individuos y así discriminar entre los diferentes materiales del banco (De Oliveira et al. 1992). Como siguiente etapa de la caracterización de los bancos de germoplasma para detectar posibles clones duplicados o para detectar más diferencias entre ellos, identificados morfológica e isoenzimáticamente, se recomienda ampliamente usar marcadores directos (ADN), dependiendo de las posibilidades económicas de que se disponga para desarrollar este tipo de análisis (Ocampo et al. 1987).

Además existen publicaciones que refieren el uso de las técnicas isoenzimáticas para evaluar la variabilidad

Cuadro 1. Clones de yuca del banco de germoplasma seleccionados para establecer la metodología de caracterización isoenzimática.

Clon	Z.C	Clon	Z.C.	Clon	Z.C.
1. Amacuro 130	Da	23. Lengua e´ pajaro	Arc	45. M 440	Su
2. Amacuro 134	Da	24. M 278	Am	46. M 478	Su
3. Amacuro 135	Da	25. M 279	Am	47. M 479	Su
4. Amacuro 144	Da	26. M 284	Am	48. MEVEN 61	Arc
5. Amacuro 145	Da	27. M 285	Am	49. MEVEN 95	Arc
6. Amacuro 166	Da	28. M 291	Am	50. MEVEN 150	Arc
7. Amacuro 168	Da	29. M 292	Am	51. MEVEN 180	Arc
8. Amazonas 188	Am	30. M 306	Am	52. México 59	A
9. Barinas 1	Ba	31. M 307	Am	53. Morichalera	Mo
10. Bolívar 43	Bo	32. M 327	Am	54. Pastorita	Ap
11. Bolívar 50	Bo	33. M 338	Am	55. Plan de Hierro	An
12. Bolívar 58	Bo	34. M 365	Su	56. Proletaria	Arc
13. Bolívar 59	Bo	35. M 366	Su	57. Querepa Blanca	An
14. Bolívar 60	Bo	36. M 388	Su	58. Remigio	Su
15. Bolívar 89	Bo	37. M 392	Su	59. Rivero	Arc
16. Bolívar 98	Bo	38. M 393	Su	60. Tempranita	Zu
17. Burrera	Mi	39. M 394	Su	61. UCV 2062	Aru
18. Brasileña 12	Bo	40. M 395	Su	62. UCV 2076	Aru
19. Chapapotera	Mo	41. M 402	Su	63. UCV 2105	Aru
20. Cogollo verde	An	42. M 422	Su	64. UCV 2129	Aru
21. Juliana	An	43. M 433	Su	65. UCV 2184	Aru
22. Juliana catira	An	44. M 434	Su		

Entidad Federal donde se efectuaron las colectas: AM = Amazonas; An = Anzoátegui; Ap = Apure; Arc = CENIAP-Maracay; Aru = UCV-Maracay; Bo = Bolívar; Da = Delta Amacuro; Mi = Miranda; Mo = Monagas; Su = Sucre; Zu = Zulia.

somaclonal inducida por cultivo *in vitro* usando sistemas electroforéticos de esterasas, peroxidasas y proteínas totales (González et al. 2002) y también se han desarrollado estudios de tolerancia al estrés hídrico haciendo uso de electroforesis de esterasas para conocer su base molecular (Lokko et al. 2007).

En este trabajo se describe la metodología empleada para caracterizar clones de yuca provenientes del Banco de Germoplasma del INIA-CENIAP utilizando marcadores isoenzimáticos.

Materiales y métodos

Selección de clones de yuca

El experimento se realizó en las instalaciones de la Unidad de Biotecnología del INIA-CENIAP, Maracay, estado Aragua, Venezuela. El ajuste del protocolo se elaboró mediante las respuestas electroforéticas de extractos derivados de tejidos de hojas apicales y puntas de raíces de 65 clones de yuca cultivados, dulces y amargos, seleccionados entre 170 entradas colectadas en Venezuela que componen el banco de germoplasma del INIA-CENIAP (Cuadro 1).

Los esquejes seleccionados fueron sembrados en bolsas de plástico, 10 estacas de aproximadamente 20 cm de longitude por clon. El sustrato empleado fue del tipo arena-tierra en proporción 1:1, bajo condiciones de umbráculo. Entre los 40 y 45 días se procedió a la cosecha de todas las hojas apicales y raíces, que fueron lavadas cuidadosamente con abundante agua corriente y posteriormente almacenadas en bolsas a punto de congelamiento hasta su procesamiento.

Protocolo de extracción

Los tejidos seleccionados de los clones de yuca se maceraron con la solución tampón Tris HCl 0,05M a pH 8,3 (Hussain et al. 1987) en frío, en las proporciones de 1:2 para hojas apicales y 1:1 para puntas de raíces. Luego se centrífugó a 10 000 rpm, 25 minutos y 7ºC; posteriormente se almacenó el sobrenadante en condiciones de –20°C hasta corrida.

Aplicación de la técnica electroforética

Las proteínas desnaturalizadas y 20 sistemas enzimáticos (Cuadro 2) fueron evaluados en los extractos provenientes de hojas y raíces. Antes de la ejecución de las corridas se procedió a la aplicación de un tampón de corrida para las muestras, constituido por 60 µl del extracto con 15 µl de azul de bromofenol y 9,6 µl de glicerol al 85%. Las proteínas fueron desnaturalizadas siguiendo el procedimiento de Laemmli (1970). El tipo de gel empleado fue poliacrilamida en un sistema discontinuo (DISCPAGE), en concentraciones de 5–10% para isoenzimas y 6–15% para proteínas desnaturalizadas-SDS. La solución tampón electrodo empleada fue Tris Glicina a pH 8,3 (Laemmli 1970), en condiciones eléctricas de 50 voltios durante los primeros 20 minutos (gel concentrador), con intensidad y poder libre, luego 15 miliamper por gel, 10 a 30 voltios y una potencia variable de 0,2 a 2 vatios (gel separador), y tiempo de corrida variable entre cinco y seis horas.

Resultados y discusión

De los sistemas evaluados fueron seleccionados: proteínas

Cuadro 2. Identificación para los sistemas enzimáticos, código enzimático (C.E.) y su correspondiente referencia.

Sistema	Iniciales	C.E.	Referencia
Fosfatasa Ácida	ACP	3.1.3.2	Wendel y Weeden (1989)
Aconitato Hidratasa	ACO	4.2.3.3	Manchenko (1994)
Alcohol Deshidrogenasa	ADH	1.1.1.1	Manchenko (1994)
Catalasa	CAT	1.11.1.6	Manchenko (1994)
Formaldehído Deshidrogenada	FDH	1.2.1.1	Vallejos (1983)
Esterasas (α y β)	EST	3.1.1.1	Wendel y Weeden (1989)
Glutamato deshidrogenada NADP	GDH	1.4.1.2-4	Wendel y Weeden (1989)
Glucosa – 6- fosfato Deshidrogenada	G6PDH	1.1.1.49	Vallejos (1983)
Glutamato Oxaloacetato Trasaminasa	GOT	2.6.1.1	Wendel y Weeden (1989)
Isocitrato Deshidrogenasa	IDH	1.1.1.42	Wendel y Weeden (1989)
Lactato Deshidrogenasa	LDH	1.1.1.27	
Malato Deshidrogenasa – NADP	ME	1.1.1.40	Wendel y Weeden (1989)
Malato Deshidrogenasa – NAD	MDH	1.1.1.37	Wendel y Weeden (1989)
Fosfogluconato Deshidrogenasa	PGD	1.1.1.44	Vallejos (1983)
Fosfoglucoisomerasa	PGI	5.3.1.9	Wendel y Weeden (1989)
Fosfoglucomutasa	PGM	5.4.2.2	Wendel y Weeden (1989)
Peroxidasa	PRX	1.11.1.7	Vallejos (1983)
Shikimato Deshidrogenasa	SDH	1.1.1.25	Manchenko (1994)
Superoxido Dismutasa	SOD	1.15.1.1	Wendel y Weeden (1989)

Cuadro 3. Composición de la solución fijadora para proteínas.

Componente	Cantidad
Acido Tricloroacético (TCA)	60 g
H_2O destilada	800 ml
Metanol	200 ml
Acido acético al 96%	70 ml

Fuente: Stegeman et al. (1985).

Cuadro 4. Composición de la solución decolorante para proteínas.

Componente	Cantidad
Etanol al 75%	300 ml
Acido acético glacial	50 ml
H_2O destilada	650 ml

Fuente: Stegeman et al. (1985).

Cuadro 5. Composición de la solución fosfato de sodio monobásico pH 7,0.

Componente	Cantidad
NaH_2PO_4	3,6 g
H_2O destilada	100 ml

Cuadro 6. Composición de la solución fosfato de sodio dibásico pH 7,0.

Componente	Cantidad
Na_2HPO_4	4,23 g
H_2O destilada	100 ml

Cuadro 7. Composición de la solución fosfato de sodio pH 7,0.

Componente	Cantidad
Solución fosfato de sodio dibásico pH 7,0	15 ml
Solución fosfato de sodio monobásico	10 ml
H_2O destilada	25 ml

Cuadro 8. Composición de la solución α y β naftil acetato.

Componente	Cantidad
α (ó β) naftil acetato	1,25 g
Acetona al 100%	50 ml

Cuadro 9. Composición de la solución de tinción α ó β-esterasas.

Componente	Cantidad
Solución fosfato de sodio pH 7,0	50 ml
α- (ó β) naftil acetato (disuelto en acetona)	1 ml
Fast Blue RR	25 mg

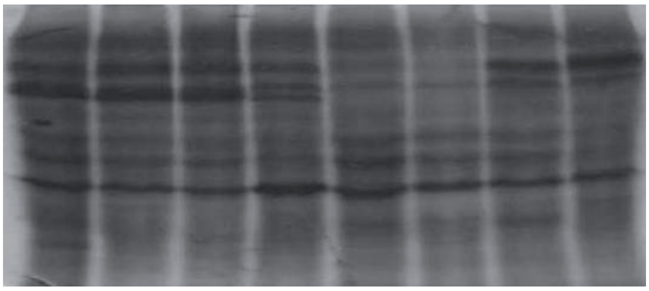

Figura 1. Ejemplo de patrones de proteínas desnaturalizadas de hojas apicales de yuca (Los carriles se enumeran de izquierda a derecha, y son: 1, 2 y 3 = Amacuro 168; 4 = Bolívar 98; 5 = Querepa Blanca; 6 = Brasileña 12; 7 = Barinas; 8 = Bolívar 58).

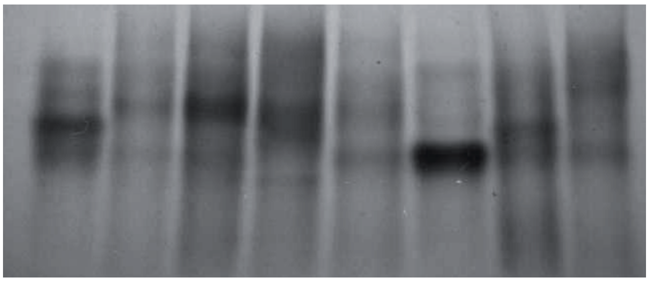

Figura 2. Ejemplo de patrones de α-esterasas en puntas de raíces de yuca (Los carriles se enumeran de izquierda a derecha, y son:1, 2 y 5 = Clon M388; 3 y 4 = Clon M366; 6 = Clon Proletaria; 7 = Clon UCV 2129; 8 = Clon MEVEN 95).

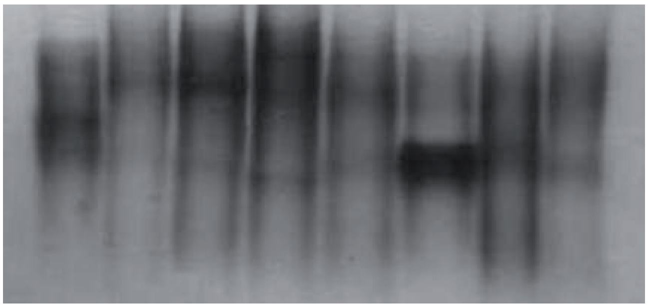

Figura 3. Ejemplo de patrones de ß-esterasa en puntas de raíces de yuca (Los carriles se enumeran de izquierda a derecha, los cuales son: 1, 2 y 5 = Clon M388; 3 y 4 =Clon M366; 6 =Clon Proletaria; 7 = Clon UCV 2129; 8 = Clon MEVEN 95).

Figura 4. Ejemplo de patrones de peroxidasa en hojas apicales de yuca (Los carriles se enumeran de izquierda a derecha, y son: 1 = Clon M279; 2 = Clon Amacuro 166; 3 = Clon Amacuro 145; 4 y 5 = Clon Amacuro 188; 6 y 7 = Clon UCV 2062; 8 y 9 = Clon M306).

desnaturalizadas, rubisco y peroxidasas para hojas jóvenes, α y β esterasas en puntas de raíces; todos estos sistemas presentaron mayor polimorfismo, repetibilidad y consistencia en los 65 clones.

Mediante el uso de estos sistemas se lograron observar diferencias en los patrones electroforéticos entre los diferentes clones evaluados, lo que nos permite señalar que estos marcadores pueden ser empleados para establecer diferencias entre clones y posibles duplicaciones dentro del banco de germoplasma de yuca evaluado.

El procedimiento de tinción utilizado para proteínas desnaturalizadas fue el recomendado por Stegeman et al. (1985), mientras que para los sistemas isoenzimáticos se emplearon los protocolos recomendados por Wendel y Weeden (1989), Vallejos et al. (1983) y Manchenko (1994) y Brune et al. (1998).

Para el caso de proteínas desnaturalizadas con SDS-mercaptoetanol, el proceso de tinción usado consistió en colocar el gel por unos segundos en una solución fijadora de proteínas (Cuadro 3) y luego se agregó azul de Coomasie al 1%, en la proporción de 1,25 ml por cada 50 ml de la solución fijadora. Después de trascurridas 12 horas de tinción se procedió a lavar el gel con una solución decolorante (Cuadro 4). La misma se debe añadir en pequeñas porciones al gel, lentamente, luego de haber retirado la solución colorante, hasta lograr la intensidad deseada de las bandas (Figura 1).

En el caso de α y β - esterasas (C.E. 3.1.1.1), el procedimiento de tinción consistió en colocar el gel en la solución tampón fosfato de sodio pH 7,0 (Cuadros 5, 6 y 7) por un mínimo de 10 minutos. Posteriormente se agregó el Fast Blue RR junto con el α ó β naftil acetato (Cuadros 8 y 9), según sea el caso. Luego se incubó con agitación a 37ºC, hasta que aparecieron las bandas negras (α-esterasas) o las rojas (β-esterasas) (Figuras 2 y 3).

Para las peroxidasas (PRX) (E.C. 1.11.1.7), el método de tinción empleado fue el recomendado por Wendel y Weeden (1989) (Cuadro 10), cuyo procedimiento de tinción fue colocar previamente el gel en la solución tampón NaH_2PO_4 1M y luego agregar rápidamente los otros componentes, dejándose en agitación en oscuridad a 37ºC (Figura 4).

En el caso de Rubisco (RBC) (E.C. 4.1.1.39), el método de tinción usado fue el recomendado por Wendel y Weeden (1989) (Cuadro 11). Se preparó una solución fijadora rubisco, compuesta de cinco partes de metanol, cinco de agua destilada y una de ácido acético glacial. El colorante Amido Black (Naftol blue black) se agregó a la solución fijadora, e inmediatamente se incorporó el gel en dicha solución por 15 a 30 minutos. Posteriormente, se aclaró tres veces seguidas, con la misma solución fijadora rubisco, por períodos de 15 minutos cada uno (Figura 5).

Cuadro 10. Solución de tinción PRX.

Componente	Cantidad
O-Dianisidina	25 mg
Metanol	15 ml
Solución tampón NaH2PO4 1M	35 ml
H_2O_2 al 3%	75 µl

Cuadro 11. Composición de la solución de tinción Rubisco.

Componente	Cantidad
Amido Black	25 mg
Solución fijadora rubisco	50 ml

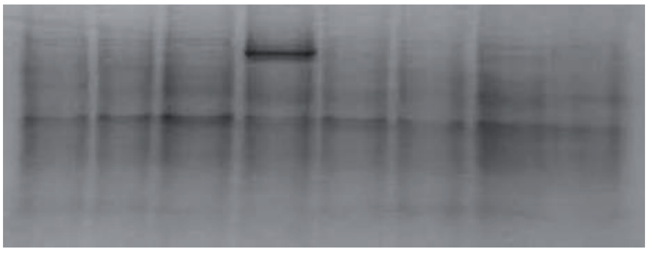

Figura 5. Ejemplo de patrones de rubisco en hojas apicales de yuca (Los carriles se enumeran de izquierda a derecha, y son: 1 = Clon M479; 2 y 6 = Clon Amacuro 135; 3 y 7 = Clon Juliana; 4 = Clon UCV 2076; 5 = Clon Bolívar 59; 8 = Clon Plan de Hierro).

Conclusiones

Se logró adaptar un protocolo electroforético a las condiciones del laboratorio. La metodología descrita es sencilla y de bajo costo, y permitirá caracterizar el banco de germoplasma de yuca del INIA-CENIAP. De los sistemas evaluados se detectaron cinco polimórficos y consistentes. El mayor polimorfismo se observó en los sistemas proteínas desnaturalizadas (PD.SDS), peroxidasas (PRX) y rubisco (RBC) para hojas apicales y en puntas de raíces, αβ-esterasas. Se observó mayor polimorfismo en los sistemas analizados en raíces; sin embargo la mayor actividad se visualizó en las hojas.

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Assembling a preliminary core collection of Tea (*Camellia sinensis* (L.) O. Kuntze) genetic resources in Sri Lanka

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Summary

Assembling a preliminary core collection of Tea (*Camellia sinensis* (L.) O. Kuntze) genetic resources in Sri Lanka

The need for a broader approach, with logical progression from conservation-management-utilization of tea genetic resources in Sri Lanka is stressed. Available information on taxonomy, pedigree, geographical origin and breeding history of 500 accessions conserved as germplasm at the Tea Research Institute of Sri Lanka was collected. Tea genetic resources comprise both beverage and non-beverage types, and most of the accessions are *C. sinensis* species, with wild and related species poorly represented. Most germplasm accessions are not adequately characterized for agronomic traits. The recurrent use of the same parents in breeding programmes has further narrowed the genetic diversity of the cultivated tea gene pool. To enhance the utilization of tea genetic resources, a diversity tree of tea germplasm has been developed as an initial step towards assembling a core collection.

Key words: ***Camellia sinensis*, conservation, core collection, diversity, germplasm.**

Résumé

Constitution d’une collection de référence préliminaire de matériel génétique de thé (*Camellia sinensis* (L.) O. Kuntze) au Sri Lanka

Cet article souligne la nécessité de développer un concept plus large, incluant une progression logique de la conservation vers la gestion, puis l’utilisation des ressources génétiques de thé au Sri Lanka. Les informations disponibles sur la taxonomie, le pedigree, l’origine géographique et l’historique de la sélection de 500 accessions conservées à l’Institut de recherche sur le matériel génétique de thé du Sri Lanka ont été rassemblées. Les ressources génétiques de thé comprennent à la fois des types utilisés ou non pour la boisson et la plupart des accessions appartiennent à l’espèce *C. sinensis*, les espèces sauvages et espèces apparentées étant peu représentées. La plupart des accessions de matériel génétique ne sont pas décrites de manière appropriée en ce qui concerne leurs caractéristiques agronomiques. L’utilisation répétée des mêmes parents dans les programmes de sélection a réduit la diversité génétique du pool de gènes des variétés cultivées de thé. Pour stimuler l’utilisation des ressources génétiques de thé, un arbre de diversité du matériel génétique du thé a été élaboré et est une première étape en vue de la constitution d’une collection de référence.

Resumen

Formación de una colección testigo preliminar de recursos genéticos de té (*Camellia sinensis* (L.) O. Kuntze) en Sri Lanka

Se destaca la necesidad de adoptar un método más amplio, con una progresión lógica que vaya de la conservación al ordenamiento y utilización de los recursos genéticos de té en Sri Lanka. Se recogió información disponible sobre taxonomía, pedigree, origen geográfico e historia del cultivo de 500 accesiones de germoplasma conservadas en el Instituto de Investigación de Té en Sri Lanka. Los recursos genéticos de té comprenden tanto tipos bebibles como no bebibles, y la mayoría de las accesiones son de la especie *C. sinensis*, con muy poca representación de especies silvestres y otras relacionadas con ellas. No se han caracterizado adecuadamente los rasgos agronómicos de la mayoría de las accesiones de germoplasma. El empleo recurrente de los mismos progenitores en los programas de cultivo ha restringido aún más la diversidad del patrimonio genético de té cultivado. A fin de promover la utilización de más recursos genéticos de esta especie, se ha desarrollado un diagrama árbol de la diversidad de germoplasma de té, como un primer paso en pos de la formación de una colección testigo.

Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze, is a national commodity of Sri Lanka, covering over 222 000 ha of the country and providing 12.8% of the export earnings of the country’s economy (Anon. 2006). Sri Lankan tea has very limited genetic diversity, as it is an introduced crop (Singh and Gunesekera 2000). Several authors have shown that the genetic base of cultivated tea is very narrow due to the recurrent use of same parents for the tea breeding programme over the years (Singh et al. 2003). The limited genetic diversity of the crop renders it more vulnerable to disease and insect epidemics, and jeopardizes the potential for sustained genetic improvement in the long term.

The exploitation of genetic potential inherent in tea germplasm is hampered by the non-availability of characterized and evaluated data. Currently, about 500 accessions are being maintained in the field genebank of the Tea Research Institute of Sri Lanka (TRI). These accessions consist of ‘introductions’, ‘estate selections’, improved cultivars and related species. The core collection approach was developed by Frankel (1984) to deal with the documentation, conservation, multiplication, evaluation and characterization of large germplasm collections. A core collection is a set of accessions selected to represent the genetic diversity of a base collection with minimum redundancies (Frankel 1984). This strategy was introduced with the intention of minimizing the cost of genetic conservation while ensuring representation of maximum genetic variation for rapid evaluation of germplasm, to provide better access to the base collection. A core collection is a starting point for screening of a base population in the search for desirable traits, allowing efficient use of land, time and money.

Traditionally, a core collection has been constructed on the basis of a variety of taxonomic, morphological, agronomic and ecogeographical criteria. The advent of DNA marker systems provided large numbers of polymorphic loci and demonstrated the suitability of DNA molecular markers as core selection criteria.

In China, a preliminary core collection of tea was constructed using place of origin, tree form and variety types (Li and Jiang 2004), along with morphological and DNA molecular markers (Li et al. 2005). To construct a core collection, it is important to compile all available information, including morphological, pedigree, passport, biochemical and molecular markers.

Singh et al. (2000) reviewed the status of tea genetic resources in Sri Lanka and stressed the need for conservation. Gunesekera and Kumara (2005) initiated the documentation of available information on estate selections since the 1930s as an initial step towards systematic maintenance of tea germplasm resources. This paper aims to collate available information to systematize and construct a preliminary core collection concept for the tea genetic resources of Sri Lanka.

Information was gathered on the 500 accessions of tea conserved in the genebank at TRI, Talawakelle. Accessions were stratified into groups based on available information on taxonomy, pedigree, geographical origin, domestication, distribution, utilization and breeding history. The division into distinct groups was achieved using a step-wise procedure (stratification) to develop a hierarchy, which was graphically represented by a 'diversity tree'. The major divisions were then successively split into subgroups until the subgroups were genetically homogeneous and no further difference were available to justify further subgrouping. The hierarchy was recorded as a descriptor called the 'path indicator', a series of ciphers that describe the subsequent divisions (Boukema et al. 1997).

Tea genetic resources in Sri Lanka

The tea genetic resources in Sri Lanka can be broadly categorized into two groups: beverage types and non-beverage types (ornamental). Beverage types are composed of *Camellia sinensis* accessions, and can be further subgrouped as introductions, estate selections and improved cultivars, based on available pedigree data and breeding history. The first tea germplasm in Sri Lanka was introduced as tea seeds from India and China during the early stages of the industry, and became limited due to increased quarantine or political restrictions and cost of importation. Currently in the TRI germplasm collection, accessions from India, Indo-China, Japan, Korea and Russia are available. The continue exploration of exotic germplasm for cultivar development should not be overlooked, as many recommended tea cultivars in use were based on germplasm from introductions.

Introductions from India

The ancestry of the popular TRI 2020 series cultivars were traced back to 30 seeds of a single tree bearing the identification number 4/10 (currently designated ASM 4/10) introduced by Dr F.R. Tubbs in 1937 from Tocklai Experimental Station of the Indian Tea Association (now Tea Research Association) near Jorhat, Assam (Richards 1965). The original parent tree, No 4/10, was noted to produce a red flush and believed to be a southern form of tea, recognized by Roberts et al. (1958). The pedigree of ASM 4/10 relates to a Cambod plant with red pigmentation of stock 124 at Tocklai, selected from open pollinated seed of stock 19/22, an introduction from Indo-China by Mrs A.C. Tunstall in 1918, and has chemical affinity to *Camellia irrawadiensis*, a species endemic to Burma. ASM 4/10 is recognized as the putative southern parent of Tocklai cultivars 107/4 and 107/5 (Roberts et al. 1958). Eight bushes were selected from 15 seedlings raised in 1946, and seven of these selections were released to the industry during 1959–1960, designated TRI 2020 to TRI 2027. Realizing the potential in the seeds of ASM 4/10, subsequent introductions of another batch was carried out in 1958. Fifteen TRI 62 series accessions were released in the 1960s (Richards 1965), out of which three are recommended for commercial cultivation (Anon. 2002).

Fourteen TRI 3000 series cultivars originating from another batch of seeds from ASM 4/10 are available as TRI germplasm. The date of introduction of the batch is unknown. The cultivars are TRI 3047, 3048, 3049, 3052, 3054, 3055, 3057, 3058, 3062, 3063, 3064, 3065, 3070 and 3071, with three of these being recommended for commercial planting (Anon. 2002).

The seedlings known as Stock 450, another introduction from India by Dr P. Sivapalan, in 1988 were evaluated in cultivar testing trials (Anandappa 1990a) and 6 selected for further evaluation for release as potential cultivars. Currently 26 accessions are available at TRI germplasm.

Introductions from Indo-China

TRI 2043, an accession popular for production of 'Silver tips', is a TRI selection made by Dr Tubbs during the 1950s from Shan Bansang No 777 seeds received from Pho Ho Station, Indo-China. A similarly high quality cultivar, TRI 777, was a selection made at TRI from the seedling progeny of Shan Cho Lang No 777 introduced from the same Station (Richards 1965).

Introductions from Japan

Six seedlings of morphologically different 'Yabukita' plants from Japan are available at TRI. The appearance of these Yabukitas resembles more the China type (Anandappa 1990a). In 2001, further seed stock of Yabukita was received and 9 Yabukita seedlings are available as germplasm at TRI.

Other Introductions

In 2001, two seed lots from the Korean Peninsula and China were received that included 4 wild types, 40 seedling of Korean cultivated types and 14 Chinese Oolong types. All these accessions are available for distribution. In addition, a small seed lot from Russia was received in 2006, and a few unknown accessions of East African origin are available in the collection.

Estate selections

With the development of vegetative propagation techniques during 1931–1935, a clonal selection programme was started as a mean of developing improved planting materials. The selection programmes were extended to old seedling and tea fields, and accessions were selected based on their field performances and released as ‘Estate selections’. Estate selections became available from the 1950s and continue to be made available. Initially only planters were involved, but then they were later assisted by TRI. Estate selections are usually named after the sites or estates where they were originally selected.

According to Gunesekera and Kumara (2005), a total of 688 tea accessions were derived from old seedling tea populations from estates in ten agro-ecological regions. TRI currently hold 217 estate selections at Talawakelle. Accessions selected at the St Coombs Estate, where TRI is located, were given a ‘TRI’ designation. There are 54 TRI accessions in the TRI germplasm collection, among which, TRI 9, a non-fermenting cultivar, is deficient in enzymic copper (Ramaswamy 1960). Other estate tea germplasm selections at TRI germplasm are summarized in Table 1.

Table 1. Origin and sources of estate selections and improved tea cultivars in Sri Lanka.

Origin/Source	No. of accessions
Estate selections (based on agro-ecological region)	
Up-country intermediate zone 2 (IU2)	3
Up-country intermediate zone 3 (IU3)	36 (1)
Up-country wet zone 1 (WU1)	104 (4)
Up-country wet zone 2 (WU2)	30 (1)
Up-country wet zone 3 (WU3)	6 (1)
Low-country wet zone 1 (WL1)	8 (3)
Mid-country wet zone 1 (WM1)	6 (1)
Mid-country wet zone 2 (WM2)	13 (1)
Mid-country wet zone 3 (WM3)	11 (2)
Half-sib selections (Open pollinated)	
ASM 4/10	2 (1)
TRI 2000 series (TRI 2024, 2025, 2026)	20 (8)
Estate selections	2
Full-sib selections	
ASM 4/10 × DT 95	6 (3)
ASM 4/10 × TRI 777	7
ASM 4/10 × CY 9	11 (6)
TRI 777 × TRI 2026	21 (1)
TRI 2000 series × TRI 2000 series	36 (10)
TRI 2000 series × Estate selections	15 (3)
Estate selections × Estate selections	8 (2)
Other	2

Note: Figures in parenthesis are number of recommended cultivars for commercial planting.

Improved tea cultivars

The growers’ ready and successful acceptance of cultivars of the TRI 2000 series encouraged plant breeders to exploit the inherent variability in open-pollinated seeds (Singh et al. 2003) and led to the development of the TRI 3000 series of cultivars during the 1980s. Accessions derived from open pollinated progenies are summarized in Table 1.

Controlled hybridization of tea with the aim of creating variations was initiated at TRI during 1961–1962 (Singh et al. 2003). Crossing selected parents for best recombinants among the progeny appears to be the most fruitful line of work. Early attempts in the controlled hybridization programme during the 1970s resulted in some of the TRI 3000 and TRI 4000 series of hybrid cultivars (Table 1). Simultaneously, non-conventional approaches to tea breeding were strengthened during this period to supplement the conventional tea breeding programme (Singh et al. 2003).

Polyploids

In Sri Lanka, natural and artificially induced polyploids have been reported by Sebastiampillai and Janikiram (1969). According to them, two cultivars (HS 10A and GF 5/01) available at TRI were triploids, and cultivar HR 1/8 was reported to be an aneuploid. All were estate selections made by early planters.

Five artificially induced tetraploid cultivars, namely TRI 2023, 2024, 2025, 2026 and DT 95 (Sebastiampillai 1976) are available at TRI, and a tetraploid of TRI 2025 was released as TRI 3069.

Other (cultivars with unknown origin)

There are an additional 11 accessions of unknown origin, designated ‘INTRI’, also available in the TRI germplasm collection.

Non beverage types

Camellia sasanqua Thunb.

Two seed batches of *C. sasanqua*, an ornamental tea, collected from Shizuoka-Ken and Kagoshima-Ken, Japan, were received from the National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT), Japan in 1990 (Anandappa 1990b). *C. sasanqua* or ‘Sazanca’ is a native Japanese species with high ornamental value and oil content. Currently, 40 *C. sasanqua* seedlings are available in the TRI germplasm collection, and it has been found to be highly resistant to blister blight disease (Balasuriya 2001).

Other *Camellia* species

Currently, seventeen non-beverage types and ornamental cultivars are available in the R.G. Coombs Camellia garden at TRI, which was established in 1950 and later augmented with new plants in 1968. The origins of these ornamentals are unknown. Species available include *Camellia lutescens*, *Pyrenaria barringtoniaefolia*, *Camellia rosaeflora*, *Gordonia zeylanica* cv. Elliptica, and various *Camellia japonica* ornamental

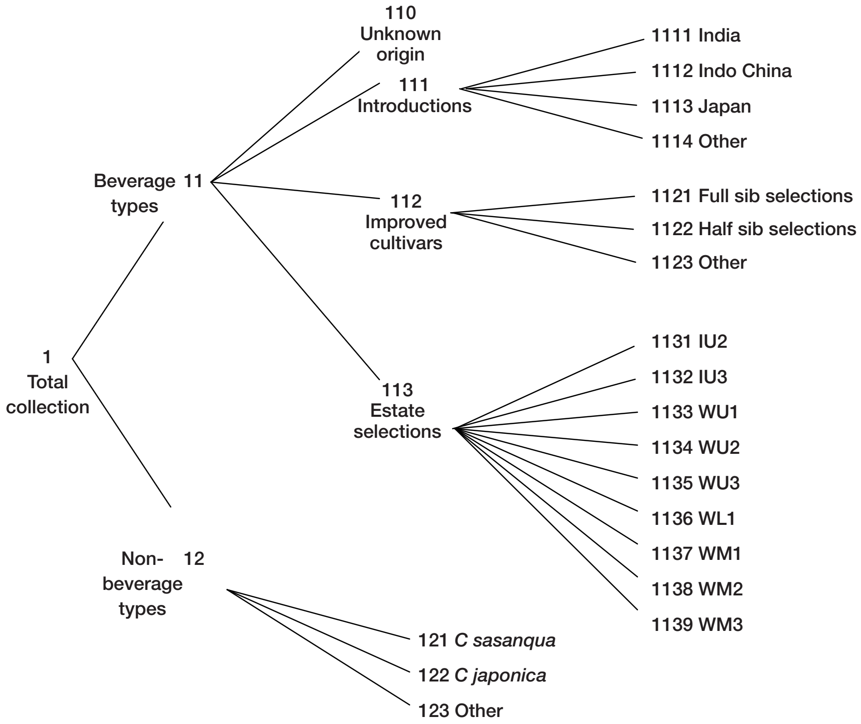

Figure 1. The first part of the diversity tree of the tea germplasm collection at TRI, Talawakelle.

cultivars (Japonica jubilee, Japonica red, Japonica albaphena, Japonica daitairin, Rosaeflora, Japonica elegans, Japonica white, Japonica donkelaari, Japonica herculea, Japonica yellow, Japonica sulphurea, Japonica diamond de bardi and Japonica old double pink).

With the information available on pedigree, breeding history, geographical origin and other parameters, the tea germplasm collection could be broadly categorized into the above mentioned groups. The resultant diversity tree of tea germplasm with its path indicator is illustrated in Figure 1 and the structure of the path indicator is given in Table 2.

Discussion

Sri Lankan tea has very limited genetic variability as it is a crop introduced to the country. Most of the early introductions from north India originated from common ancestors and they had similar characteristics (Singh et al. 2003). Collections from other gene-rich sources, such as Indo-China and China, are negligible, and most are *C. sinensis* species with little or no representation of wild tea species. The results imply that subsequent crop improvement programmes used only a narrow genetic base. Recent Coefficient of Parentage (COP) analysis of some tea cultivars revealed that most of the commercial tea cultivars are derived from two parental lines: Assam/Cambod introductions, and Estate cultivar CY 9 (Ariyaratne and Gunesekera 2007). Using isozymes, analysis of genetic diversity among known TRI cultivars demonstrated the narrow genetic base of the cultivated gene pool (Liyanage et al. 2003). The close genetic relationship of TRI cultivars was further confirmed using RAPD markers (Mewan et al. 2005). Recent surveys revealed that over 55% of the extent tea area

Table 2. Structure of the 'path indicator'

Path	Group	No. of entries
1	Total collection	482++
11	Beverage types (*C. sinensis*)	437++
12	Non beverage types	45
110	Unknown origin	15
111	Introductions	70++
112	Improved cultivars	135
113	Estate selections	217
1111	India	62
1112	Indo China	2
1113	Japan	6++
1114	Other	
1121	Full sib selections	106
1122	Half sib selections	24
1123	Other	5
1131	Selected from IU2 zone	3
1132	Selected from IU3 zone	36
1133	Selected from WU1 zone	104
1134	Selected from WU2 zone	30
1135	Selected from WU3 zone	6
1136	Selected from WL1 zone	8
1137	Selected from WM1 zone	6
1138	Selected from WM2 zone	13
1139	Selected from WM3 zone	11
121	*C. sasanqua* Thunb.	40
122	*C. japonica*	13
123	Other *Camellia* spp.	4

is under clonal plantings, of which over 80% is limited to a few cultivars (Singh et al. 2000). Introduction of foreign germplasm from centres of diversity and germplasm-rich countries is difficult due to competition among tea growing countries and legislation governing importation. Therefore, the available germplasm is the only source for future crop improvement programmes and it is important to develop effective conservation strategies.

The systematic conservation and maintenance of genetic resources followed by their proper characterization and evaluation is of paramount importance if they are to be used effectively in crop improvement programmes. The systematic conservation of tea genetic resource was initiated in 1987, and 260 accessions were initially conserved in a field genebank at TRI, Talawakelle, where the main research station is located (Anandappa 1987). During subsequent years a limited number of accessions has been duplicated at the TRI regional stations (Ratnapura, Passara and Kottawa), representing major agroclimatic regions in Sri Lanka. Acquisition of new material for the germplasm collection, mainly from old seedling tea populations, has continued for the last century. The characterization and evaluation of some accessions of tea germplasm were attempted by several authors using morphological markers (Gunesekera et al. 2001; Gunesekera and Peiris 2006; Piyasundara et al. 2006; Sundaravathany et al. 2007), isozyme markers (Liyanage et al. 2003) and molecular markers (Mewan et al. 2005). However, it would be highly desirable to have germplasm characterized for morphological, biochemical and molecular markers, which could then be utilized in tea crop improvement programmes.

Though a germplasm collection or genebank is meant to conserve the diversity of the genetic resources for the benefit of plant breeders for germplasm enhancement through variety development, unavailability of evaluation data hampers the utilization of a germplasm collection for crop improvement. Most of the tea germplasm accessions are not adequately evaluated for morphological, agronomical and biochemical characteristics due to time and financial limitations. Tea breeders are confronted with the difficulty of selecting a diverse and representative selection of accessions for cultivar development from a long list of essentially anonymous accessions. This could be one reason for tea breeding relying on such on a narrow genetic base. Therefore, to meet the challenges arising from tea genetic resources conservation and utilization, construction of a core collection could be an attractive option.

The present study is a first step towards a construction of a core collection in tea genetic resources conserved in Sri Lanka. Characterization and evaluation of accessions for morphological and agronomical traits and molecular characterization of accessions are in progress. Once the evaluations are completed it should be possible to construct a well characterized core subset of tea germplasm that provides a proper working collection for the subsequent search for desired alleles for crop improvement programmes, and a point of entry to the entire germplasm collection.

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Germplasm management of *Vicia faba* L.: Comparative study of the mating system of local and common cultivars growing under different agro-ecological conditions

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Summary

Germplasm management of *Vicia faba* L.: Comparative study of the mating system of local and common cultivars growing under different agro-ecological conditions

Knowledge about patterns of variation in the mating system of partially allogamous species is essential for germplasm management. To elucidate patterns of variation, we tested two different types of cultivars, one locally adapted to the area and the other more widely adapted, in two different European agro-ecological conditions to help in developing effective germplasm management strategies. Agro-ecological conditions were: two locations and winter cultivars in southwest Europe; and five locations and spring cultivars in northeast Europe. We analysed enzyme polymorphisms and applied multilocus biometrical models to determine mating system parameters. Outcrossing ranged from 0.14 to 0.72. These results were strongly influenced by agro-ecological conditions. Southwestern cultivars showed a consistently higher outcrossing rate than did northeastern cultivars. No significant differences were found among local and widely adapted cultivar outcrossing rates under northeastern conditions. However, under southwestern conditions, we found a tendency to higher selfing rates in the local cultivars than in the more widely adapted ones. Based on the degree of variation among the mating systems of faba bean cultivars, serious genetic erosion is a threat if the same regeneration and management protocols are used for all genebank accessions. Our data not only provide useful information to help in developing effective strategies for germplasm regeneration but also for the development of pre-breeding strategies and landrace enhancement.

Key words: faba bean; genetic resources conservation; genetic integrity; outcrossing; regeneration.

Résumé

Gestion du matériel génétique de *Vicia faba* L. : étude comparative du système de croisement de cultivars locaux et communs croissant dans différentes conditions agro-écologiques

La connaissance des profils de variation dans le système de croisement d'espèces partiellement allogames est essentielle pour la gestion du matériel génétique. Deux types de cultivars (adaptés à une zone limitée ou vaste) ont été testés dans deux conditions agro-écologiques différentes : deux localités et des cultivars d'hiver dans le sud-ouest de l'Europe ; et cinq localités et des cultivars de printemps dans le nord-est de l'Europe. Les polymorphismes enzymatiques ont été étudiés et des modèles biométriques multilocaux ont été appliqués pour déterminer les paramètres du système de croisement. Les taux de fécondation croisée vont de 0,14 à 0,72, ceux des cultivars du sud-ouest étant systématiquement plus élevés que ceux du nord-est. Il n'y a pas de différences significatives entre les taux de fécondation croisées des cultivars adaptés à une région limitée ou vaste dans le nord-est. Cependant, dans le sud-ouest, les taux d'autofécondation sont plus élevés parmi les cultivars locaux que chez ceux qui sont adaptés à une région plus vaste. Le degré de variation observé dans les systèmes de croisement de cultivars de fèves indique qu'il existe une grave menace d'érosion génétique si l'on utilise les mêmes protocoles de régénération et de gestion pour toutes les accessions de banques de gènes. Non seulement nos données fournissent des informations utiles contribuant à la régénération du matériel génétique, mais elles permettent également d'élaborer des stratégies de pré-sélection et d'utilisation des variétés locales.

Resumen

Ordenamiento de germoplasma de *Vicia faba* L.: estudio comparativo del sistema de fecundación de cultivares locales y comunes bajo diferentes condiciones agroecológicas

Conocer las pautas de variación del sistema de fecundación de especies parcialmente alógamas es esencial para el ordenamiento del germoplasma. Para examinar las pautas de variación y desarrollar estrategias efectivas de ordenamiento del germoplasma se probaron dos tipos de cultivares, uno adaptado localmente y otro adaptado más ampliamente en dos condiciones agroecológicas diferentes de Europa: dos ubicaciones y cultivares de invierno en Europa sudoccidental y cinco ubicaciones y cultivares de primavera en Europa nordoriental. Se analizaron los polimorfismos enzimáticos aplicando modelos biométricos multilocus para determinar los parámetros de los sistemas de fecundación. La fecundación por cruza iba de 0,14 a 0,72. Los resultados dependían fuertemente de las condiciones agroecológicas. Los cultivares sudoccidentales mostraron un índice de cruza más alto que los nordorientales. No había diferencias significativas de índices de cruza entre los cultivares locales y los adaptados en amplia escala en las condiciones nordorientales, pero bajo las condiciones sudoccidentales se halló tendencia a mayores índices de autofecundación en los cultivares locales que en los adaptados más extensamente. Usar los mismos protocolos de regeneración y ordenamiento para todas las accesiones del banco de genes es una grave amenaza de erosión genética. Estos datos proveen información útil para desarrollar estrategias eficaces de regeneración de germoplasma y estrategias previas a la fecundación y el mejoramiento de las variedades locales.

Introduction

Vicia faba L. has been considered as a partially allogamous species in all genetic and breeding studies carried out to date. The level of allogamy can be both considerable and variable, ranging from 4 to 84% with a mean around 30 to 40%. (Holden and Bond 1960; Link et al. 1994a; Suso and Moreno 1999; Suso et al. 2001; Gasim et al. 2004).

Observed differences in outcrossing rate apparently depend on genetic and environmental factors as well as on the different methods and markers used in their estimation. Our perception of mating systems depends on the technology at hand, the availability of markers and methodologies. Thus, it is valuable to reflect on what we know based on the technology that was employed to generate the information. Without that context, the biological meaning of the estimates can be so unclear that they cannot be effectively compared.

Most studies in this field have been limited to statistical approaches based on the classical tools of morphological markers (Xanthopoulos et al. 1986; Metz et al. 1993; Link et al. 1994a; Gasim et al. 2004). For instance, flower and seed pigmentation differences, which usually have a single-gene inheritance, have been used as markers in populations. Procedures used to estimate the degree of cross-pollination in these studies refer to the frequency of hybrids that would have resulted from crossing between pollen donor plants offering the dominant marker and from crossing among different pollen recipients with the recessive marker. Furthermore, the recessive allele is often rare, so that the locus can be used only in experimental situations. And more importantly, these markers may directly affect pollinator behaviour, in turn biasing the estimates (Carre et al. 1993).

Molecular markers and more elaborate bio-informatic models allowed the development of multilocus approaches (Cruzan 1998). The multilocus approach uses information from all genotypes across various loci, better reflecting the true amount of crossing in open-pollinated populations (Ritland 2002). This can be especially valuable in partially allogamous species, such as *Vicia faba*, consisting of mixtures of inbred and hybrid individuals that differ in their degree of heterozygosity.

The level of outcrossing potentially determines the level of heterozygosity and the amount of realized heterosis, and therefore the mating system has an important impact on the performance of open-pollinated populations. Previous studies indicated that yield, yield stability and drought tolerance of faba bean cultivars could be improved by increasing the level of heterozygosity (Link et al. 1994b, 1996; Abdelmula et al. 1999), which could be achieved through increased outcrossing. Thus, knowledge of the levels and variability of outcrossing in populations is an important first step for developing pre-breeding strategies to increase heterozygosity. This knowledge-based approach was proposed by Suso et al. (2005a) specifically for faba bean, and has been employed profitably to improve yield of wind-pollinated cereals (Virmani 1996; Ghani et al. 2003).

From the germplasm management point of view, various reasons justify additional studies of the mating patterns of *Vicia faba* cultivars under current field conditions, including (1) a compelling interest in regenerating faba bean samples held in genebanks (Global Crop Diversity Trust 2007), which requires careful planning of regeneration procedures; and (2) a clearer understanding of our ability to manage the amount of heterozygosity in establishing reliable conservation and pre-breeding strategies. To these ends, the objectives of our research were to obtain estimates of major mating system parameters in faba bean cultivars cultivated under standard field conditions in two different European agro-ecological conditions and to evaluate differences between geographical locations and cultivar type.

Materials and methods

Plant materials and field trials

Faba bean is mostly grown as spring crop in northeastern Europe and as winter crop in southwestern Europe. So, in order to better mirror the actual conditions of European populations (and, by extension, landraces), spring cultivars in northeastern Europe and winter cultivars in southwestern Europe were used. The experiments were designed to investigate mating system variation in seven locations: five locations with northeastern European agro-ecological conditions and two locations in southwestern European agro-ecological conditions. In addition, we characterized major parameters of the mating system of two kinds of cultivars, one locally adapted, maintained as open-pollinated populations under local environmental conditions, and the other more-broadly adapted. The widely adapted cultivar for the northeastern agro-ecological condition was cv. Gloria, and that used in the southwestern condition was cv. Alameda (Table 1).

Mating system parameters were estimated from plots of 600 plants sown at 10 cm intervals in rows 70 cm apart. Plots were spring sown in the northeast and winter sown in the southwest. We evaluated 'progeny arrays', which are collections of genotypes descended from a common mother parent. Such data are easily obtained by sampling individual plants at maturity. At harvest, around 50 plants of each cultivar and location were collected and their seeds analysed

Table 1. Country, location and names of local and common cultivars studied for the mating system paramenters.

Location	Cultivars	
	Local	Common
Northeastern conditions		
Gleisdorf, Austria	Valeria	Gloria
Sumperk, Czech Republic	Borek	Gloria
Roslev, Denmark	Marcel	Gloria
Jõgeva, Estonia	Jogeva	Gloria
Göttingen, Germany	BasisPopulation [BPop]	Gloria
Southwestern conditions		
Elvas, Portugal	Favel	Alameda
Córdoba, Spain	Syn-4	Alameda

electrophoretically. We assayed the following enzyme staining systems: 6 Phosphogluconate dehydrogenase (one locus); shikimic dehydrogenase (one locus); superoxide dismutase (3 loci); esterase (one locus); isocitrate dehydrogenase (one locus); and malate dehydrogenase (one locus). Extraction buffers, their running conditions and scoring systems followed those described by Suso et al. (1993).

Mating system estimation

We estimated the following mating system parameters: mutilocus outcrossing (tm), single locus outcrossing (ts), bi-parental inbreeding (tm−ts) and inbreeding coefficient of maternal parents (F), for each cultivar by using maximum-likelihood methods based on a mixed mating model (Ritland and Jain 1981). This model assumes that every individual in the population produces a proportion of its seeds through self-fertilization and a proportion t = 1 - s through cross-fertilization with pollen randomly dispersed from other plants. Analyses were conducted by employing the polymorphic allozyme markers and the MLTR-program supplied by Ritland (2002). Standard errors were calculated from 500 bootstrap replicates with re-sampling among maternal plants within populations. Estimates differing by more than ±2 SE were considered significantly different.

The equilibrium value of the inbreeding coefficient (Feq) was also estimated from multilocus outcrossing rate estimates by using the relationship 1-tm / 1+tm. Feq represents the level of inbreeding expected from selfing alone under mixed mating. The inbreeding coefficient of maternal parents, F, is the heterozygosity observed relative to random mating (F = 1-Ho/He, where Ho is the observed heterozygosity and He the heterozygosity predicted under Hardy-Weinberg equilibrium). Thus, the relationship between F and Feq is an indication of the influence of factors other than selfing on genotypic frequencies. The inbreeding coefficient estimated from heterozygosity, was compared to Feq derived from the outcrossing rate. The difference between the two measures should be negligible if the mating system is the sole determinant of inbreeding in a population.

Bi-parental inbreeding represents the impact of matings between related individuals. The level of bi-parental inbreeding can be estimated as the difference between multilocus and single locus estimates of outcrossing rate (tm−ts). In the absence of bi-parental inbreeding, the estimates will be the same in each comparison; with bi-parental inbreeding the single-locus outcrossing rate will be less than tm because outcrossing events that are not detected at a single locus due to alleles that are identical by descent have a higher probability of being detected as more loci are examined (Ritland 1990; Shaw and Allard 1982).

Results and discussion

Extraordinary variation of the mating system

Parameters describing the mating systems in the seven locations for local and more-widely adapted cultivars of *Vicia faba* are shown in Table 2.

The extent of total variation in multilocus outcrossing ranged from 0.14 for cv. Valeria in Austria to 0.72 for cv. Alameda in Portugal. Among local cultivars, a strong influence of the cultivar was observed in northeastern agro-ecological conditions. The two estimates of the lowest outcrossing rates were found in the local cultivars, cv. Valeria and cv. Marcel,

Table 2. Parameters of the mating system of *Vicia faba* cultivars at seven sites under two broad agro-ecological conditions in Europe.

Location	Cultivar	Feq	F (SE)	tm (SE)	ts (SE)	tm-ts (SE)
Northeastern conditions						
Gleisdorf, Austria	Valeria	0.75	0.66 (0.13)	0.14 (0.04)	0.13 (0.04)	0.01 (0.01)
	Gloria	0.36	-0.09 (0.06)	0.47 (0.08)	0.40 (0.07)	0.07 (0.02)
Sumperk, Czech Rep.	Borek	0.54	0.23 (0.06)	0.30 (0.04)	0.23 (0.04)	0.06 (0.01)
	Gloria	0.61	0.45 (0.05)	0.24 (0.03)	0.12 (0.03)	0.07 (0.01)
Roslev, Denmark	Marcel	0.72	0.66 (0.11)	0.16 (0.04)	0.13 (0.04)	0.02 (0.01)
	Gloria	0.49	0.11 (0.08)	0.34 (0.05)	0.31 (0.05)	0.03 (0.01)
Jõgeva, Estonia	Jogeva	0.49	0.09 (0.09)	0.34 (0.05)	0.29 (0.05)	0.04 (0.01)
	Gloria	0.59	0.32 (0.09)	0.26 (0.04)	0.22 (0.05)	0.04 (0.01)
Göttingen, Germany	Bpop	0.50	0.49 (0.08)	0.33 (0.03)	0.24 (0.03)	0.09 (0.01)
	Gloria	0.52	0.29 (0.11)	0.32 (0.07)	0.27 (0.06)	0.05 (0.02)
Southwestern conditions						
Elvas, Portugal	Favel	0.20	0.43 (0.12)	0.67 (0.05)	0.56 (0.06)	0.11 (0.03)
	Alameda	0.16	0.08 (0.07)	0.72 (0.05)	0.62 (0.04)	0.10 (0.02)
Córdoba, Spain	Syn-5	0.32	0.15 (0.05)	0.51 (0.06)	0.45 (0.06)	0.06 (0.02)
	Alameda	0.18	0.38 (0.08)	0.70 (0.04)	0.69 (0.05)	0.01 (0.01)

Key: Feq = expected equilibrium coefficient; F = inbreeding coefficient of maternal parents; tm = multilocus outcrossing rate; ts = singlelocus outcrossing rate; SE = standard error from 500 bootstrap replicates (in parentheses).

and these were significantly different from estimates of the Estonian and German local cultivars, cv. Jogeva and cv. BPop. No significant differences in outcrossing were found among locals cultivars in the southwestern conditions.

The experimental design of the present study enabled us to compare the outcrossing rates of widely adapted common cultivars over multiple locations, cv. Gloria for northeastern agro-ecological conditions and cv. Alameda for the southwestern agro-ecological conditions. In contrast to outcrossing among local cultivars, outcrossing rates of these common cultivars did not differ significantly among locations. In five locations across the northeast, excluding the Austrian location, there was no significant difference between locations for cv. Gloria, with values ranging from 0.24 to 0.34. No significantly different outcrossing rates were observed for cv. Alameda in the southwestern locations.

Differences in outcrossing rates between local cultivars and cv. Gloria were not significant in northeastern locations. However, in the southwest, when averaged over cultivars, the outcrossing rate tended to be higher in cv. Alameda than in the local ones, 0.71 versus 0.59.

A strong agro-ecological effect on outcrossing was apparent. Southwestern outcrossing rates showed consistently higher values. On average, 29% of the progeny from northeastern locations resulted from outcrossing events, whereas the average for progenies from southwestern locations was 65%. The mean outcrossing rate for northeastern cultivars lies within the range of outcrossing rates typically reported for faba bean varieties (Crofton 1996).

Cultivar multilocus estimates (tm) were slightly larger than single-locus estimates (ts) in nearly all cultivars indicating that most of the apparent selfing is actual self-fertilization and not the result of mating between related individuals. However, tm−ts comparisons for cultivars growing in Elvas, Portugal, indicated that other types of consanguineous mating may occur in this location.

Estimates of the inbreeding coefficient, F, of maternal plants were indistinguishable from the predicted equilibrium coefficient, Fe, in the local cultivars Valeria, Marcel and BPop, indicating that the level of inbreeding is indeed the level as predicted by the mating system, demonstrating that levels of heterozygosity in these cultivars can be accounted for solely by the mating system. However, in other cultivars, such as Borek, Jogeva and Gloria, F was smaller than Fe; thus, substantially more heterozygotes were observed among the mother plants than would be predicted if the mating system were the only factor affecting genotypic frequencies, suggesting a certain heterozygote selective advantage. Thus, natural selection may act against selfed individuals during pollination, and against homozygotes derived from selfing and consanguineous matings both during and after seed maturation. These results indicate that it is important to prevent increases in inbreeding and to maintain pollinator communities to facilitate outcrossing. The opposite held true in cvs. Favel and Alameda (in Spain), where a significant deficiency of heterozygotes was observed relative to the mating system equilibrium.

The comparisons of multilocus outcrossing rate, the inbreeding coefficient of maternal plants and the equilibrium inbreeding coefficient indicated that cultivars with higher estimates of tm exhibit a deficiency of heterozygotes, whereas cultivars with lower tm exhibit an excess of heterozygotes, providing evidence for selection against selfed offspring. This observation is consistent with the ‘heterozygosity paradox’ (Brown 1979), where outbreeding species tend to show a deficit of heterozygotes and inbreeders show an excess when compared to panmictic expectations. Brown (1979) proposed several genetic and ecological mechanisms to adequately explain this phenomenon. Among these mechanisms, he suggested, in accordance with Nei (1975), that spatial and temporal variations in outcrossing could lead to apparent excess heterozygosity among self-pollinated plants. Interestingly, Drayner (1956) reported this fluctuating mating system pattern for faba bean, noting that plants arising from outcrossing in the previous year will set more seed by selfing, and selfed plants will set more seed by outcrossing. The overall effect will be to maintain the population in a stable state of intermediate heterozygosity despite disturbing factors. This regulating mechanism represents a means of maintaining a moderate degree of heterozygosity without strong dependence on insect pollinators.

The patterns of variation we observed indicate that the level of outcrossing alone does not determine the level of heterozygosity and that the mating system is controlled by a complex interplay of genetic and environmental factors. These factors operate within contrasting regional contexts. Conservation biologists have recognized that preservation efforts must involve different levels of biological organization from ecosystems and communities to genes and genomes (Hamilton 1994). Thus, additional studies should include estimates of the rates of outcrossing in populations under different conditions, but in conjunction with observations on the diversity, abundance and distribution of populations of pollinators and a close examination of the role of variation in floral characteristics. With these results in mind, it seems reasonable to suggest that the proposed research should be undertaken in parallel to future regeneration activities.

Relevance to germplasm management

Contrasting patterns of mating system variation among cultivars and regions can have profound implications for the management of germplasm (Widrlechner 1987; Engels and Rao 1998; Engels 2002). The importance of mating systems in structuring genetic variation within and among populations is well documented (Hamrick and Godt 1989, 1996). Predominantly outcrossing faba bean populations, such as those from the southwest, typically have most of their diversity residing within populations, with relatively little differentiation among populations; highly selfing populations of the northeast will show just the opposite pattern. Considering that substantial variation in outcrossing rate is associated with agro-ecological conditions, different procedures for germplasm management should be established for each region.

Thus, the germplasm diversity of southwestern cultivars could be effectively conserved by maintaining a few populations of sufficient size. Additional populations are not likely to capture much additional diversity. In contrast, capturing the range of genetic diversity present in the northeastern cultivars, with higher degrees of selfing, will require a larger number of populations to conserve the same amount of genetic diversity, even if these populations are somewhat smaller.

In both regions, there is a general need to establish isolation strategies between accessions, in order to minimize gene flow. In fact, the risk of contamination in the multiplication field is greater in the southwest than in the northeast. Suso et al. (2006) described similar geographical patterns of variation in inter-plot crossing.

A major objective of genebanks is to maintain the genetic integrity of accessions as far as possible (van Treuren et al. 2006). Thus, international guidelines have been established for the maintenance of germplasm collections (Breese 1989; Sackville Hamilton and Chorlton 1997; Engels and Visser 2003). Maintaining the genetic integrity of faba bean genebank accessions proves more complex than expected. These accessions exist in genebanks as heterogeneous mixtures comprising seeds of differing inbreeding status. Population genetic theory predicts that major threats—such as drift, selection and contamination—are greater in highly heterogeneous populations. In general, accessions from northeastern Europe display a mating system theoretically less vulnerable to loss of genetic diversity, while, in contrast, southwestern accessions, as a consequence of the level of outcrossing, display substantial heterozygosity and heterogeneity, and therefore will be more vulnerable to genetic loss. However, the general expectation does not hold true everywhere: for some cultivars, the comparisons of multilocus outcrossing rate, the inbreeding coefficient of maternal plants, and the equilibrium inbreeding coefficient indicated that cultivars with higher estimates of tm exhibited a deficiency of heterozygosity, whereas cultivars with lower tm exhibited an excess of heterozygosity. Thus, to preserve the genetic integrity of northeastern selfed accessions with levels of heterozygosity substantially above theoretical expectations, taking into account the outcrossing level alone, will be more difficult than expected, and efforts to preserve the genetic integrity of southwestern accessions with levels of heterozygosity substantially below theoretical expectations, taking into account the outcrossing level alone, could be easier than expected.

Additionally, the fact that the 'heterozygosity paradox' affects faba bean cultivars also complicates the development of effective pre-breeding strategies that exploit the level of heterosis by increasing the level of heterozygosity. To some extent, this paradox might hamper the increase of heterozygosity by intentional outcrossing selection.

Based on the amplitude of variation in faba bean's mating system, the use of the same regeneration and management protocols for all genebank accessions can threaten genetic integrity (Widrlechner 1987), underscoring the need for collecting baseline information on the actual mating behaviour of faba bean by location and accession, and for developing appropriate regeneration techniques. These main conclusions also emerged from a survey recently conducted by a task force of members from the ECP/GR Working Group on Grain Legumes (Suso et al. 2005b) designed to develop guiding principles to address the maintenance of grain legumes.

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Locating and managing the mango (*Mangifera indica* L.) genetic resources in Nepal

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Summary

Locating and managing the mango (*Mangifera indica* L.) genetic resources in Nepal

Mango diversity in Nepal is increasingly threatened by the commercialization of the production systems, changes in land use, habitat loss and population pressure. An ecogeographic survey was conducted to locate, analyse and assess the current status of mango genetic resources, and suggested conservation and sustainable utilization measures. Fruit samples were collected to characterize 19 qualitative and quantitative characteristics to assess genetic variation and establish relationships. A total of 132 different mango cultivars were identified from 11 districts surveyed. A rich diversity of both commercial and local cultivars was found. Local mango cultivars possess unique characteristics, having both economic and cultural value. Important traits identified included ability to grow in marginal and drought areas, adaptation to high altitude, high rate of fruit setting, fruiting in off-season, special aroma, and fibreless pulp. Introduction of Indian commercial mango varieties and a high rate of logging of old mango trees are major threats to maintenance of mango genetic resources. The paper identified Dadeldhura, Parbat, Dhading, Kavre and Sirah districts as major areas of mango diversity and suggests potential methods of community-based conservation action in Nepal.

Key words: Genetic resources, *in situ* conservation, on-farm conservation, mango, Nepal

Résumé

Localisation et gestion de ressources génétiques de manguier (*Mangifera indica* L.) au Népal

Au Népal, la diversité des manguiers est de plus en plus menacée par l'orientation commerciale de production, les changements d'occupation des sols, la disparition d'habitats et la pression démographique. Une étude éco-géographique a été réalisée afin de localiser, analyser et évaluer le statut actuel des ressources génétiques de manguier et de proposer des mesures de conservation et d'utilisation durable. Des échantillons de fruits ont été collectés afin de déterminer 19 caractères qualitatifs et quantitatifs en relation avec la variation génétique. Au total, 132 cultivars différents de manguier de 11 districts ont été identifiés, témoignant d'une riche diversité de cultivars commerciaux ou locaux. Les cultivars locaux de manguier possèdent des caractéristiques uniques et ont une valeur à la fois économique et culturelle. Des caractères importants ont été identifiés, parmi lesquels, la capacité de croître dans des zones marginales et sèches, l'adaptation à une altitude élevée, un taux de nouaison important, la fructification hors saison, un arôme particulier, et une pulpe non fibreuse. L'introduction de variétés commerciales indiennes de manguier et un taux élevé d'abattage des vieux manguiers constituent des menaces importantes. Dans cet article, les districts de Dadeldhura, Parbat, Dhading, Kavre et Sirah sont identifiés comme les principales régions de diversité du manguier et des mesures possibles de conservation s'appuyant sur les communautés sont proposées.

Resumen

Ubicación y ordenamiento de recursos genéticos de mango (*Mangifera indica* L.) en Nepal

La diversidad de mangos en Nepal está amenazada cada vez más por la mercantilización de los sistemas de producción, cambio de uso de los suelos, pérdida de hábitat y presión de la población humana. Se realizó un examen ecogeográfico para analizar y evaluar el estado actual de los recursos genéticos de mango, y se sugirieron medidas de conservación y utilización sustentable. Se recogieron muestras del fruto a fin de examinar 19 características cualitativas y cuantitativas útiles para establecer la variación genética y las interrelaciones. En los 11 distritos examinados se identificaron 132 cultivares diferentes de mango. Hay una rica diversidad de cultivares, tanto comerciales como locales. Los cultivares locales presentan características únicas que tienen valor económico y cultural. Los rasgos de importancia incluyen capacidad de crecimiento en zonas secas y marginales, adaptación a las grandes alturas, elevado porcentaje de rendimiento de frutos, fructificación fuera de estación, aroma especial y pulpa no fibrosa. La introducción de variedades comerciales de mango de la India y un alto índice de madereo de árboles viejos son las mayores amenazas para la conservación de los recursos genéticos de mango. El documento identifica a los distritos de Dadeldhura, Parbat, Dhading, Kavre y Sirah como las principales zonas de diversidad de mangos y sugieren métodos potenciales para la actividad de conservación de base comunitaria en Nepal.

Introduction

The genus *Mangifera* belongs to the order Sapindales in the family Anacardiaceae, with more than 40 species around the world, and 15 species bear edible fruits. Common mango (*Mangifera indica* L.) originated as an alloploid from eastern India, Assam and Burma (Popenoe 1920). Mango has rich intraspecific diversity and there are about 1600 cultivars in the world (Pandey 1998), of which some 350 cultivars are in commercial production and the rest are limited to mixed orchards or home gardens.

Mango is one of the important tropical fruits of Nepal, covering the 9% of total fruit cultivated area, and is ranked second among all fruits cultivated in Nepal (Gautam and Dhakal 1994). The total area under mango cultivation in the country is about 14 000 ha, with annual production exceeding 100 000 t (ASD 2005) mostly in the *tarai*, low-hills, mid-hills and mountains regions. Home gardens, village gardens, commercial orchards, religious or cultural places and river gorge areas are the major habitats, where both local and commercial cultivars of mango are either cultivated or harvested from escapes from controlled cultivation (Subedi et al. 2005a). Notable local cultivars are *Sindhure, Kali, Supare* and *Lohare* from the lower hills, and *Chinia, Sipiya, Chausa* and *Safeda* from the *tarai* and hills region (NARC 2003).

In recent years the habitats of local mangoes (such as village orchards in communal land or along pilgrim trail) have been affected by various factors, and their existence is threatened by genetic erosion. As part of the 2001–2005 project on *Strengthening the scientific basis of in situ conservation of agricultural biodiversity on-farm*, an ecogeographical survey was funded by IDRC, Canada, to characterize and evaluate mango genetic resources and to understand the extent and distribution of their diversity. This paper presents results of this genetic diversity study and of efforts to locate potential sites for *in situ* conservation of mango genetic resources for future use in research and development (Subedi et al. 2004, 2005a,b).

Materials and methods

Ecogeographical surveys

A literature review, consultation workshops with national crop experts and staff of Horticulture Programme of the Department of Agriculture formed preliminary steps for understanding the mango genetic resources in Nepal, and identification of preliminary sites for field surveys. A multidisciplinary team, consisting of a taxonomist-*cum*-ethnobotanist, social scientists and horticulturalists, carried out surveys in target districts representative of the eastern *tarai* (Saptari, Siraha), central *tarai* (Sarlahi, Dhanusa), central hills (Kavre, Dhading), western hills (Baglung, Parbat), mid-western hills (Surkhet) and far-western hills (Dadeldhura, Doti). According to the literature review, few potential valley bottoms of Panchkhal, Sera, Trishuli, etc., could not be surveyed.

During this period checklists were developed to record the mango samples and establish coordinate values, and short interview were conducted with key informants of the survey sites. 'Farmers' descriptors were documented with the help of the IBPGR Mango Descriptors (IBPGR 1989). Ecogeographical surveys of the target areas (Figure 1) were then undertaken, covering home gardens, village gardens, river gorges, roads and highways, and scattered populations of mango in villages. Mango populations were found to be maintained in sacred groves with cultural and religious importance.

Characterization and evaluation

Fruits from 216 mango cultivars were collected during the surveys, and evaluated for qualitative and quantitative morphological characteristics to assess the genetic variation and relationships (Subedi et al. 2005a). Three fruits of each sample were characterised for 11 quantitative and 8 qualitative

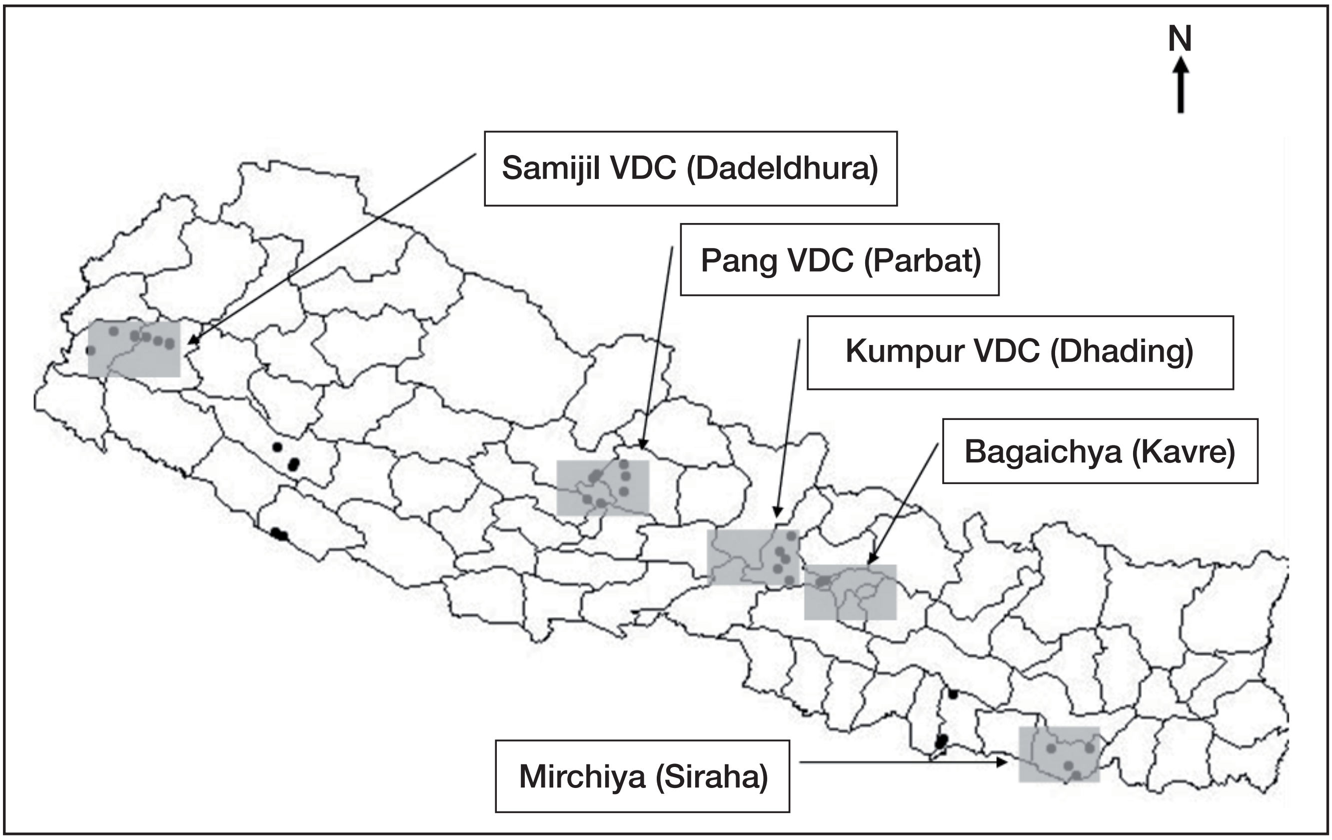

Figure 1. Collecting sites of mango (solid circle) and diversity-rich areas for mango (grey blocks) in Nepal.

traits (selected on the basis of farmer's descriptors used to distinguish cultivars at the community level) following the IBPGR Mango Descriptor list (IBPGR 1989). Descriptive statistics of quantitative traits using two diversity indices (Shannon-Weaver and Simpson) of qualitative traits were estimated across the collection sites (Shannon 1948; Magnussen and Boyle 1995). Analysis of variance and principal component analysis (PCA) using both quantitative and qualitative traits were performed. Significantly different quantitative and qualitative data were standardized for PCA. The importance of traits in determining the genetic variation among the accessions under study was ascertained through their loading values.

Results and discussion

Structure and organization of diversity

The farming communities in Nepal maintain a rich mango genetic diversity resource, both commercial and local cultivars. Local cultivars were often maintained in village orchards and sacred groves, as well as found growing along riverbanks and forest edges. Farmers' varieties were mainly distinguished by fruit morphology (size, shape, colour) and qualitative traits (fruiting time, fibre content in flesh, aroma and taste) (Figure 2). Tree canopy structure and shape and size of leaf were also important characteristics to farmers in distinguishing cultivars. Based on these, farmers have precise names for mango varieties in their own language. A fairly high degree of consistency was noted with respect to the local names given by farmers, whether within or between communities. For example, some cultivars growing in mid-hills and low-hills regions were known as *Supare*, *Lohare* and *Sindhure,* whereas cultivars growing in *tarai* region were named *Supariya*, *Lohariya* and *Sindhuriya,* respectively, which implies the specific cultural influence of *tarai* communities. Nevertheless, the root meaning is the same. A similar case was found with local cultivars *Kali*, *Kathe* and *Dhaule* of central and mid-western hills, known as *Kalya* or *Kathya* in far-western mountains. In contrast, in some cases the same cultivars are known by different names, such as *Bhonth* in eastern *tarai*, *Pharsi* in central hills, *Fajeri* in far-western mountains and *Fajali* in various parts of Nepal: all these apply to the Indian cultivar *Fajli*. In addition, in the mid-western hills, many local cultivar names were found to be very odd and pronounced peculiarly. This highlights the manner in which farmers maintain their cultivars distinct from others. An isozyme study in 2003 also showed genetic variation among cultivars with the same and different names collected from two adjoining districts of Nepal (Subedi et al. 2005b).

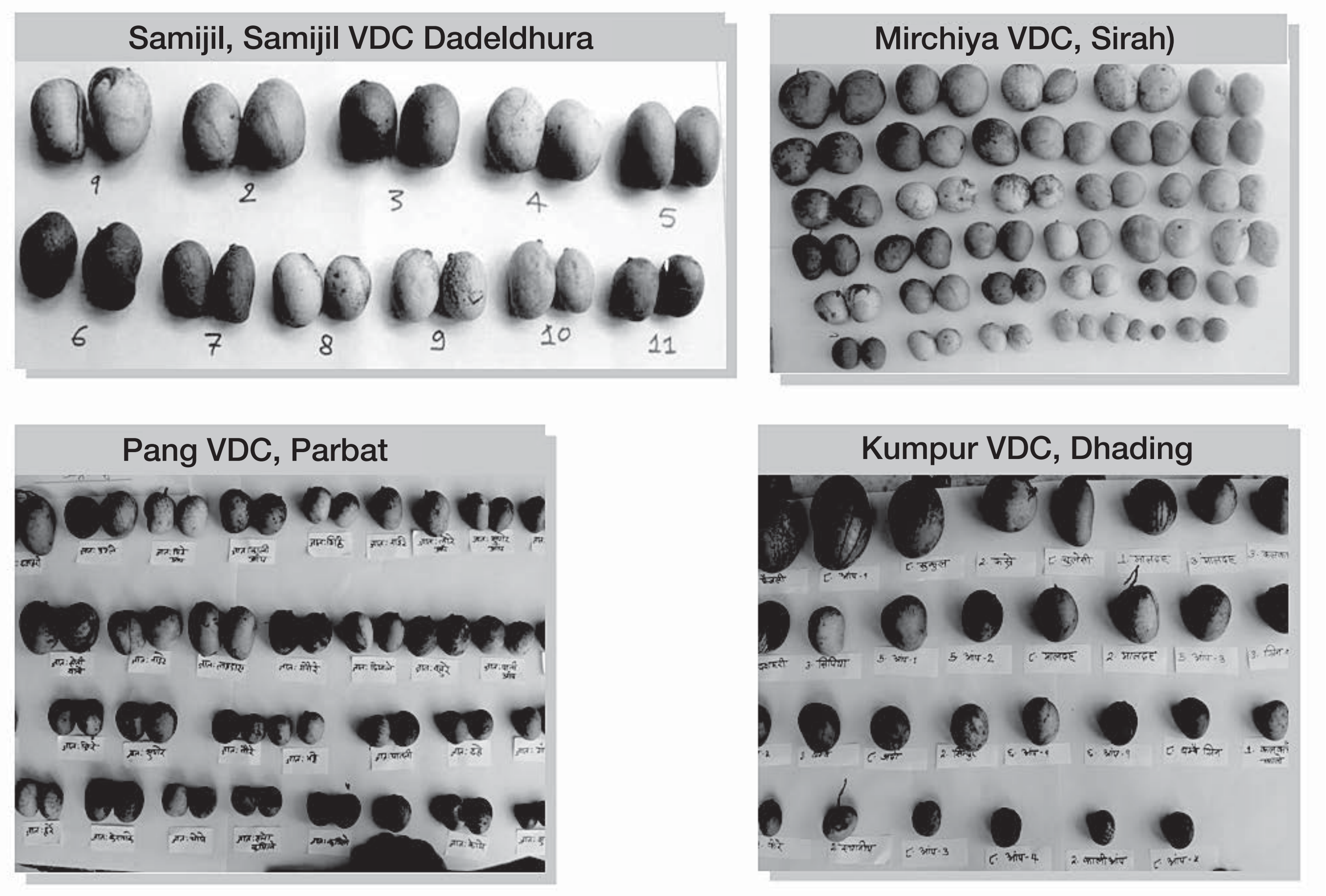

Figure 2. Comparison of mango fruit diversity collected from selected villages in Nepal.

Table 1. An inventory of mango cultivars collected from *tarai*, hills and mountains of Nepal.

Districts	Location(s)‡	Altitude (masl.)	Mango cultivars		
			Local	Indian or commercial	*Bijju* †
Eastern *tarai*					
Saptari	Kanchanpur-6 Kalyanpur-5 Jandole-6 Rajdevi, Rajbiraj-9	90–150	Barbariya, Bathuwa, Bhadaiya, Darmi, Jalmare, Karelwa, Kapuriya, Keruwa, Chiniya, Laduwa, Maldhua, Mishrikand, Rathi, Sindhuriya, Sridhanka, Supariya, Suryapuri	Amarpali, Alponoso, Bhonth (Fajli), Kalkatiya Krishnabhog, Bombay, Malika, Neelam, Sipiya,	Krishnabhog bijju 1,2,3 & 4, Lakhnau Bombay, Nawaras Bombay, Bombay bijju 1 & 2
Siraha	Bishnupur Mirchaiya, Ramnagar-9 Lahan, Lahan-4	100–125	Barbariya, Bathuwa, Bhadaiya, Darmi, Jalmare, Karelwa, Kapuriya, Keruwa, Chiniya, Laduwa, Maldhua, Mishrikand, Rathi, Sindhuriya, Sridhanka, Supariya, Suryapuri	Amarpali, Alphonso, Bhonth (Fajli), Kalkatiya Krishnabhog, Bombay, Malika, Neelam, Sipiya,	Krishnabhog bijju Lakhnau Bombay, Nawaras Bombay, Bombay bijju
Central *terai*					
Dhanusa	Devpuri-rupani-2 Devpuri-rupani-3 Kuwa, Janakpur-12 Ghodghans-1 Ghodghans-2	90	Dudhi-Kerwa, Madhuwa, Gopiya, Kapuriya		Chakariya, Thala, Sarahi, Kunaliya
Mid-western *terai*					
Banke	Bhauniyapur- 5 & 7 Suryapur, Udaipur-5	130–150	Gola, Kapuri, Lakhnaw safeda, Sinduriya		
Central hills					
Kavre	Aanpghari, Baluwa -3 Balakhe, Shikharpur-1 Jadetar, Baluwa-9 Kharkachowr-Baluwa-9 Bagaicha, Kharketar-6	700–900	Banarasi, Bellure, Bhadaure, Chuche kali, Diyale, Dhupi, Dhobi kali, Jhuttre, Kali, Kere, Kari, Kakre, Labate, Lohare, Lokharke, Maldawa, Malta-Bijju, Mishree, Pharse, Saune, Saune malta, Saune maldawa, Sindhure, Sindhure Kali, Supare, Thulo Kali	Bombay, Bombay green	Bijju-1, 2, 3, Kali bijju, Malta-Bijju
Dhading	Baireni, Malang -7 Baharbote, Nalang-6 Muralibhanjyang-3 Majhitar, Kumpur-3 Sayale, Khanikhola-1 Seplaji, Khanikhola-9 Badritar, Jibanpur-8	450–1020	Alini, Aama-bubu, Boke Aanp, Bombay-local, Bombay-old, Budhi Aanp, Chauthe-bali, Chulshi, Farsi, Hade Aanp, Hattijula, Jarda, Jayashree, Jhutte Aanp, Juwane Anp, Kali Bombay, Lohre, Malbhog, Maldawa, Maldhawa-bijju, Mishri-Bombay, Rato-tauke, Saune-aanp, Seti-aanp, Sindure, Sipiya, Sukul, Supare	Bombay-green, Dasahari, Fajli, Kalkatiya, Krishnabhog,	Bombay-bijju, Maldhawa-bijju
Western hills					
Baglung	Baglung Municipality-1 Kalika mindir, Baglung Niraye, Baglung Municipality-11	760–920	Dalle, Gitthe, Kali, Lohare, Mitthe, Naite, Supare	Aamrapali, Bombay	
Parbat	Dimuwa, Tilahar-6 Saharshadhara Pang-1 Pang-2 Patedhunga Mudkuwa-5 Dharmasala Devisthan-9 Rale Devisthan-8 Ekghare Mudkuwa-1	730–850	Bahure, Bhatte, Bhettne, Chope, Dahe, Diyale, Dudule, Dum Gande, Genaurre, Harre, Hade, Jirre, Kamile, Kawale, Kera pake, Koye, Lamadaya, Lamche, Mitthe, Naite, Pani aanp, Patali, Rato kupu, Rato chake, Sano kamile, Supare (28)	Bombay	Seti Bombay

Table 1. An inventory of mango cultivars collected from *tarai*, hills and mountains of Nepal.

Districts	Location(s)‡	Altitude (masl.)	Mango cultivars		
			Local	Indian or commercial	*Bijju* †
Mid-western hills					
Surkhet	Chhinchu-7 Ramghat-6 Birendranagar	490–590	Safeda	Bombay, Chausa, Dasahari, Kalkatiya,	Local Dasahari bijju,
Far-western hills					
Doti	Punnagaun, Silgadi-5 Sungada Dipayal-7 & 8 Talkot Pachnali-6 Bandumrisain Banlekh-4	560–1490	Dhaulya, Kalya, Kokya, Kathi, Maldaha	Bombay, Bombay-green, Dasahari, Dasahari, Dhaulya, Fajri (Fajari), Langda	
Dadeldhura	Samaiji-1 Samaiji-2 Mastamandu-3	1400–1430	Achare, Chaksa, Kathya, Rithya	Bombay, Bombay-green, Dasahari, Fajri, Langda	Dasahari bijju, Langdi-bijju, Local bijju, Local golakar bijju

Notes: † = chance seedling; ‡ number refers to ward number of each village development committee-(VDC) – the smallest political unit of Nepal.

Origin and ecogeographical distribution of mango in the study area

Based on farmer's descriptors, 132 different mango cultivars were characterized from the 11 districts of Nepal, representing *tarai*, low-hills, mid-hills and mountains (Table 1). Characterization of all 216 accessions was not possible as some of the collected germplasm died. Broadly three groups of mango cultivars can be recognised in Nepal, all mono-embryonic in origin: (1) Commercial cultivars of Indian origin; (2) 'Bijju' (chance seedling); and (3) Local cultivars (Kashkush et al. 2001; NARC 2003). In Nepal, most mango diversity is mono-embryonic and therefore needs to be propagated vegetatively from the best tree.

Indian cultivars

Many cultivars are of Indian origin and most of them are the result of open pollination leading to chance seedling and then further maintained asexually. Noteworthy cultivars are *Alphonso, Bombay, Bombay green, Chausa, Chiniya, Dashehari, Langara, Fajli, Kalkatiya, Kishenbhog, Neelum, Sipiya* and *Zardalu*. Two common hybrid cultivars are *Amarapali* and *Mallika.* The cultivars are found in home gardens, commercial nurseries and community gardens throughout the country, within an altitude range of 100 to 800 masl, with a rich diversity of commercial cultivars that are maintained to meet home and commercial demands. Eastern and central *tarai,* namely the districts of Siraha, Sarlahi, Mahotari, Sunsari, and Dhanusa, are known for their high production of commercial mango. Many Indian commercial cultivars, such as *Bombay, Dashehari* and *Langara,* are also cultivated above 1300 masl in far-western mountains.

Bijju (seedling)

These *Bijju* cultivars are chance local seedlings, found mainly in the lower hills and *tarai* plain regions. These are mono-embryonic and need to be propagated vegetatively. Some of these cultivars are the chance seedlings of Indian commercial cultivars. This category includes *Lucknow Bombay, Kali Bombay, Mishri-Bombay, Nawaras Bombay, Saune Bombay, Lucknow-safeda, Dasahari Bijju, Kali Bijju, Krishnabhog Bijju, Local golakar Bijju* and *Malta-Bijju*.

Local cultivars

These seedling cultivars can be found along riverbanks and tropical/sub-tropical forest areas; with time some have been gradually domesticated in village gardens, in diverse ecosystems, including mountains, mid-hills and *tarai*. The most popular local cultivars are *Supare* (a small fruit with a betel-nut shape), *Lahare* (a cluster of chained fruits) found from 200 to 1000 masl across the country. About 60% of all local cultivars were documented from central and mid-western hills (Kavre, Dhading and Parbat districts). In far-western mountains, the local cultivars *Rithya*, *Achare*, *Kathi* and *Local golakar* were found at high altitude (>1400 masl), representing the highest-altitude distribution of mango genetic resources in Nepal.

Extent and distribution of genetic diversity in mango in Nepal

The coefficient of variation (CV%) was found to be high for most fruit characteristics among the accessions studied. The range of variation for fruit weight and length among the accessions within and between surveyed districts was high, along with fruit and kernel (seed) measurements. Fruit shape, flesh colour, pulp taste and seed shape were found to be significant when assessing the level of variation among mango cultivars (Figure 2). Samples from eastern *tarai* and central hills were found to be more diverse, with high coefficients of variation and high diversity indices (Tables 2 and 3), especially for skin colour.

Table 2. Means and coefficient of variation (CV%) of quantitative characters among mango fruit collected from different regions of Nepal.

Character	Statistics	Eastern tarai	Central hills		Western hills		Far-western hills	*F* value
		Siraha (n=62)	Kavre (n=31)	Dhading (n=34)	Baglung (n=10)	Parbat (n=29)	Dadeldura (n=50)	
Fruit weight (g)	Mean	217.5	133.9	206.0	101.2	69.4	160.7	1.10 ns
	CV	0.85	0.58	1.01	0.60	1.50	0.61	
Fruit length (cm)	Mean	8.9	10.1	8.3	7.0	5.8	8.2	1.55**
	CV	0.23	1.89	0.33	0.18	0.29	0.21	
Fruit width (cm)	Mean	6.5	5.6	6.2	5.0	4.3	5.7	1.55**
	CV	0.23	0.18	0.28	0.16	0.27	0.23	
Fruit thickness (cm)	Mean	6.0	5.2	5.8	4.6	3.9	5.4	1.42*
	CV	0.19	0.19	0.26	0.20	0.31	0.23	
Skin thickness, (cm)	Mean	0.21	0.21	0.22	0.25	0.17	0.22	0.78 ns
	CV	0.34	0.17	0.25	0.21	0.30	0.42	
Flesh thickness, (cm)	Mean	1.9	1.6	1.7	1.3	1.2	1.6	2.33***
	CV	0.30	0.23	0.37	0.21	0.17	0.32	
Seed length (cm)	Mean	7.2	6.2	6.9	5.9	4.9	6.8	1.66 **
	CV	0.20	0.26	0.33	0.19	0.30	0.21	
Seed width (cm)	Mean	3.5	3.2	3.6	3.2	2.8	3.3	1.10 ns
	CV	0.18	0.14	0.28	0.14	0.19	0.18	
Seed thickness (cm)	Mean	2.1	1.9	2.0	2.0	1.9	1.9	2.19***
	CV	0.17	0.25	0.17	0.20	0.19	0.17	
Seed weight (g)	Mean	27.0	18.9	28.4	22.1	17.1	22.4	1.27 ns
	CV	0.37	0.58	0.63	0.39	0.42	0.34	
Brix (%)	Mean	16.6	15.6	14.2	13.4	14.8	15.2	0.88 ns
	CV	0.24	0.23	0.24	0.31	0.22	0.29	
Average	CV	0.30	0.43	0.38	0.23	0.36	0.29	

Notes: CV = coefficient of variation (%). Figure in parenthesis is sample size (n). For *F* value: ns = not significant; **p* = >0.05; ***p* = >0.01; ****p* = >0.001.

Table 3. Shannon-Weaver and Simpson Indices based on qualitative traits showing diversity of fruit types across the collection sites.

Character	*F* value	Eastern tarai	Central hills		Western hills		Far-western hills
		Siraha (n=62)	Kavre (n=31)	Dhading (n=34)	Parbat (n=29)	Baglung (n=10)	Dadeldhura (n=50)
Fruit shape	2.58*	0.73 (0.39)†	0.24 (0.12)	0.30 (0.16)	0.33 (0.19)	0.00 (0.00)	0.59 (0.31)
Skin colour	1.77 ns	1.07 (0.65)	0.73 (0.46)	1.01 (0.61)	1.03 (0.63)	1.03 (0.62)	1.03 (0.62)
Flesh colour	4.97***	0.83 (0.46)	0.68 (0.49)	0.81 (0.49)	0.55 (0.37)	0.61 (0.42)	0.84 (0.48)
Fibre content	2.67	0.55 (0.61)	0.53 (0.64)	0.61 (0.60)	0.46 (0.53)	0.50 (0.56)	0.37 (0.63)
Fruit aroma	0.03 ns	1.01 (0.37)	1.05 (0.35)	1.00 (0.42)	0.87 (0.29)	0.95 (0.32)	1.05 (0.21)
Pulp taste	0.23 ns	0.88 (0.53)	0.91 (0.55)	0.96 (0.58)	0.80 (0.51)	1.03 (0.62)	1.03 (0.62)
Pulp colour	5.12***	0.86 (0.50)	0.67 (0.48)	0.65 (0.46)	0.68 (0.49)	0.61 (0.42)	0.85 (0.50)
Seed shape	8.23***	0.99 (0.54)	0.14 (0.06)	0.49 (0.26)	0.40 (0.19)	0.00 (0.00)	0.60 (0.09)
Mean		0.87 (0.51)	0.62 (0.39)	0.73 (0.45)	0.64 (0.40)	0.59 (0.37)	0.79 (0.43)

Notes: data figures in parentheses are the Simpson indices. ns = not significant; **p* = >0.05; ***p* = >0.01; ****p* = >0.001.

These results show that quantitative characters are the most influential for selection under heterogeneous environments of the eastern *tarai* and central hills regions (Tables 2 and 3). In contrast, it was quality traits that mattered most in the western hills (*Parbat* and *Baglung* districts). This finding supports the earlier studies of fruit tree species where qualitative traits have been found useful in identification and assessment of varieties for fruit production on large scale (Leakey et al. 2000). In addition, earlier studies on diversity of crop species within centres of diversity have demonstrated the importance of quantitative traits outside the centre of diversity, and qualitative traits within the centre of diversity (Tolbert et al. 1979; Witcombe and Gilani 1979). In comparison, the accessions from western hills are less variable than eastern *tarai* or central hills regions. At the same time, the large variation in the eastern *tarai* and central hills are limited by natural selection.

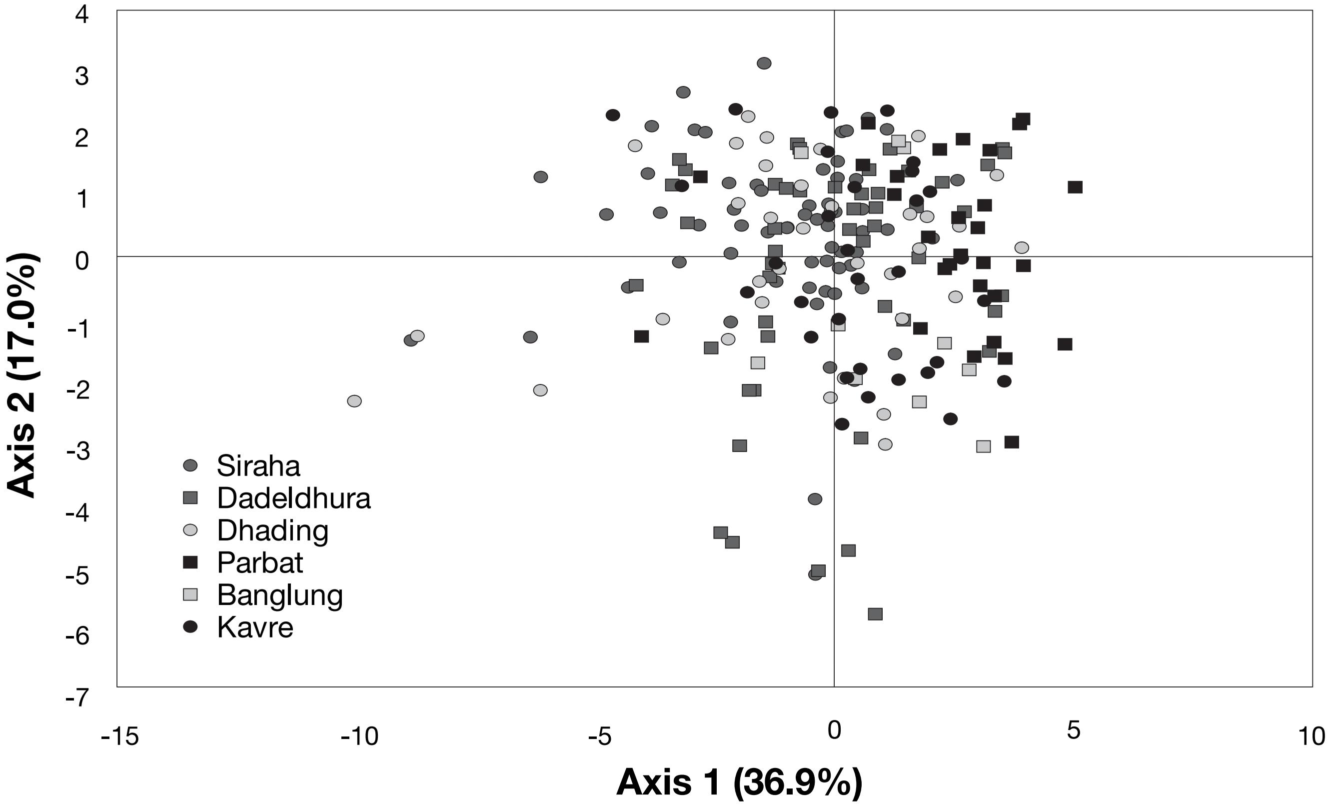

Figure 3. Scatter plot of the accessions of mango based on significantly different quantitative and qualitative characters.

Production areas and maintenance of mango diversity in Nepal

Historically, farmers grew local Nepali cultivars, but abandoned them in favour of more lucrative commercial cultivars. Farmers with access to the research system have been systematically maintaining commercial mango cultivars for fresh consumption and income generation. This influence is clearly seen throughout the *tarai* and lower-hills, where most commercial Indian cultivars were found, originating from research stations. This is because many farmers depend upon informal exchange within the community or across the border with nearby Indian villages, or obtain their seedling from private nurseries or government horticultural farms. Elsewhere, in the areas with limited access to the formal research system and where the environment is less favourable for commercial mango production, such as dry or marginal lands or mountainous areas, local cultivars are grown for fruit, fodder, fuelwood and shade purposes. Generally, old mango cultivars are maintained in either home or village gardens, within the precincts of temples and on public sites such as schools. This system continues due to the mango's religious and cultural importance. According to Hindu mythology, mango leaves are part of auspicious offering in worship as *Panchapalav*. Village gardens (*bagichas*) represent social prestige, and hence old mango orchards have been maintained in these *bagaicha* for hundreds of years. This phenomenon is unique to Nepal, where hundreds of hectares in the hills and *tarai* have been maintained as *bagaicha* under the protection of the elite royal caste, such as *Shah* and *Rana*.

Conservation of mango genetic resources is intimately interlinked with the perspectives of the farmers who grow, use and market them. The formal system emphasizes production countrywide of commercial mango of Indian origin. For example, Nawalpur Horticultural Farm of the Department of Agriculture (DoA) and Tarahara Research Station of Nepal Agricultural Research Council (NARC) promoted commercial mango cultivation. Major commercial production areas of mango are located in the Central and Eastern Development Regions of Nepal (ASD 2005). Comparatively, the *Sarlahi, Siraha, Kavilbastu, Mahotari* and *Dhanusa* districts have a high proportion of commercial mango production, with the main cultivars being *Bombay, Bombay Green, Chausa, Dashehari, Fazli, Kishen Bhog, Langra, Neelum* and *Chausa,* alongside hybrid cultivars such as *Amarapali* and *Malika*.

Appreciating the use-values of mango diversity

Table 5 shows that local mango cultivars possess various positive characteristics, such as ability to grow in dry and marginal areas or at high altitude, absence of fibre in pulp, high fruit setting rate and off-season fruiting. Production of these local cultivars is based upon intended use. For example, local cultivars like *Lahare* are produced on a large scale because of high market demand, while *Jarmale* of *tarai* region is popular for its sweet taste, even at the green stage, and used as as a salad or pickle. Fibrous cultivars are used for pickling or making fresh local juice. Some mango diversity is conserved because of its association with the ethnic food culture of *tarai* and mid-

Table 4. Possible *in situ* conservation sites for mango genetic resources in Nepal.

Site	Eco-geographical characteristics	Total number of cultivars	Unique local cultivars
Samijil, Samijil Village Development Committee VDC, Dadeldhura district (Far-western Nepal)	Middle mountain with valley bottoms (1400–1430 masl)	21	*Achare, Kathe, Kathya* and *Rithya*
Pang, Pang VDC, Parbat district (Mid-western Nepal)	Middle mountain (900 masl)	25	*Bahure, Bhetne, Bhatte, Chope, Dayhe, Diyale, Doom Harre, Kamile, Lamche, Naite, Rato-kupu, Patali* and *Supare*
Majhitar, Kumpur VDC & *Badritar* Jibanpur VDC, Dhading district (Western Nepal)	Middle mountain with river valleys (550–1000 masl)	33	*Alini, Aama-bubu, Boke tauke, Budhi, Hade, Jhutte, Kali, Lohare, Supare, Jwane, Seti* and *Saune*
Bagaichya, Kharketar-6, Kavre District (Central Nepal)	Middle mountain (700–900 masl)	30	*Bellure, Chuche kali, Diyale, Dhupi, Dhobi Kali, Jhuttre, Kere, Kari, Kakre, Labate, Lohare, Lokharke, Supare* and *Thulo Kali*
Mirchiya, Ramnagar -9, Siraha district (Eastern Nepal)	Indo Gangetic plain with river bottom (100 masl)	35	*Barbariya, Bathuwa, Bhonth, Darmi, Jarmale, Karelwa, Keruwa, Laduwa, Sridhanka* and *Supariy*

Table 5. Use value of local mango cultivars in Nepal.

Fruit value	Juice value	Pickle and salad value
Chinia, Chuche kali, Chulesi, Kalame, Kalapahad, Kali, Lamdaya, Lohare, Mithhe, Naite, Safeda, Sindhure, Sipiya, Pharse, Tamburiya, Thulo kali	Harre, Kalya, Kathe, Kerapake, Koke, Pani aanp, Rato tauke, Supare	Achare, Bhatte, Gande, Githhe, Gola, Jarmale, Jhutte, Jhuttre, Rithya, Thulo kamile

hills. Other typical products are *Amchurna,* made of immature mango; *Mada* is made of ripe pulp, cooked, and which may be salted, sun-dried and kept for long-term storage purposes; and *Chutney,* made of peeled, sliced green mangoes, parboiled and combined with sugar, salt and spices.

Implications for conservation of mango genetic resources

Local mango genetic resources have been found to be community assets for fulfilling the nutritional and other local requirement in Nepal, such as fuelwood and shade. Maintenance of local mango orchards is a relatively cost-effective strategy since many of the cultivars are adapted to marginal conditions. In contrast, the original habitats of local mango have been rapidly changing in response to biotic, economic and other pressures in recent years (Subedi et al. 2004).

Results of this present study point towards two important facets that have influenced the genetic erosion of local mango genetic resources as well as the loss of potential local cultivars.

Firstly, there is an alarming increase in the tendency of farming communities to replace the local mango by commercial Indian mangoes. Many historical village gardens—the *Bagaincha*—in *tarai* and mid-hills have been severely threatened due to conversion to modern orchards, and logs from local mango trees are extensively used the furniture industry, and as fuel wood, such as in brick factories. This situation is more severe in the *tarai* region of Nepal due to easy access to improved or commercial cultivars and the economic incentive to sell old mango trees for timber and fuelwood.

Secondly, the national research system has yet to exploit available mango diversity in the country through identification, evaluation, characterization and promotion of good local mango cultivars. Rather, the extension programme focuses on promoting a few commercial mango cultivars under the 'mango pocket programmes' in *tarai,* aiming to increase income generation for farmers, but affecting the existence of various local mango genetic resources. Moreover, there is a profound lack of access to information and technology to harness the large amount of local mango genetic resources in order to meet consumer demands. The existing markets for mango are largely for fresh fruit consumption; farmers do not have access to alternative value addition options, such as further processing or juice preparation.

Ways forward

Ex situ conservation

Previous studies indicate that *ex situ* conservation of mango is difficult because of the recalcitrant nature of mango seed, so that it can not be stored in conventional genebanks (Bompard 1995). At best, mango seeds of can be stored for about 100 days (Chin and Roberts 1980). Therefore, conservation needs to focus on the establishment of field genebanks in *tarai* and hills of Nepal. NARC has initiated work on the collection of different local mango cultivars at Tarahara Horticultural Farm in eastern *tarai,* where some 80 different mango accessions have been maintained (NARC 2003). A field genebank has both advantages and disadvantages but the system can complement with *in situ* conservation method effectively. High maintenance costs, large land area requirement, the limited amount of genetic variation that can be stored and vulnerability of natural and human disasters are often barriers to this approach (Hodgkin et al. 2003). At the same time, a field genebank is readily accessible and useable for characterization, evaluation and crop improvement. For really long-term storage of genetic resources of species like mango, on-farm and cryopreservation are the only options, and these have yet to be systematically developed.

Field genebanks of mango should focus on core germplasm maintenance, and conduct research and development work such as stock-scion relations, use local cultivars in mango breeding programmes, and produce planting materials of local cultivars. For sustainability, the field genebank should be made self-sustaining by integrating with commercial fruit sapling nurseries, so that income can be generated to meet the cost of its maintenance. An urgent need is to salvage the mango diversity from the old village orchards (*Bagaichya*) and conserve them in field genebanks to maximize the use of diversity on-farm.

In situ *conservation in natural habitat or sacred groves*

In situ conservation is a viable tool for recalcitrant-seeded species. It extends the conservation of a species beyond the level of the individual to the habitat or ecosystem. Protected areas system scattered through out the *tarai*, hills and mountains are those areas where long-term *in situ* conservation of mango genetic resources is possible in Nepal. However, there is inadequate information on the extent, distribution and diversity of mango genetic resources in the protected areas. Table 1 illustrated some of potential low cost options for *in situ* conservation of mango genetic resources in Nepal. These sites are characterized by high intraspecific diversity and representative of different ecogeographical regions. Figure 1 shows suggested sites, including as *Mirchiya,* Ramnagar VDC, Siraha district; *Bagaichya,* Kharketar VDC, Kavre District; Trisuli valley of Nuwakot district; Kumpur and Jibanpur VDC, Dhading district; *Pang,* Pang VDC of Parbat district; and *Samijil,* Samijil VDC of Dadeldhura district. The concept of a Forest Genebank (Umashaanker and Ganeshaiah 1997) is being employed in the Western Terai Landscape Project of Nepal after locating source and sink of mango diversity in protected and natural forest ecosystems.

On-farm conservation

On-farm conservation of existing mango diversity could be promoted by improving access to diverse germplasm and creating incentive mechanisms for use of different types of mangoes in the market. This process will lead to dynamic exchange of materials and selection in a decentralized manner, which will continue to shape the genetic diversity. Home gardens, semi-commercial orchards, old sacred groves and community orchards, such as mango orchards on school premises, are strategic sites where mango diversity has been maintained for generations and needs to be conserved for future use. The potential local mango cultivars *Chinia, Chuche Kali, Chulesi, Kalame, Kalapahad, Kali, Lamdaya, Lohare, Mithhe, Naite, Safeda, Sindhure, Sipiya, Pharse, Tamburiya* and *Thulo Kali* can be immediately promoted for home gardens and commercial mango orchards. An important aspect of on-farm management is to promote and improve the locally available technologies for processing of mango for various mango products, with linkages to the market, which could play important roles in generating incentives for conservation and maintenance of germplasm in the long term.

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Collecting crop genetic resources in the Mediterranean agricultural islands: Lefkada, Ithaca and Kefalonia (Greece)

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Summary

Collecting crop genetic resources in the Mediterranean agricultural islands: Lefkada, Ithaca and Kefalonia (Greece)

In September 2006, a collecting mission explored three Greek Ionian islands (Lefkada, Ithaca and Kefalonia), within the framework of an agreement among the Institute of Plant Genetics of the CNR, Bari (Italy), the University of Kassel (Germany) and the Greek Gene Bank of Thermi-Thessaloniki (Greece), targeted at safeguarding autochthonous crop genetic resources still present in Mediterranean islands. Strong genetic erosion was noted for landraces of vegetables and cereals, while legumes, forages, spices and other crops were less affected. During this mission, 148 accessions of landraces and wild crop relatives were collected, belonging to 68 taxa of pulses, vegetables, cereals, forages and spices. Nowadays, Greek agriculture is an intensive, market oriented, competitive economic activity, aimed at both the domestic and the global market; nevertheless, in some areas (e.g. the Ionian islands), several valuable landraces are still present and used by local communities.

Key words: agricultural biodiversity, collecting, crop genetic erosion, Lefkada, Ithaca, Kefalonia

Résumé

Collecte de ressources génétiques de plantes cultivées dans les îles méditerranéennes : Leucade, Ithaque et Céphalonie (Grèce)

En septembre 2006, une mission de collecte a exploré trois îles ioniennes grecques (Leucade, Ithaque et Céphalonie), dans le cadre d'un accord entre l'Institut de phytogénétique du CNR, à Bari (Italie), l'Université de Kassel (Allemagne) et la banque grecque de gènes de Thermi-Thessalonique (Grèce). Cette mission avait pour objectif de sauvegarder les ressources génétiques autochtones encore existantes dans les îles méditerranéennes. Une forte érosion génétique a été observée en ce qui concerne les variétés locales de légumes et de céréales, tandis que les protéagineux, plantes fourragères, épices et autres plantes cultivées étaient moins affectés. Au cours de cette mission, 148 accessions de variétés locales et d'espèces sauvages apparentées aux plantes cultivées ont été collectées, appartenant à 68 taxons de protéagineux, de légumes, céréales, plantes fourragères et épices. Aujourd'hui, l'agriculture grecque est un système de production de nature intensive, à orientation commerciale et compétitive, tournée à la fois vers le marché national et international. Néanmoins, dans certaines régions (par exemple, les îles ioniennes), plusieurs variétés locales intéressantes sont encore présentes et utilisées par les communautés locales.

Resumen

Recolección de recursos genéticos de cultivos en islas agrícolas del Mediterráneo: Lefkada, Itaca y Cefalonia (Grecia)

En septiembre de 2006 una nueva misión de recolección exploró tres islas griegas jónicas (Lefkada, Itaca y Cefalonia) en el marco de un acuerdo entre el Instituto de Fitogenética del CNR de Bari (Italia), la Universidad de Kassel (Alemania) y el Banco Griego de Genes de Termi-Tesalónica (Grecia), dirigido a salvaguardar recursos genéticos de cultivos autóctonos aún presentes en las islas del Mediterráneo. Se notó una fuerte erosión genética en las variedades locales de hortalizas y cereales, mientras que las legumbres, forrajes, especias y otros cultivos resultaban menos afectados. En esta misión se recogieron 148 accesiones de variedades nativas y parientes silvestres de cultivos pertenecientes a 68 taxa de hortalizas, legumbres, cereales, forrajes y especias. La agricultura griega actual es una actividad económica competitiva, intensiva y orientada tanto al mercado nacional como al mundial. No obstante en algunas zonas (por ejemplo en las islas jónicas) siguen existiendo numerosas variedades nativas valiosas que son utilizadas por las comunidades locales.

Introduction

An exploration and collecting mission was carried out in the Greek Ionian islands of Lefkada, Ithaca and Kefalonia in September 2006, within the framework of an agreement among the Institute of Plant Genetics (IGV) of the National Research Council (CNR), Bari, Italy; the University of Kassel, Germany; and the Greek Gene Bank (GGB) of Thermi-Thessaloniki, Greece. This mission was the fifth of a project that started in 2002 with the exploration of the Maltese archipelago (Laghetti et al. 2004; Bullitta et al. 2005) jointly by IGV and University of Kassel. The overall aim of this project is the safeguard of autochthonous crop genetic resources still present in Mediterranean islands through their collecting, characterization, conservation and exploitation. This activity represents the extension of a similar research programme, already concluded, addressing the crop germplasm grown in minor Italian islands of agricultural interest (Hammer and Laghetti 2006).

In Greece, a new National Programme was initiated in 2003 by the Directorate of Physical Planning and Environmental Protection of the Ministry of Rural Development and Food, as the Competent Body for the protection of agricultural biodiversity, and the GGB, titled *Establishment of a new National Plant Gene Bank*, Measure 6.3/Action B in the Operational Programme of Rural Development-Reconstruction of Countryside 2000-2006 (3rd Cohesion Fund Support). This project is being co-funded with 69.7% by the European Agricultural Guidance and Guarantee Fund (FEOGA) and the rest by the National Programme of Public Investment.

This project intends to create a Plant Gene Bank with suitable facilities (building, modern laboratories, etc.) in Thermi, Thessaloniki (Subprojects I, II and IV) as well as the collection of all the remain genetic material in Greece. This task has been entrusted to GGB, to execute a large number of exploratory missions around Greece (Subproject III, under the supervision of the National Agricultural Research Foundation – N.AG.RE.F.). The total budget of the project is €2.42 million, and due to terminate on 31 December 2008.

Today's agriculture in Greece is an intensive, market oriented, competitive sector of the economy, aiming at both domestic and global markets. Exports of farm products have been the backbone of the national economy in the past, and an important source of foreign currency. Although the contribution of agriculture to the gross national product and exports has dropped to less than 13% in recent years, certain crops and agricultural products still maintain a significant position as sources of income (e.g. tobacco, cotton, olives and olive oil, fruits, vegetables) or as essential raw materials for the domestic processing industry (e.g. textiles, cigarettes, sugar, wines, juices, soft drinks) (Stavropoulos 1996). The wealth in cultivated germplasm diversity is analogous to the wild one, due to diverse natural habitats, intense trading with neighbouring and remote lands since ancient times, massive populations movements, etc. The existence of a favourable natural environment for the cultivation of a broad spectrum of species in conjunction with an agricultural system based on subsistence, as was prevalent in the early twentieth century, has resulted in the cultivation of a large number of landraces, well adapted to the local conditions and human preferences. These local varieties include both genera originated or diversified in Greece (e.g. *Cicer*, *Lens*, *Vicia*, *Pisum, Lupinus*, *Brassica*, *Lactuca*, *Cichorium*, *Beta*, *Olea*, *Ficus*, *Vitis*), and genera of more recent introduction, which have afterwards adapted to the local conditions (e.g. *Malus*, *Pyrus*, *Prunus, Triticum*, *Hordeum*, *Secale, Phaseolus*, *Lycopersicon*, *Solanum*, *Capsicum*). Approximately 4400 seed samples of local varieties are conserved in the GGB, plus some minor specific *ex situ* seed or field collections. In this collection, germplasm of cereals (730), tobacco (488), cotton (305), pulses (932), forages, grapevine (567) and *Prunus* (142) are particularly well represented (Stavropoulos 1996). Early collections in Greece, funded by FAO and IBPGR (now Bioversity International), targeted FAO priority species (Galanopoulou and Skorda 1981). Later expeditions were theoretically multicrop ones (e.g. Cortessi 1986; Reid et al. 1989; Vaitsis et al. 2000) focusing on wild and cultivated cereals (Furuta and Ohta 1991; IBPGR 1981; Zamanis et al. 1992). GGB therefore currently stores a good collection of cereals, but is rather poor in other species of agricultural interest, particularly vegetables, fruit trees, aromatic plants and ornamentals (Stavropoulos 1996). Recent expeditions organized in the framework of the new National Programme *Establishment of a new National Plant Gene Bank*, Measure 6.3/Action B in the Operational Programme of Rural Development-Reconstruction of Countryside 2000–2006 (3rd Cohesion Fund Support) in 2004–2006 throughout almost all Greece have yielded another 4000 accessions of the most important crops and wild relatives in Greece, in particular cereals, forage crops and pulses, and vegetables. Most of this germplasm is currently under multiplication and characterization-evaluation.

With the shift to modern agriculture, Greece suffered a dramatic loss of its traditional agricultural germplasm, which was displaced by modern varieties produced by local breeders or imported. Genetic erosion was particularly intense and rapid in cereals, since local germplasm cultivated today covers hardly 1–2% of the total cereal acreage. An analogous, but less dramatic, trend is now becoming evident for vegetable crops, where local landraces have been rapidly displaced in recent years, even from home gardens. Traditional varieties of many tree crops (e.g. olive, apples, cherry, apricots, pears, nuts) and grapevine still endure, although the number of varieties used on a large scale has been substantially reduced. The main reason for this genetic erosion has been the unquestionable superiority of the modern varieties over their traditional counterparts, their suitability to intense farming systems and their conformity to market requirements. GGB has identified and proposed for protection certain areas where traditional agricultural systems still survive, resisting modern pressures. These areas where a significant number of old local varieties are still grown could be protected through a system of on-farm conservation based on national or international support so that both the endangered local landraces and the associated traditional agricultural systems and landscapes are rescued from the imminent threat of extinction. In these areas, the protection of traditional systems and landraces could be further enhanced by combining it with parallel support schemes for ecological agriculture, given the strong affinity of ecological to traditional agriculture.

There are seven important islands in the Ionian Sea, and are known as Eptanissa ('seven islands') in Greek. The islands are Kefalonia, Corfu, Lefkada, Ithaca, Zakynthos, Kythira and Paxi. The present mission covered three of these islands, namely Lefkada, Ithaka and Kefalonia. The histories of these islands are largely similar, especially in modern times. They have been inhabited since prehistoric times.

Lefkada

The island of Lefkada, also called Lefkas, is the fourth-largest island of the Ionian Islands, after Zakynthos, Kefalonia and Corfu. It has an area of 303 km^2, a coastline of 117 km and a population of about 22 000; it is separated from the coast of Akarnania on the mainland by a narrow isthmus and a floating bridge 50 m long. The weather is typically Mediterranean, characterized by hot and sunny summers, and mild winters with high rainfalls. An important characteristic of Lefkada's environment is its wide biodiversity. Agricultural buildings, such as dozens of watermills, the olive presses, threshing floors, the simple stone-built houses and the unique domed stone stores, are evidence of the importance of farming on the island. Agriculture had been for centuries the most important occupation of Lefkada's inhabitants, the activity that ensured their survival. The cultivation of olive (e.g. cvs. Asprolia and

Prevezana) is the most profitable agricultural activity on the plains, with viniculture on the highlands, activities practised from ancient times. Other profitable activities include stock breeding, fishing, trade and crafting. Lefkada also produces a large variety of agricultural products, such as thyme honey in Dragano and Athani; liqueurs like 'rozoli', 'mint' and 'rose'; virgin olive oil; and wines. Wine making is mostly based on the varieties Vardea, Laorkos and Vertzami (Marzemino in Italian); this last one is very rare and considered one of the best varieties of Greece. It was probably brought by the Venetians in the seventeenth century, and it is cultivated in the towns of Sfakiotes, Karya, Apollon and Ellomenos between 200 to 700 masl. Vertzami is late-ripening variety, extremely high in anthocyanins, which prefers lower elevations (below 500 masl) where it can ripen enough to produce wines of deep extract and full body.

Ithaca

This island is separated from Kefalonia by a narrow, 3-km wide channel. The east coast of the island is harsh and steep, contrasting with the west coast, which is verdant and soft, gently sloping down to the Ionian Sea. The interior of the island is quite mountainous, covered with olive trees, vineyards and forests of pine and cypress.

Ithaca is the second smallest inhabited island of the Eptanissa group, covering an area of about 95 km^2, with a maximum length of approximately 29 km, width of about 6.5 km and a coastline measuring 100 km. Long and narrow in shape, the island is almost divided into half by the deep bay of Molos. The island's capital is Vathy since Venetian times. The population of Ithaca stabilized after 1985 at 3000 inhabitants, but decreased to 2500 during the 1990s. Today, a number of new residents have come from all around Greece, because they have either inherited land or seek a more natural quality of life, and from abroad, and the population stands now at around 3700 inhabitants.

The main local source of income is tourism, even though the island has not been spoiled yet by mass tourism. This is one of the reasons why young people remain, maintaining the population stable. Agriculture is quite limited, and a large percentage of people are sailors by tradition.

Kefalonia

Kefalonia is the largest among the Ionian Islands in western Greece. It is a mountainous island with dense vegetation, long sandy beaches, small traditional villages, several Venetian castles, and a National Park for the protection of the fauna and flora. Kefalonia island is very fertile and offers a variety of excellent agricultural products. Cheese, olives and wines are the island's specialities. In general, the food in Kefalonia is similar to that of the other Ionian Islands, but small variations can be noticed, as well as local island specialities. The food specialities of Kefalonia are the *bourbourelia* (a soup of mixed beans), excellent hare or rabbit stew, *riganada* (bread slices with oregano), and fine egg and potato soup. Kefalonia island is also a candy paradise. Its most famous pastries are the *mandoles* (toasted almonds), the *confetto* (baked quince), anise biscuits, *barboule* (caramelized almonds), *nougat*, *pastelli* (a thick and hard sweet made of caramelized sesame seeds), and *amygthalopita* (almond cake). Kefalonia is very famous for its excellent wine and has three appellations of origin, namely *Robola* (a dry white wine), *Mavrodafni* (a sweet, thick and dark red wine) and *Muscat* (a sweet white wine). Kephalonian wines are made of many varieties of grapes. The grapes used for the Robola wine are the most famous, and must be cultivated in specific areas of the island in order to qualify for the official appellation of origin.

Collecting methods

The main sources of the collected seed samples were farmers' stores and their fields, and, in a few cases, also from seed and fruit sellers. At each collecting site, and for each sample, a passport data sheet was filled in, using data from a hand-held GPS system and information from the farmers. In addition to the data recorded directly by the collecting team, further information on local agriculture was obtained from specialists and through several interviews with growers. Further details on the sampling methods and exploration strategy used are reported elsewhere (Hammer and Laghetti 2006; Hammer et al. 1997; Laghetti et al. 1996, 2004).

Results and discussion

A total of 148 samples were collected from 37 sites within the three islands (Table 1). Samples of wild cardoon (*Cynara cardunculus* L. var. *sylvestris* Lam.) were also collected from the Ionian mainland during the journey to reach the islands due to the interest of the IGV team in wild artichoke resources (Pignone and Monti 2005).

Lefkada

Fourty-four accessions were collected (9 cereals, 9 legumes, 15 vegetables and 11 other crops) (Table 1).

The 9 samples of cereals consist of one wild relative (*Dasypyrum villosum* (L.) P. Candargy) and five cultivated *taxa*: oat, bread wheat (three samples, of which one is an old type with reddish seeds), durum wheat and maize (three samples).

Among the 9 landraces of pulses, two were common bean, two broad bean (*Vicia faba* L. var. *faba* named 'eptakukia'), two grass peas (*Lathyrus* sp.), one of which is locally named 'psarra', one chickpea with white seeds, one lentil and one pea.

Today in Lefkada many vegetables are cultivated on an industrial scale, but some landraces are still present, and 15 of them were collected, such as a bottle gourd (*Lagenaria siceraria* (Molina) Standl.), the cabbage 'frio' (*Brassica oleracea* L.), three samples of garlic (*Allium sativum* L.) with variable bulb colour and shape, one endive (*Cichorium endivia* L.), a lettuce (*Lactuca sativa* L.), together with one sample of the wild relative 'prickly lettuce' (*L. serriola* L.), one of wild radish (*Raphanus raphanistrum* L.) and one of wild beet (*Beta vulgaris* L. subsp. *vulgaris*).

Table 1. Accessions collected in 2006.

Species	Greek Ionian islands			Greek mainland	Total
	Lefkada	Kefalonia	Ithaca		
Avena byzantina K. Koch	1	–	–	–	1
Dasypyrum villosum (L.) P. Candargy	1*	–	–	–	1
Hordeum vulgare L.	–	1	–	–	1
Triticum aestivum L.	3	–	–	–	3
Triticum durum + aestivum	1	–	–	–	1
Triticum durum Desf.	–	–	2	–	2
Zea mays L.	3	–	1	–	4
Total cereals	9	1	3	–	13
Cicer arietinum L.	1	–	–	–	1
Lathyrus spp.	2	3	1	–	6
Lens culinaris Medik.	1	1	–	–	2
Phaseolus vulgaris L.	2	–	–	–	2
Pisum sativum L.	1	–	–	–	1
Vicia faba L. var. *faba*	2	2	1	–	5
Vicia faba L. var. *minor* (Peterm. em. Harz) Rothm.	–	–	1	–	1
Vigna unguiculata subsp. *sesquipedalis* (L.) Verdc.	–	1	–	–	1
Vigna unguiculata (L.) Walp. subsp. *unguiculata*	–	1	–	–	1
Total pulses	9	8	3	–	20
Allium ampeloprasum L.	–	–	1	–	1
Allium cepa L.	–	1	–	–	1
Allium fistulosum L.	–	–	1	–	1
Allium sativum L.	3	1	–	–	4
Amaranthus cruentus L.	1	2	1	–	4
Amaranthus retroflexus L.	1	1	1 + 2	–	5
Apium graveolens L.	–	2	–	–	2
Beta maritima L.	–	1	–	–	1
Beta vulgaris L.	1	4	2 + 1	–	8
Brassica nigra (L.) Koch in Röhling	–	2	1	–	3
Brassica oleracea L.	1	1	–	–	2
Brassica rapa L. em. Metzg. ssp. *rapa*	–	1	–	–	1
Brassica sp.	–	1	–	–	1
Capsicum baccatum L.	–	1	–	–	1
Cichorium endivia L.	1	–	2	–	3
Cichorium intybus L.	–	1	–	–	1
Cichorium sp.	–	1	–	–	1
Citrullus lanatus (Thunb.) Matsumara & Nakai	–	2	–	–	2
Cucumis melo L.	–	3	–	–	3
Cucumis sativus L.	–	1	1	–	2
Cucurbita maxima Duch. ex Lam.	–	1	–	–	1
Cucurbita pepo L.	–	1	1	–	2
Cynara cardunculus L. var. *sativa* Moris	3	–	3	–	6
Cynara cardunculus L. var. *sylvestris* Lam.	–	1	–	5	6
Daucus carota L.	–	2	–	–	2
Foeniculum vulgare Mill.	–	1	–	–	1
Lactuca saligna L.	–	–	1	–	1

Table 1. Accessions collected in 2006.

Species	Greek Ionian islands			Greek mainland	Total
	Lefkada	Kefalonia	Ithaca		
Lactuca sativa L.	1	1	1	–	3
Lactuca serriola L.	1	–	1	–	2
Lagenaria siceraria (Molina) Standl.	1	–	–	–	1
Lycopersicon esculentum Mill.	–	8	5	–	13
Raphanus raphanistrum L.	1	–	–	–	1
Raphanus sativus L.	–	1	–	–	1
Solanum melongena L.	–	–	1	–	1
Sonchus sp.	–	–	1	–	1
Total vegetables	15	42	27	5	89
Astragalus sp.	1	–	–	–	1
Calamintha nepeta (L.) Savi	–	–	1	–	1
Cardamine sp.	–	1	–	–	1
Crithmum maritimum L.	1	–	–	–	1
Hyoscyamus albus L.	1	–	–	–	1
Ipomoea sp.	1	–	–	–	1
Linum usitatissimum L.	–	–	1 + 1	–	2
Medicago truncatula Gaertn.	–	1	–	–	1
Ocimum basilicum L.	–	4	1	–	5
Ocimum basilicum subsp. *minimum* (L.) Danert	1	1	1	–	3
Origanum majorana L.	3	–	–	–	3
Origanum vulgare L.	–	2	–	–	2
Ornithopus sp.	1	–	–	–	1
Petroselinum crispum (Mill.) Nym.	–	1	–	–	1
Pimpinella anisum L.	–	1	–	–	1
Rumex sp.	1	–	–	–	1
Solanum pseudocapsicum L.	–	–	1	–	1
Solanum villosum Mill.	–	–	1	–	1
Trigonella balansae Boiss. & Reuter	1	–	–	–	1
Total forages, spices and others	11	11	7	–	29
Grand Total	44	59	40	5	148

Note: numbers underlined are of wild or weedy accessions.

The churches and the monasteries provided support to the Greek and Christian culture of the island during the difficult years of the Tourkokratia (Turkish domination). Still today, many monasteries are present on Lefkada, and it was noted that some old monks safeguard ancient traditional seeds with endless care. In fact, some old landraces of common bean with red testa (called ʼbarbouniʼ), tomato (e.g. ʼkarambolaʼ: a semiflat type introduced in 1940 from Mesolonghi) and the typical red amaranth ʼblito kalliergomenoʼ (*Amaranthus cruentus* L.) were collected from a monastic store. During the mission, a sample of the redroot amaranth ʼblito trahyʼ (*Amaranthus retroflexus* L.) was also collected; more details about this Greek traditional neglected crop is reported by Pignone et al. (2007). At three sites, many interesting accessions of cultivated artichoke (*Cynara cardunculus* L. var. *sativa* Moris) were gathered, characterized by some wild traits, such as the presence of prickles on stems and leaves. This occurrence might be due to the fact that in this area this crop is traditionally propagated by seeds instead of vegetatively; the resulting character segregation might produce the variation observed in the artichoke plots.

Eleven accessions of plants for other uses were also gathered, among which four accessions of aromatic plants: sweet marjoram (*Origanum majorana* L.) and the traditional bush basil (*Ocimum basilicum* L. subsp. *minimum* (L.) Danert), bearing small, narrow leaves with a sweeter, less pungent smell than the large-leaf types. Two wild vegetables frequently used by local people were collected: marine fennel (*Crithmum maritimum* L.) and a dock type (*Rumex* sp.), probably escaped from former cultivation and naturalized. A sample of the medicinal plant white henbane (*Hyoscyamus albus* L.) and three populations of pasture plants were sampled from the wild (Table 1).

Ithaca

Forty accessions were collected from Ithaca island (3 cereals, 3 legumes, 27 vegetables and 7 of other crops) (Table 1).

Two old landraces of durum wheat (called ′mavragagli′ and ′xilocastro′) were collected, together with a local type of maize reported to be drought resistant. Among the legumes collected there were also the traditional grass pea ′psarra′ and the so-called ′microcucchia′ field bean (*Vicia faba* L. var. *minor* (Peterm. em. Harz) Rothm.).

Marked genetic erosion was noted for local vegetables, but some landraces were found, such as the tomatoes ′voidocardia′, ′greza′ (rosy and ribbed fruits, with prickles on calyx) and ′panda rosa′ (very small fruits); the typical local cucumber (*Cucumis sativus* L.) with long fruits; an old variety of Italian squash (*Cucurbita pepo* L.) (very productive with ca. 40 fruits per plant, with two fruits per axil); a white eggplant (*Solanum melongena* L.); two populations of chard (*Beta vulgaris* L. var. *cicla* L. s.l.) cultivated for their leaves, with variable stem colour (white and red) showing introgression with semi-wild beets (Hammer et al. 1987), a population of which was also sampled; a cultivated lettuce (′Romana′ type) and its wild relative ′willow-leaf lettuce′ (*Lactuca saligna* L.); and a rare accession of black mustard (*Brassica nigra* (L.) Koch in Röhling) that local people use for its leaves, both fresh and cooked, even though this plant is more commonly cultivated for its seeds, used as a spice.

Among the other crops reported in Table 1, the most important are a relic accession of flax (*Linum usitatissimum* L. convar. *usitatissimum*) together with a sample of its wild progenitor (*L. usitatissimum* subsp. *angustifolium* (Huds.) Thell.), and a wild population of calamint (*Calamintha nepeta* (L.) Savi) used at Ithaca as condiment. Recently, it has been described that in some Mediterranean areas this plant is undergoing a domestication process that might still involve introgression from wild plants surrounding the cultivation area (Hammer et al. 2005).

Kefalonia

Fifty-nine accessions were collected (1 cereal, 8 legumes, 42 vegetables and 11 other crops) (Table 1).

Only one accession of a six-rowed barley, escaped from cultivation, was found on the side of a country road, but no other landraces of cereals could be collected.

Three populations of grass pea, here locally also called ′psarra′, were collected. These are characterized by round, light or dark seeds; curiously, the flowers of one accession are also used for salad. A typical small local lentil (′fachì molùs′) together with two high-yield landraces of broad beans (*V. faba* L. var. *faba*) were gathered; according to local farmers, one type of these broad beans is broomrape (*Orobanche* spp.) resistant. From a family garden an old variety of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) and one of asparagus bean (*V. unguiculata* subsp. *sesquipedalis* (L.) Verdc.), mainly utilized as green pods, were sampled.

Among the vegetables collected on Kefalonia, tomato showed the widest variation from a phenotypic point of view. Some of the tomato landraces still cultivated, such as the so-called ′comidori′ (a variable pointed type used to prepare concentrate) and ′pomodori′ (a flat ′Roma′ type), were introduced by Venetians ca. 300 years ago; indeed, those names are of Italian linguistic root (pomidori or pomodori in Italian). Some other uncommon local varieties of tomato were found, such as ′coriatico seariufeliatosma′; ′gresa′ (characterized by big fruits with some splitting of fruits, and very high flower fertility); a type with a very irregular shape; an old round-shaped variety, used for stuffed tomatoes and very tasty if fried when still green; also very particular was an orange tomato characterized by seed pre-germination in the fruit, which is not much appreciated by local farmers and undergoing rapid elimination from cultivation.

Two samples of onion (*Allium cepa* L.), with large, flat and white bulbs, and garlic (*Allium sativum* L.), named ′rischianico′ and reported as rust resistant, were collected in the north of the island together with three typical ′blito′ (*Amaranthus cruentus* L. and *A. retroflexus* L.). Three accessions of local parsley (*Petroselinum crispum* (Mill.) Nym. called ′maidanò′) and celery (*Apium graveolens* L., named ′selino′, with small leaves and much aroma) were collected. Three local melons (*Cucumis melo* L.) were also sampled: the ′smirneco′, a type with very small fruits, yellowish rind and greenish pulp, not good for storage, and the ′cassada′, with a yellow-green rind and white-greenish pulp, usually sown in March and harvested in August, that is traditionally lopped to stimulate production of a higher number of fruits. Two accessions of the water melon (*Citrullus lanatus* (Thunb.) Matsumara & Nakai) ′carputi′ with dark bark, five of summer squash (*Cucurbita maxima* Duch. ex Lam. called ′fragkalokyfo′), cucumber, zucchini, radish (*Raphanus sativus* L., a small and red type called ′cochinogu′) and the ornamental (more rarely also eaten for its hot taste) ′bishops crown′ (*Capsicum baccatum* L.), were collected in the southern part of the island. In other sites closer to the sea, the following accessions were sampled: wild fennel ′marafò′ (*Foeniculum vulgare* Mill.), wild beet (*Beta maritima* L.), 4 beets (*Beta vulgaris* L.) cultivated for roots and leaves, curly lettuce ′maruli sgurò′ (*Lactuca sativa* L.), two samples of red carrots (*Daucus carota* L.), cauliflower ′mapà′ (*Brassica oleracea* L. var. *botrytis* L.), one accession of ′Italian turnip broccoli′ (*Brassica rapa* L. em. Metzg. ssp. *rapa*) and two very hot black mustards (*Brassica nigra* (L.) Koch in Röhling). In five family gardens, an equal number of accessions of basil (*Ocimum basilicum* L.) were collected; this germplasm was very variable for leaf colour (green, red), size (small, medium and large) and shape. Among other aromatic crops traditionally used on Kefalonia, one accession of anise (*Pimpinella anisum* L.) and two of origanum (*Origanum vulgare* L., cultivated starting from wild plants) were gathered. A sample of the forage and model legume *Medicago truncatula* Gaertn. was also collected, because in recent years IGV is dedicating a special section of its germplasm collection to the model plants (Maruca et al. 2006).

During the mission, as the occasion arose, some traditional medicinal plants were also collected, such as the typical climbing 'aurogna' (still under classification), whose red fruits are used 'to clean the blood'.

Conclusions

In conclusion, in these first three Ionian Greek islands visited, it was found that many crops are today cultivated at an industrial scale, but valuable landraces are still present and are being used by the local people. After this collecting mission, another one should be carried out on Corfu, Kythira, Zakynthos and Paxi to complete the investigation.

Additional data and details about the present collecting mission are reported in the exploration registers stored and available through IGV and GGB.

Availability of germplasm

The material collected is being stored at the Greek Gene Bank of Thermi-Thessaloniki, and duplicated at the IGV of Bari, for further classification and characterization. After its multiplication, small seed samples of this germplasm will be available for distribution to scientists.

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Ex situ and *in situ* management of wild and weedy rice in Nepal using a geographical information system

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Summary

Ex situ and *in situ* management of wild and weedy rice in Nepal using a geographical information system

The study describes distribution of wild and weedy rice in Nepal, and analyses the collection and conservation status of these genetic resources to help identify priorities for further collection. Using 144 accessions collected from farmland and natural habitats, mapping and diversity analyses were performed using DIVA-GIS and FloraMap. Over 50% of the accessions were *Oryza sativa f. spontanea*, while about 28% were *O. rufipogon*. Only a single accession was *O. officinalis*. Two districts (Rupandehi and Kapilvastu) showed the highest level of diversity, and these are considered, along with locations at the extremes of latitude and altitude, as targets for *in situ* conservation. The study suggests that further collections for *ex situ* conservation purposes should be made from the far western region, the high altitude site (Palpa), remote areas, the northernmost limit area, and extreme sites in terms of temperature, rainfall and soil type.

Key words: conservation, geographical information system (GIS), germplasm collection, Nepal, wild rice, weedy rice

Résumé

Gestion *ex situ* et *in situ* au Népal d’espèces sauvages de riz et adventices apparentées à l’aide d’un système d’information géographique

L’étude décrit la distribution au Népal d’espèces sauvages de riz et adventices apparentées et présente une analyse de la collection et l’état de conservation de ces ressources génétiques afin d’identifier des priorités pour les futures collectes. En utilisant 144 accessions collectées dans des zones cultivées et des habitats naturels, une cartographie et des analyses de diversité ont été effectuées à l’aide du SIG DIVA et de FloraMap. *Oryza sativa f. spontanea* représente plus de 50 % des accessions et *O. rufipogon,* près de 28 %. *O. officinalis* n’est représenté que par une seule accession. Le degré de diversité le plus élevé est observé dans deux districts (Rupandehi et Kapilvastu). Ils sont considérés pour cette raison et du fait de leur situation à des latitudes et altitudes extrêmes, comme des cibles pour une conservation *in situ*. L’étude suggère d’entreprendre d’autres collectes destinées à une conservation *ex situ* dans la région le plus à l’ouest, au site d’altitude extrême (Palpa), dans des régions isolées, dans la région à la limite la plus septentrionale et dans des sites extrêmes en termes de température, précipitations et type de sol.

Resumen

Ordenamiento *ex situ* e *in situ* de arroz silvestre y arroz enmalezado en Nepal, empleando un sistema de información geográfica

Este estudio describe la distribución de arroz silvestre y arroz enmalezado en Nepal, y analiza el estado en que se encuentra la recolección y conservación de esos recursos genéticos a fin de establecer criterios de prioridad para su ulterior recolección. Se llevaron a cabo análisis de diversidad y mapeos de 144 accesiones recolectadas en granjas y hábitats naturales empleando DIVA-GIS y FloraMap. Más del 50% de las accesiones eran *Oryza sativa f. spontanea*, mientras que un 28% eran *O. rufipogon*. Solo una accesión era *O. officinalis*. El mayor nivel de diversidad se registró en dos distritos (Rupandehi y Kapilvastu), que junto con las ubicaciones extremas en latitud y altitud se consideran como puntos adecuados para la conservación *in situ*. El estudio considera que se deberían realizar ulteriores recolecciones destinadas a la conservación *ex situ* en la región occidental más lejana, el sitio de gran altura (Palpa), áreas remotas, la zona del extremo límite norte y sitios extremos en términos de temperatura, precipitaciones y tipo de suelo.

Introduction

Nepal lies on the southern slope of the Hindukush-Himalayan region between 26°22′N and 30°27′N, and 80°4′E and 88°12′E, with elevations ranging from 60 to 8848 masl. Three agro-ecological zones have been defined: mountain, mid-hill and *tarai*. Rice is a staple food crop, and is cultivated up to 3050 masl (Shahi and Hue 1979; Mallick 1981/82), the highest recorded altitude for rice cultivation in the world.

Two cultivated and over 20 wild rice species are widely distributed in the pantropics and subtropics (Lu 1998). The Asian cultivated rice *Oryza sativa* originated in South and Southeast Asia (Chang 1985), whereas the African cultivated rice, *O. glaberrima,* was domesticated in western Africa. Four wild species, *O. granulata, O. nivara, O. officinalis* and *O. rufipogon*, one weedy rice, *O. sativa f. spontanea,* and two wild relatives: *Hygroryza aristata* and *Leersia hexandra* have been recorded in the tropical to subtropical regions of Nepal (Shrestha and Vaughan 1989; Shahi 1999; Shrestha and Upadhyay 1999; Shrestha 2002; Upadhyay and Joshi 2003). *Oryza rufipogon* has been found in the Bulbule Park of Birendra Nagar at 28°56′N and in Tansen, Palpa, at an altitude of 890 masl, which represent, respectively, the northernmost limit and the highest altitude for *O. rufipogon* (Shahi 1999; Sthapit 1999; Shrestha and Upadhyay 1999). The role of wild rice in crop improvement is well understood (Heinrichs et al. 1985; Shahi 1995; Sitch et al. 1989), but to date, only one wild rice

(*O. nivara*) features as an ancestor of Nepali rice cultivars (Joshi 2004). Exploration and conservation of wild rice was initiated in Nepal in 1978 (Shrestha and Upadhyay 1999).

Geographical information systems (GIS) are designed to facilitate the integration and analysis of geographically referenced data (Mallawaarachchi et al. 1996). Its usefulness for plant genetic resources management has been recognized by a number of studies (e.g. Jarvis et al. 2002; Aspinall and Mathews 1994; Hijmans and Spooner 2001; Huaccho and Hijmans 2000; Greene et al. 1999a, b; Pollak and Corbett 1993; Ravikanth et al. 2002; Ganeshaiah et al. 2002; Guarino et al. 2002). The mapping and spatial analysis of genebank data allowed by GIS, are valuable for the development of long-term *in situ* and *ex situ* conservation strategies. The objectives of the present study were to describe the distribution of wild and weedy rice in Nepal and to analyse the collection and conservation status of these genetic resources to help to identify priorities for further collection.

Materials and methods

The Agriculture Botany Division (ABD) under the Nepal Agricultural Research Council (NARC) has 144 accessions, with passport descriptors, of wild rice, weedy rice and wild relatives of rice stored in a medium-term genebank. The materials were collected by four exploration missions between 1988 and 1999. Only 74% of the accessions have longitude and latitude of collection, but all have locality names. These names have been verified (Shrestha and Vaughan 1989; Shahi 1999; Shrestha and Upadhyay 1999; Joshi 2004). Mapping and diversity analysis was performed using DIVA-GIS (see http://www.diva-gis.org/).

During the mapping of collection sites by DIVA-GIS, four accessions appeared to be located in India. This might be due to in typing error in latitude or longitude during data entry. We therefore corrected those accession points using DIVA-GIS. We used FloraMap (see http://www.floramap-ciat.org/) to map the probability of occurrence of wild or weedy rice. FloraMap uses a PCA analysis of the climate data associated with the collection sites to predict occurrence in all sites (pixels). Five principal components were included that explained a total variance of 99.57%. Uses and assumptions of FloraMap and DIVA-GIS were described by Jarvis et al. (2002), Jones et al. (2002) and Hijmans et al. (2001). Based on the collection sites and probability of occurrence of wild and weedy rice, further potential collections sites were identified.

Results

The altitude range for the collection is 100 to 1640 masl (Figure 1), but the majority of the accessions were collected at sites below 300 masl. The weedy rice accessions derived from sites at about 150 masl. The collection ranged in latitude from 26° 22′ 38.81″N to 28° 35′ 00″N, and in longitude from 81° 16′ 06.1″E to 88° 00′ 00″E. The collection involved 89 sites (Figure 2), concentrated in the lower belt of eastern, central, western and mid-western Nepal. Soil type at the collection site was not available for 93 accessions, and of the remainder, only loamy and sandy soils are noted.

Wild rice and wild relatives of rice are found in 29 of the 75 districts, but the study collection covered only 24 districts (Table 1). Most of the collection sites were in road-accessible districts, primarily in the *tarai* and mid-hill zones. Weedy rice is present in most of the rice growing areas. The most frequently observed species are *O. sativa f. spontanea* and *O. rufipogon*. A small number of districts feature many species, but most districts have only a few species. As a proportion of the overall collection, 16% of accessions were from the Banke and Kapilbastu districts, and 12% from Rupandehi. The highest number of species was found in Rupandehi and Kapilvastu districts. The topography of the collection site for 92 accessions was not known, but of the remainder, 1 accession is from mountainous, 21 from level plain, and 30 from swampy areas. Of the 48% of the collection for which this was recorded, 19% were collected from wild habitats. Four wild rice species, *O. granulata, O. nivara, O. officinalis* and *O. rufipogon*, one wild relative, *L. hexandra,* and weedy rice were collected and conserved. Over 50% of the collection consists of *O. sativa f. spontanea*, and 28% *O. rufipogon*. Just one

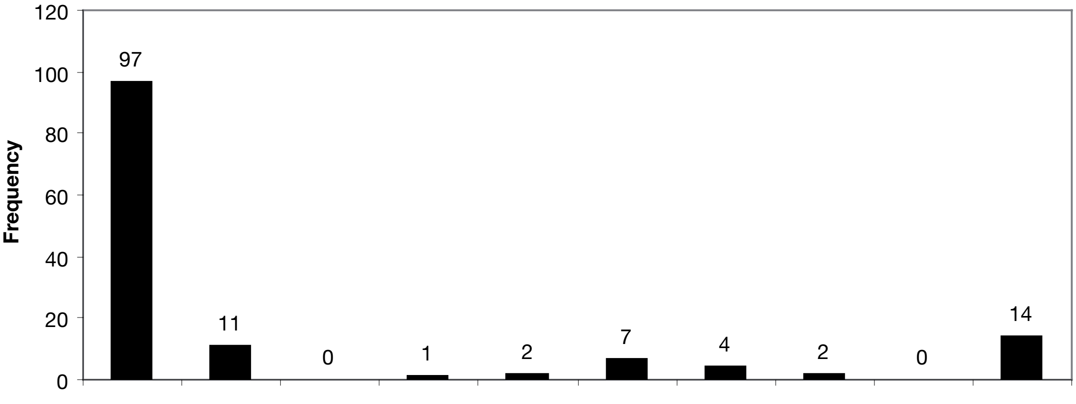

Figure 1. Frequency of wild and weedy rice collections based on altitude (altitude of 6 collections was not known).

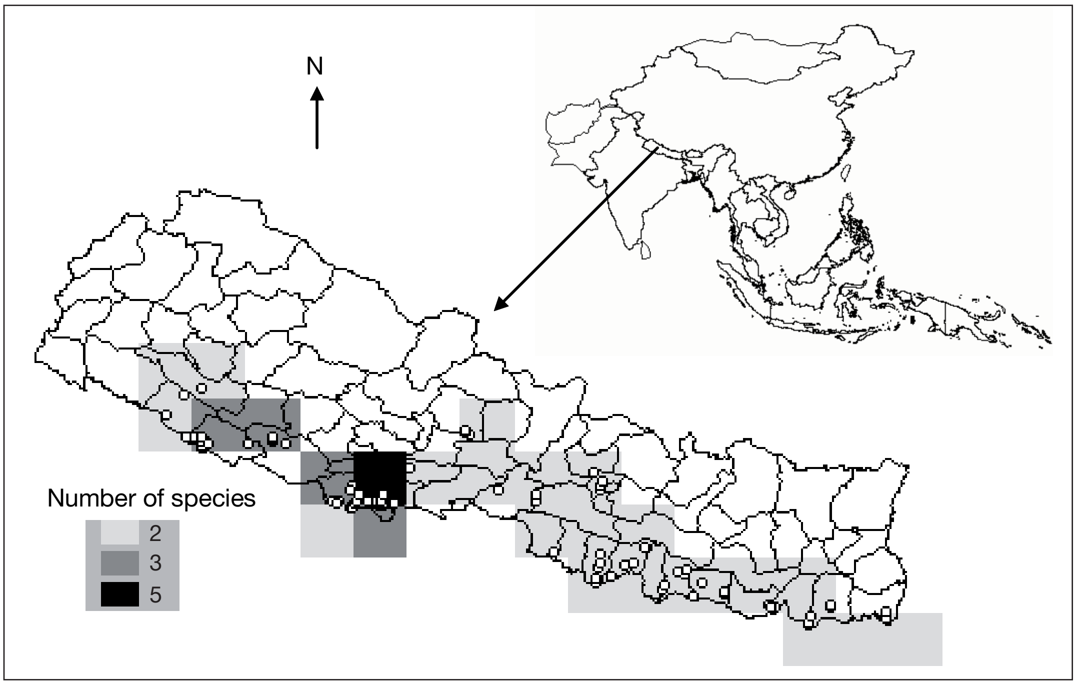

Figure 2. Collection sites and mapping species richness area of wild and weedy rice species in Nepal (white circles are collection sites).

accession is *O. officinalis* (Table 1). Farmers have 16 different local names for wild rice (Table 2), of which Jhara (15%) and Tinni (9%) are the most common. Most of the local names relate to shattering and God. There are eight different local names for a single weedy rice.

The probability of matching sites, i.e. the probability of occurrence of wild and weedy rice based on these 89 observations, are identified, which will be very useful for further exploration. Sites most matching these collection areas have been found in far western Nepal and in the northern part of India (Figure 3). Even so, *O. rufipogon* was not found in far western Nepal during exploration, but the possibility of *O. officinalis* in this region is high.

Discussion

Farmers generally distinguish wild and weedy rice by their local name. The most common local name for *O. rufipogon* is Tinni in western Nepal and Oiri in eastern Nepal, while *O. nivara* is called Tinna and the weedy types Jharanga. These names are determined by locality, farmer group and language, as well as by morphological type. Different names have been used by farmers for the same species across the country. This may be due to variation in ethnic group and language. The diverse local names given by Nepalese farmers reflect the diversity of the wild and weedy rices in Nepal.

Oryza rufipogon was less common in central and eastern Nepal than in mid-western and western Nepal, while *Oryza nivara* was uncommon in central and eastern Nepal. Weedy rice was very common in the Kathmandu valley, and *tarai* areas of the central and eastern Nepal. Significant diversity was reported among the *O. sativa f. spontanea* populations (Shrestha and Upadhyay 1999; Shrestha 2002). Diversity studies by Shahi (1999), Shrestha and Upadhyay (1999) and Shrestha (2002) suggest that the *tarai* region is the centre of diversity for wild and weedy rice species. Dr D.A. Vaughan says the Ajigara Lake, which lies in the *tarai* region, is heaven for wild rice, *O. nivara* and O. *rufipogon* (Shrestha 2002). These diversities are to some extent being maintained because the habitats for wild rice in Nepal are less disturbed compared with many other countries.

Some of the collecting localities are far outside the areas considered likely to contain wild rice by FloraMap. This may be due to variation in adaptability traits of wild rice found in Nepal and the great geographical variation in Nepal. Climatically, western Nepal is dry and receives low rainfall compared with eastern Nepal. Equally, factors considered in FloraMap might not account for possible environmental parameters, at least in the case of wild rice in Nepal.

These probability-matching sites, i.e. lower parts of western, mid- and far western Nepal, are suitable for either exploration or introduction of these wild rice species, because the probability of occurrence of wild and weedy rice in these areas are very high. Introducing the pre-existing species in western Nepal, particularly in National Parks, could be an effective tool for conservation. For *in situ* conservation, the diversity-rich Kapilvastu and Rupandehi districts, and the extreme latitude (Bulbule Park of Birendra Nagar) and altitude (Palpa district) sites should be considered. Protected areas for wild rice, such as the Lumbini Garden and Chitwan National Park, offer a low cost *in situ* conservation strategy. In addition, other National Parks could be allowed to grow these economically important plant genetic resources (wild and wild relatives) for long-term *ex situ* conservation. Public areas, ponds and lakes, where large populations of wild species exist, are good targets for *in situ* conservation. Particular

Table 1. Frequency of wild and weedy rice collections based on district, local name and species in Nepal

District	Freq.	%	Local name	Freq.	%	Species	Freq.	%
Banke	23	16.0	Adara	2	1.4	*O. granulata*	2	1.4
Bara	1	0.7	Anga	1	0.7	*L. hexandra*	3	2.1
Bardiya	3	2.1	Bagar	4	2.8	*O. nivara*	24	16.7
Chitwan	3	2.1	Chhita	1	0.7	*O. officinalis*	1	0.7
Dang	6	4.2	Jhara	21	14.6	*O. rufipogon*	40	27.8
Dhanusha	8	5.6	Jharang	2	1.4	*O. sativa f. spontanea*	74	51.4
Ilam	1	0.7	Jharanga	2	1.4			
Jhapa	1	0.7	Khera	1	0.7			
Kapilvastu	23	16.0	Nabo/Thimaha	6	4.2			
Kaski	4	2.8	Oiri	6	4.2			
Kathmandu	4	2.8	Parwal	1	0.7			
Lalitpur	3	2.1	Ram dhan	1	0.7			
Makwanpur	1	0.7	Singra	1	0.7			
Morang	8	5.6	Sintharo	1	0.7			
Nuwakot	7	4.9	Tinna	5	3.5			
Palpa	2	1.4	Tinni	13	9.0			
Parsa	1	0.7	Tumba	2	1.4			
Rautahat	8	5.6	Not known	74	51.4			
Rupandehi	17	11.8						
Saptari	5	3.5						
Sarlahi	5	3.5						
Siraha	6	4.2						
Sunsari	3	2.1						
Surkhet	1	0.7						
Total	144	100	Total	144	100	Total	144	100

Table 2. Wild rice species and the local names used by Nepalese farmers.

Species	Local name	Meaning	District
O. rufipogon	Chhita	Name only	Palpa
	Jhara	Shattering	Kapilbastu, Dang
	Jharanga	Shattering rice	Kailali
	Nabo	Grown automatically	Kaski
	Naga	Name only	Kaski
O. nivara	Tinni	Name of female	Rupandehi, Kapilbastu, Banke
	Ban dhan	Forest rice	Kanchanpur
	Jhara	Shattering	Kapilbastu, Dang
	Jharanga	Shattering rice	Bardiya, Kailali
	Tinna	Name of a male	Rupandehi, Kapilbastu, Banke
O. sativa f. spontanea	Jhara	Shattering rice	Kapilbastu, Dang
	Jharanga	Shattering rice	Rupandehi
	Jharuwa dhan	Shattering rice	Kanchanpur
	Ram dhan	Rama rice [given by Lord]	Banke
	Talani dhan	Lake rice	
	Tinna	Name of a male	Rupandehi
	Tinni	Name of a female	Rupandehi
	Tumba	Shattering rice	Rupandehi
O. granulata	Sitarani dhan	Lord Queen Sita rice	Makwanpur

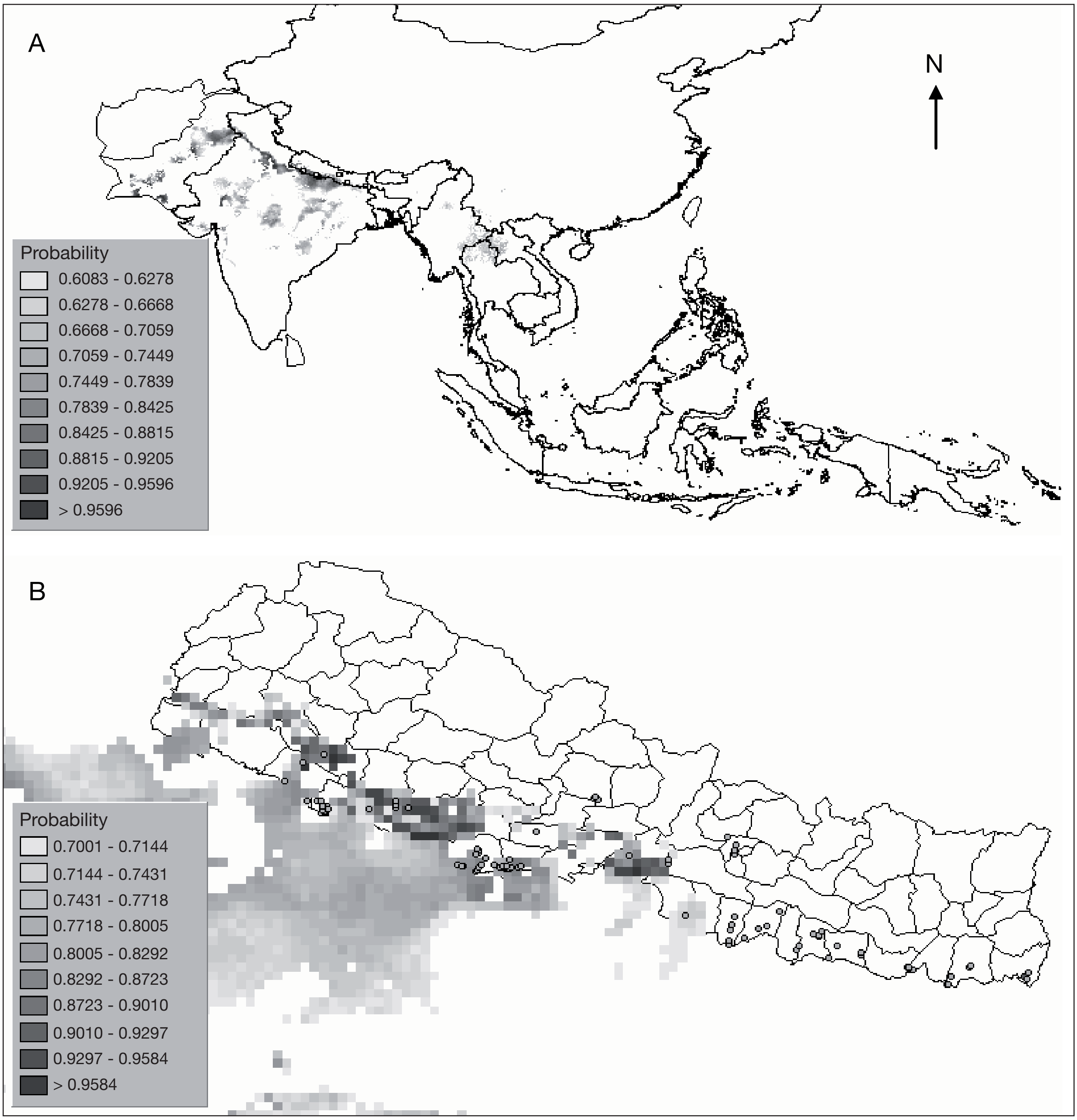

Figure 3. Probability distribution of wild and weedy rice in Asian countries (A) and Nepal (B).

examples of these sites are Ajigara Lake (Kapilvastu district) and Koshi River (Morang district). Shrestha and Upadhyay (1999) have proposed the Pokhara Valley lakes as an *in situ* conservation site for wild rice, *O. nivara* and *O. rufipogon*. *O. officinalis* was not found in the area from where the collection originated done (Shrestha and Upadhyay 1999), therefore the one accession of *O. officinalis* conserved *ex situ* should be multiplied and returned to its original collection site (Kapilvastu) for *in situ* management. A high priority should be given to *O. officinalis, O. granulata* and *L. hexandra,* of which only 1, 2 and 3 accessions, respectively, are conserved.

Conclusions and recommendations

In these collecting missions, only the districts near to roads were explored. Remote areas, in terms of road accessibility, should also be studied. During the collection, soil and climatic data should be reported. *Oryza officinalis* needs to be further explored in far western Nepal. In addition to this, considerable morphological variation within these species exists in different localities and habitats. Therefore, a study on economically important traits is necessary and should be linked with GIS for better utilization and conservation of wild rice genetic resources. Utilization of these species should be strengthened and their contribution to rice crop improvement should be popularized for effective wild genepool management.

The habitats for wild rice have deteriorated significantly because of human population growth and development activities (Shrestha and Upadhyay 1999; Shahi 1999). If no immediate action is taken for the protection of the wild rice species, then major wild rice populations in central and eastern Nepal will be extinct in the near future. Major threats are land encroachment, draining of swampy areas, and conversion of swampy areas to cultivated land. Therefore, specific matching sites for each wild rice species should be identified on government farms using GIS for *ex situ* conservation of these wild and weedy rice species. These farms should have a strategy to manage these species *ex situ* within the farm. In other words, part of these farms should be allocated to grow suitable wild rice and be maintained as a natural habitat. The sites most matching the ecological criteria, such as in the far western region, should be studied and particular sites for *in situ* conservation identified.

This study suggest that collections should be made from the far western region, the highest altitude site (Palpa), areas located remote from highways, northernmost limit area i.e. Bulbule Park, and extreme sites in term of temperature, rainfall and soil type. Wild rice in some of these areas is endangered, e.g. *O. rufipogon* in Bulbule Park is almost extinct because of increased urbanization and human activities. Identification of potential areas for wild rice collecting, exploration missions and screening of duplicates are some actions that should be immediately carried out. In addition, areas and species at risk should be assessed and prioritized accordingly for *in situ* and *ex situ* conservation.

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