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Cover: A community seed bank in Bara, Nepal. Photo: A. King/IPGRI.

Couverture : Une banque des semences de la communauté de Bara, Nepal. Photo: A. King/IPGRI.

Portada: Un banco de semillas de la comunidad en Bara, Nepal. Foto: A. King/IPGRI.

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International Treaty on Plant Genetic Resources for Food and Agriculture

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Introduction

The International Treaty on Plant Genetic Resources for Food and Agriculture entered into force in June 2004.

Agricultural biological diversity, or more specifically, genetic resources for food and agriculture, is the storehouse that provides humanity with food, clothes and medicines. It is essential in the development of sustainable agriculture and food security.

Matters related to the conservation and sustainable use of genetic resources and the management of related biotechnologies may appear to be technical, but they have strong socioeconomic, political, cultural, legal and ethical implications, in that problems in these fields could put at risk the future of humanity.

According to present estimates, food production in developing countries will have to increase more than 60% in the next 25 years just to keep pace with population growth. The possibilities for expanding the areas used for terrestrial and aquatic farming are relatively limited and most of the world's wild fish stocks are already overexploited. Production must therefore be intensified, productivity increased and productive natural systems must be optimally managed all in a sustainable manner. This will require the combined application of new and old biotechnologies, including innovative approaches to plant and animal breeding and to farming practices. Success in this endeavour will depend on the sustainable utilization of a broader range of species, and of the genetic material within each species, including genes from the wild relatives of domesticated species.

In spite of its vital importance for human survival, agricultural biodiversity is being lost at an alarmingly increased rate. It is estimated that some 10 000 species have been used for human food and agriculture. Currently no more than 120 cultivated species provide 90% of human food supplied by plants, and 12 plant species and 5 animal species alone provide more than 70% of all human food. A mere 4 plant species (potatoes, rice, maize and wheat) and 3 animal species (cattle, swine and chickens) provide more than half. Within the so-called 'main food species', a tremendous loss of genetic diversity has occurred in the last present century. Hundreds of thousands of farmers' heterogeneous plant varieties and landraces that existed, for generations, in farmers' fields until the beginning of the 20th century, have been substituted by a small number of modern and highly uniform commercial varieties. In the USA alone, more than 90% of fruit trees and vegetables that were grown in farmers' fields at the beginning of the 20th century can no more be found and only a few of them are maintained in genebanks. Similar alarming figures can be given for the genetic erosion of domestic animal breeds and varieties. The picture is much the same throughout the world. The loss of agricultural biological diversity has drastically reduced the capability of present and future generations to face unpredictable environmental changes and human needs.

The rapid process of globalization and economic integration is creating an increasing number of situations where nations and regions are interdependent. This can raise important ethical questions. One of the oldest forms of interdependence, going right back to the spread of crops from their centers of origins since the Neolithic, is in relation to agrobiodiversity. Appendix 1 shows the origins of cultivated plants, and their regions of diversity.

In general, we can say that no country on the planet is today self-sufficient with respect to the genetic resources for food and agriculture they are using, and the average degree of interdependence among countries with regard to the most important crops is 70%. Paradoxically, the countries which are poorest from the economic point of view, and which are in general located in tropical or subtropical zones, are also richest in terms of genetic diversity needed to ensure human survival. International cooperation should lead to a more fair and equitable sharing of the benefits derived from the use of genetic resources, providing an essential incentive to ensure that countries continue developing, conserving and making available to humanity their genetic diversity.

There is also interdependence between generations. Agricultural biodiversity is a precious inheritance from previous generations, which we have the moral obligation to pass on, intact to coming generations to help them to face unforeseen needs and problems. However, up to now, the interests of future generations, who neither vote nor consume, have not been adequately taken into account by our political and economic systems.

It is the inescapable responsibility of our generation to develop ethical solutions to the problems and implications mentioned above, within an overall political framework to allow and equitable sharing of benefits for all countries, and ensure food and agriculture for future generations. For this task, the United Nations, as universal inter-governmental forum, has a fundamental role to play in the facilitation of the necessary inter-governmental negotiations.

In the 1970s, worldwide systematic actions began within the Food and Agriculture Organization of the United Nations (FAO) resulting in the establishment, in 1983, of the first permanent inter-governmental forum on this subject: The Commission on Genetic Resources for Food and Agriculture (CGRFA), which is currently composed of 164 Member Nations and the European

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Community. This forum made possible in the 1980s the negotiation and development of an International Undertaking on Plant Genetic Resources which recognized Farmers' Rights as being complementary to Plant Breeders' Rights. Farmers' Rights were adopted by the FAO Commission in 1999 as "rights arising from the past, present and future contributions of farmers in conserving, improving, and making available plant genetic resources, particularly those in the centers of origin/diversity", with the aim of allowing "farmers, their communities, and countries in all regions, to participate fully in the benefits derived, at present and in the future, from the improved use of plant genetic resources". The members of the Commission then negotiated a revision of the International Undertaking in harmony with the Convention on Biological Diversity (CBD), which allows the regulation of access to genetic resources for food and agriculture, the fair and equitable sharing of the benefits derived from their use and the realization of Farmers' Rights. This international agreement, which is binding, was adopted by consensus by the FAO Member Nations on 3 November 2001, with the name of International Treaty on Plant Genetic Resources for Food and Agriculture.

The uniqueness of plant genetic resources for food and agriculture

In the long negotiating process to develop and agree on the International Treaty on Plant Genetic Resources for Food and Agriculture, countries took into consideration the fact that plant genetic resources for food and agriculture differ substantially from other plant genetic resources, and therefore that specific solutions—which are not necessarily similar to those required for other kinds of biodiversity—were needed for their conservation and development, as well as for their availability, and the fair and equitable sharing of the benefits derived from their use. Among these differences are the following:

1. They are essentially *man-made*, that is, biological diversity developed and consciously selected by farmers since the origins of agriculture, who have guided the evolution and development of these plants for over 10 000 years. In recent times, scientific plant breeders have built upon this rich inheritance. Much of the genetic diversity of cultivated plants can only survive through continued human conservation and maintenance.
2. They are not randomly distributed over the world, but rather concentrated in the so-called "centres of origin and diversity" of cultivated plants and their wild relatives, which are largely located in the tropical and sub-tropical areas (see Appendix 1).
3. Because of the diffusion of agriculture all over the world, over the past 10 000 years, and because of the association of major crops with the spread of civilizations, many crop genes, genotypes and populations have spread, and continue to develop, all over the planet. Moreover, plant genetic resources for food and agriculture have been systematically and freely collected and exchanged for over 200 years, and a large proportion have been incorporated in *ex situ* collections.[2]
4. There is much greater inter-dependence among countries for plant genetic resources for food and agriculture than for any other kind of biodiversity (see Appendix 1). Continued agricultural progress implies the need for continued access to the global stock of plant genetic resources for food and agriculture. No region can afford to be isolated, or isolate itself, from the germplasm of other parts of the world.

For such reasons, the second session of the Conference of the Parties to the Convention of Biological Diversity, in 1995, adopted decision II/15, "recognizing the special nature of agricultural biodiversity, its distinctive features and problems needing distinctive solutions". The Conference of the Parties also supported the negotiations for the International Treaty on Plant Genetic Resources for Food and Agriculture, in order to provide such solutions.

The International Treaty on Plant Genetic Resources for Food and Agriculture

On 3 November 2001, the Thirty-first Session of the Conference of the FAO adopted, by consensus and as a binding international agreement, the International Treaty on Plant Genetic Resources for Food and Agriculture. Only two and a half years later, the Treaty entered into force on 29 June 2004 and the first meeting if its Governing Body can now be convened.

The Treaty is the outcome of many years of intense negotiations in FAO's inter-governmental Commission on Genetic Resources for Food and Agriculture, to revise the voluntary International Undertaking on Plant Genetic Resources for Food and Agriculture. As the Thirtieth Session of the Conference of the FAO noted, in 1999 these negotiations were at the meeting point between agriculture, the environment and commerce, and agreed that there should be consistency and synergy in the agreements being developed in these different sectors.

The Treaty is available at http://www.fao.org/ag/cgrfa/itpgr.htm#text.

Article 1 states that the Treaty's objectives are *"the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security"*. Article 3 establishes that its scope *"relates to plant genetic resources for food and agriculture"*.

Article 9, for the first time in a binding international agreement, makes provision for Farmers' Rights, in recognition of the collective innovation on which agriculture is based, as follows:

[2] These collections were made before the entry into force of, and hence outside the Convention on Biological Diversity, as Resolution 3 of the Nairobi Conference for the Adoption of the Agreed Text of the Convention on Biological Diversity recognized.

"9.1 The Contracting Parties recognize the enormous contribution that the local and indigenous communities and farmers of all regions of the world, particularly those in the centres of origin and crop diversity, have made and will continue to make for the conservation and development of plant genetic resources which constitute the basis of food and agriculture production throughout the world.

"9.2 The Contracting Parties agree that the responsibility for realizing Farmers' Rights, as they relate to plant genetic resources for food and agriculture, rests with national governments. In accordance with their needs and priorities, each Contracting Party should, as appropriate, and subject to its national legislation, take measures to protect and promote Farmers' Rights, including:

(a) protection of traditional knowledge relevant to plant genetic resources for food and agriculture;

(b) the right to equitably participate in sharing benefits arising from the utilization of plant genetic resources for food and agriculture; and

(c) the right to participate in making decisions, at the national level, on matters related to the conservation and sustainable use of plant genetic resources for food and agriculture.

"9.3 Nothing in this Article shall be interpreted to limit any rights that farmers have to save, use, exchange and sell farm-saved seed/propagating material, subject to national law and as appropriate."

A further important and innovative part of the Treaty is contained in Articles 10 to 13 (Part IV), which establish a Multilateral System of Access and Benefit-sharing, which applies to a list of crops in Annex I to the Treaty, established according to criteria of food security and interdependence. The crops in question cover about 80% of the world's food calorie intake from plants. In accordance with Article 11.2, Contacting Parties will bring into the Multilateral System all such resources that are under their management and control and in the public domain, and will, in accordance with Article 11.3, encourage natural and legal persons within their jurisdiction to include the resources they hold in the Multilateral System. Article 11.5 provides that the plant genetic resources for food and agriculture listed in Annex I and held in the *ex situ* collections of the International Agricultural Research Centres of the Consultative Group on International Agricultural Research (CGIAR) will also be brought into the Multilateral System, by agreements which they are invited to sign with the Treaty's Governing Body, in accordance with Article 15.

Article 12.3a provides that *"Access shall be provided solely for the purpose of utilization and conservation for research, breeding and training for food and agriculture, provided that such purpose does not include chemical, pharmaceutical and/or other non-food/feed industrial uses"*. Article 12.3b provides that *"Access shall be accorded expeditiously, without the need to track individual accessions and free of charge, or, when a fee is charged, it shall not exceed the minimal cost involved"*. Further clauses of Article 12 provide that:

"(d) Recipients shall not claim any intellectual property or other rights that limit the facilitated access to the plant genetic resources for food and agriculture, or their genetic parts or components, in the form received from the Multilateral System;

(e) Access to plant genetic resources for food and agriculture under development, including material being developed by farmers, shall be at the discretion of its developer, during the period of its development;

(f) Access to plant genetic resources for food and agriculture protected by intellectual and other property rights shall be consistent with relevant international agreements, and with relevant national laws;

(g) Plant genetic resources for food and agriculture accessed under the Multilateral System and conserved shall continue to be made available to the Multilateral System by the recipients of those plant genetic resources for food and agriculture, under the terms of this Treaty..."

Article 12.4 provides for access to be provided

"pursuant to a standard material transfer agreement (MTA), which shall be adopted by the Governing Body and contain the provisions of Articles 12.3a, d and g, as well as the benefit-sharing provisions set forth in Article 13.2d(ii) and other relevant provisions of this Treaty, and the provision that the recipient of the plant genetic resources for food and agriculture shall require that the conditions of the MTA shall apply to the transfer of plant genetic resources for food and agriculture to another person or entity, as well as to any subsequent transfers of those plant genetic resources for food and agriculture, will be contained in a standard Material Transfer Agreement to be agreed by the governing body. Its conditions shall apply to the transfer of the resources to subsequent persons".

By Article 12.5:

"Contracting Parties shall ensure that an opportunity to seek recourse is available, consistent with applicable jurisdictional requirements, under their legal systems, in case of contractual disputes arising under such MTAs, recognizing that obligations arising under such MTAs rest exclusively with the parties to those MTAs".

Article 13 deals with benefit-sharing in the Multilateral System. By Article 13.1:

"The Contracting Parties recognize that facilitated access to plant genetic resources for food and agriculture which are included in the Multilateral System constitutes itself a major benefit of the Multilateral System and agree that benefits accruing therefrom shall be shared fairly and equitably..."

Article 13.2 states that

"The Contracting Parties agree that benefits arising from the use, including commercial, of plant genetic resources for food and agriculture under the Multilateral System shall be shared fairly and equitably through the following mechanisms: the exchange of information, access to and transfer of technology, capacity-building, and the sharing of the benefits arising from commercialization, taking into account the priority activity areas in the rolling Global Plan of Action, under the guidance of the Governing Body".

Article 13.2d deals with the sharing of monetary and other benefits of commercialization. In particular, Article 13.2d(ii) provides that:

"the standard Material Transfer Agreement referred to in Article 12.4 shall include a requirement that a recipient who commercializes a product that is a plant genetic resource for food and agriculture and that incorporates material accessed from the Multilateral System, shall pay to the mechanism referred to in Article 19.3f,[3] an equitable share of the benefits arising from the commercialization of that product, except whenever such a product is available without restriction to others for further research and breeding, in which case the recipient who commercializes shall be encouraged to make such payment.

"The Governing Body shall, at its first meeting, determine the level, form and manner of the payment, in line with commercial practice. The Governing Body may decide to establish different levels of payment for various categories of recipients who commercialize such products; it may also decide on the need to exempt from such payments small farmers in developing countries and in countries with economies in transition. The Governing Body may, from time to time, review the levels of payment with a view to achieving fair and equitable sharing of benefits, and it may also assess, within a period of five years from the entry into force of this Treaty, whether the mandatory payment requirement in the MTA shall apply also in cases where such commercialized products are available without restriction to others for further research and breeding".

Article 13.3 provides that:

"benefits arising from the use of plant genetic resources for food and agriculture that are shared under the Multilateral System should flow primarily, directly and indirectly, to farmers in all countries, especially in developing countries, and countries with economies in transition, who conserve and sustainably utilize plant genetic resources for food and agriculture".

These mandatory payments foreseen in Part IV of the Treaty form part of the Treaty's funding strategy—which is detailed in Article 18—whereby the Governing Body periodically sets a funding target, within which to mobilize funds from a wide variety of sources for agreed projects and programmes aimed particularly at farmers in all countries, especially in developing countries and countries with economies in transition, who conserve and sustainably utilize plant genetic resources for food and agriculture.

The Treaty—in Article 15—deals with *ex situ* collections of plant genetic resources for food and agriculture held by the International Agricultural Research Centres (IARCs) of the Consultative Group on International Agricultural Research (CGIAR) and other international institutions. Article 15.1 recognizes the importance to the Treaty of the *ex situ* collections of plant genetic resources for food and agriculture held in trust by the IARCs of the CGIAR, and calls upon the IARCs to sign agreements with the Governing Body with regard to such *ex situ* collections. In doing so, the IARCs undertake to make their plant genetic resources for food and agriculture of the crops listed in Annex I of the Treaty available in accordance with the provisions of the Multilateral System. Their other plant genetic resources for food and agriculture collected before the entry into force of the Treaty *"shall be made available in accordance with the provisions of the MTA currently in use pursuant to agreements between the IARCs and the FAO.[4] This MTA shall be amended by the Governing Body no later than its second regular session, in consultation with the IARCs, in accordance with the relevant provisions of this Treaty..."* The IARCs will recognize the authority of the Governing Body to provide policy guidance relating to *ex situ* collections held by them and subject to the provisions of the Treaty. Article 15.5 provides that *"the Governing Body will also seek to establish agreements for the purposes stated in this Article with other relevant international institutions".*

These provisions of the Treaty—which are very innovative—provide for facilitated access to plant genetic resources for food and agriculture and an agreed way of benefit-sharing, without deriving these benefits from individual negotiations, on a case by case basis, between the provider and the user of these resources. They provide for both access and benefit-sharing to be through multilateral arrangements. This avoids the high transaction costs that such bilateral contracts involve, which are hard to justify in the context of plant breeding, which has for thousands of years been characterized by repetitive exchange, crossing, selection and local adaptation of the intra-specific genetic resources of

[3] Article 19.3f provides that a function of the Treaty's Governing Body is to *"establish, as needed, an appropriate mechanism, such as a Trust Account, for receiving and utilizing financial resources that will accrue to it for purposes of implementing this Treaty".*

[4] These agreements are available from http://www.fao.org/ag/cgrfa/exsitu.htm. They were signed in October 1994.

crops, within and between countries and regions. This ensures that plant breeders, in both the public and private sectors, can have access to the widest possible range of the resources crucial for world food security. This will benefit consumers, by providing a stream of improved and varied agricultural products. And it will benefit the seed and biotechnology industries, by providing an agreed international framework, within which to plan their investments. It also provides a firm international framework for IARCs of the CGIAR and other international organizations, whereby they hold plant genetic resources for food and agriculture in trust, under the Treaty.

What is next?

The adoption of the International Treaty marks a milestone on international cooperation. Following its entering into force this year, the first meeting of the Governing Body is to be convened, likely in 2005.

In adopting the Treaty, the FAO Conference decided that the Commission on Genetic Resources for Food and Agriculture would act as Interim Committee for the preparation of the first meeting of the Governing Body for the Treaty. The first meeting of the CGRFA acting as Interim Committee took place in October 2002, and the second meeting is planned to take place in November 2004.

The Governing Body of the Treaty will be composed of all states that have ratified the Treaty at least 90 days before the meeting. At this meeting, the Governing Body will decide on important questions, such as the level, form and manner of monetary payments on commercialisation, the standard material transfer agreement for plant genetic resources, mechanisms to promote compliance with the Treaty, and the funding strategy. A country may therefore consider it important to ratify the Treaty soon, so as to ensure that its national interests can be taken into account at the Governing Body's first meeting.

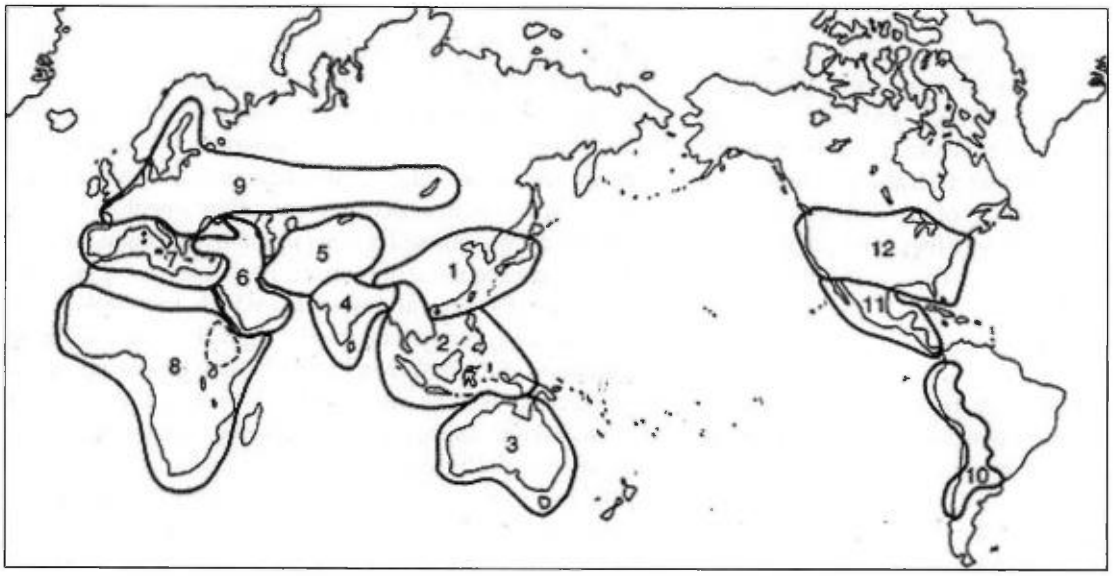
The Sixth Meeting of the Conference of the Parties to the Convention on Biological Diversity (7–19 April 2002, The Hague) in its Ministerial Declaration, which was agreed by the delegations of 176 countries, including some one 130 Ministers, also "urged all States to ratify and fully implement [...] the International Treaty on Plant Genetic Resources for Food and Agriculture". The Declaration adopted by the World Food Summit: five years later (10–13 June 2002, Rome) recognizes the importance of the International Treaty on Plant Genetic Resources for Food and Agriculture, in support of food security objectives, and calls upon countries to consider signing and ratifying the Treaty as soon as possible. In the Johannesburg Declaration on Sustainable Development, countries participating in the World Summit on Sustainable Development (26 August to 4 September, 2002), stated that "we commit ourselves to the Johannesburg Plan of Implementation", in which they "invite countries that have not yet done so to ratify the International Treaty on Plant Genetic Resources for Food and Agriculture".

An up-to-date listing of governments that have signed or ratified the Treaty is available at http://www.fao.org/Legal/TREATIES/033s-e.htm.

The Treaty will also need to be enforced at national level. The development of national legislation for implementation of its provisions will be essential in deterring genetic erosion, protecting indigenous germplasm and Farmers' Rights, facilitating access to plant genetic resources for food and agriculture, and ensuring benefit-sharing. In this endeavour, national legislations are to ensure harmony, coordination and synergy with other international agreements in all relevant sectors, especially in agriculture, the environment and trade.

In any event, it should not be forgotten that genetic erosion is but one consequence of humankind's abusive exploitation of the planet's natural resources, which has broken the balance of many ecosystems and brought about an increasing degradation of the biosphere. Safeguarding genetic resources by protecting them *ex situ* or *in situ* is crucial, if the process that has been unleashed is to be reversed, or controlled. The fundamental problem remains humankind's lack of respect for the rest of nature, and any lasting solution will have to involve establishing a new relationship with our small planet, in full understanding and recognition of its limitations and fragility. If humanity is to have a future, it is imperative that children learn this in primary schools, and that adults make it part of their life.

Appendix 1. Cultivated plants and their regions of diversity[5]



1. Chinese–Japanese Region
Prosomillet, Fox tail millet, Naked oat
Soybean, Adzuki bean
Leafy mustard
Orange/*Citrus*, Peach, Apricot, Litchi
Bamboo, Ramie, Tung oil tree, Tea

2. Indochinese–Indonesian Region
Rice
Rice bean, Winged bean
Cucurbits/Ash gourd
Mango, Banana, Rambutan, Durian, Bread fruit, *Citrus*/Lime, Grapefruit
Bamboos, Nutmeg, Clove, Sago-palm, Ginger, Taros and Yams, Betel nut, Coconut

3. Australian Region
Eucalyptus, Acacia, Macadamia nut

4. Hindustani Region
Rice, Little millet
Black gram, Green gram, Moth bean, Rice bean, *Dolichos* bean, Pigeonpea, Cowpea,
Chickpea, Horse gram, Jute
Eggplant, Okra, Cucumber, Leafy mustard, Rat's tail radish, Taros and Yams
Citrus, Banana, Mango, Sunnhemp, Tree cotton
Sesame, Ginger, Turmeric, Cardamom, Arecanut, Sugarcane, Black pepper, Indigo

5. Central Asian Region
Wheat (Bread/Club/Shot), Rye
Allium/Onion, Garlic, Spinach, Peas, Beetroot, Faba bean
Lentil, Chickpea
Apricot, Plum, Pear, Apple, Walnut, Almond, Pistachio, Melon, Grape, Carrot, Radish
Hemp/*Cannabis*, Sesame, Flax, Safflower

6. Near Eastern Region
Wheat (Einkorn, Durum, Poulard, Bread), Barley, Rye/*Secale*
Faba bean, chickpea, French bean, Lentil, Pea
Brassica oleracea, Allium, Melon, Grape, Plum, Pear, Apple, Apricot, Pistachio, Fig,
Pomegranate, Almond
Safflower, Sesame, Flax
Lupins, Medics

7. Mediterranean Region
Wheat (Durum, Turgidum), Oats
Brassica oleracea, Lettuce, Beetroot, Colza
Faba bean, Radish
Olive, *Trifolium*/Berseem, Lupins, *Crocus*, Grape, Fennel, Cumin, Celery, Linseed

8. African Region
Wheat, (Durum, Emmer, Poulard, Bread)
African rice, Sorghum, Pearl millet, Finger millet, Teff
Cowpea, Bottle gourd, Okra, Yams, Cucumber
Castor bean, Sesame, Niger, Oil palm, Safflower, Flax
Cotton, Kenaf, Coffee
Kola, Bambara groundnut, Date palm, Ensete, Melons

9. European–Siberian Region
Peach, Pear, Plum, Apricot, Apple, Almond, Walnut, Pistachio, Cherry
Cannabis, Mustard (black), Chicory, Hops, Lettuce

10. South American Region
Potato, Sweet potato, *Xanthosoma*
Lima bean, Amaranth, *Chenopodium, Cucurbita*, Tomato, Tobacco, Lupin
Papaya, Pineapple
Groundnut, Sea island cotton
Cassava, Cacao, Rubber tree, Passion fruit

11. Central American and Mexican Region
Maize, French bean, Potato, *Cucurbita*, Pepper/Chilli, Amaranth, *Chenopodium*, Tobacco,
Sisal hemp, Upland cotton

12. North American Region
Jerusalem artichoke, Sunflower, Plum, Raspberry, Strawberry.

[5] Source: Esquinas-Alcázar JT (1993) Plant Genetic Resources. In Hayward MD, Bosemark NO, Romagosa I, editors. Plant Breeding: Principles and Prospects. Chapman and Hall, London, UK, pp. 38–39. Based on Zeven and Zhukovsky (1975) and Zeven and de Wet (1982).

The Global Crop Diversity Trust: a foundation for food security

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Introduction

Perhaps the single most important goal on the development agenda is the achievement of food security for all. At the World Food Summit in 1996, the global community agreed to the target of halving the number of hungry people in the world by 2015. Since then, the right of every human being to live in a world free from hunger has been reemphasized and is enshrined in the Millennium Development Declaration that guides international cooperation today.

Crop diversity underpins food security. The hundreds of thousands of different crop varieties that have been developed over the centuries by farmers and professional plant breeders, together with the wild relatives of these crops, contain within them the genetic variability that is vital to achieving food security—the genes that will allow humanity to tailor its crops to meet new challenges and opportunities. But these priceless genetic resources are being eroded—in nature, as wild relatives succumb to environmental pressures, and in the field as farmers switch to new varieties that better meet their immediate needs. The extent of this erosion began to be widely recognized in the late 1960s and 1970s when scientists came to realize that the race against genetic erosion was a race against time. Their response was to mount collecting missions and to establish genebanks in which to conserve this vast genetic wealth.

These efforts eventually led to the establishment of almost 1500 collections of plant genetic resources around the world, comprising more than 6 million plant samples. 83% of these are maintained in national government genebanks and a further 10%, are held in trust for the world community by the Future Harvest Centres of the Consultative Group on International Agricultural Research (CGIAR)—a collection that ranks as one of the world's single most important resources for future food security.

Despite their foresight in establishing *ex situ* collections, a large number of governments failed to make sufficient provision for their ongoing financial support. Some genebanks have now closed and others are in a severe state of deterioration. Thus, alarmingly, crop diversity remains not only under threat on farmers' fields and in the wild but is also under threat in many of the genebanks that were intended to be safe havens for the future, providing long-term security removed from the threats posed by human development and modern agriculture.

Recognizing the seriousness of the situation, the Food and Agriculture Organization of the United Nations (FAO) together with the International Plant Genetic Resources Institute (IPGRI) acting on behalf of the Future Harvest Centres of the CGIAR, agreed to establish a funding mechanism that would help salvage of the world's most important collections and provide a stable source of funding to help ensure their long-term survival. This mechanism, now established as an independent international fund, is known as the Global Crop Diversity Trust.

The mission of the Global Crop Diversity Trust

The mission of the Trust is to ensure the long-term conservation and availability of crop diversity for food security worldwide. The Trust addresses two of the Millennium Development Goals: Goal 1 to *eradicate extreme poverty and hunger*, and Goal 7 to *ensure environmental sustainability*. It also responds to the calls made within a number of international agreements and conventions which recognise the need to conserve crop diversity and the critical role of *ex situ* collections:

- Convention on Biological Diversity (1992). The Trust addresses to a greater or lesser extent all three of the objectives of the Convention on Biological Diversity: the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising from the use of genetic resources.
- Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (1996). The technical framework for the Trust is provided by the Global Plan of Action which includes among its priorities the development of an efficient and effective system of *ex situ* conservation. The Trust contributes to at least half of the 20 Priority Activities of the Global Plan of Action.
- International Treaty on Plant Genetic Resources for Food and Agriculture (2001). The policy framework for the Trust is provided by the International Treaty, and the Trust operates in accordance with the overall policy guidance provided by the Governing Body of the International Treaty. The Trust serves as an element of the Treaty's funding strategy, and it is envisaged that its status will be recognized in a relationship agreement to be signed by the Executive Board of the Trust and the Governing Body of the International Treaty.

The goals of the Global Crop Diversity Trust

The goals of the Trust are to:

- promote an efficient, goal-oriented, economically efficient and sustainable global system of *ex situ* conservation in accordance with the International Treaty and the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture;
- safeguard collections of unique and valuable plant genetic resources for food and agriculture held *ex situ*, with priority

being given to those that are plant genetic resources included in Annex I to the International Treaty or referred to in Article 15.1(b) of the International Treaty;
- promote the regeneration, characterization, documentation and evaluation of plant genetic resources for food and agriculture and the exchange of related information;
- promote the availability of plant genetic resources for food and agriculture;
- promote national and regional capacity building, including the training of key personnel, with respect to the above.

The centrepiece of the Trust will be a $US260 million endowment fund, the proceeds of which will provide a permanent source of financial support to help develop and maintain efficient and effective conservation systems that will help ensure the survival of the world's most important collections. In addition, short-term funding will be provided to those collections most in need of immediate technical and capacity-building assistance.

Creating efficient and effective conservation systems

The creation of efficient and effective systems for conserving and making available genetic resources will involve not only identifying key collections but also defining responsibilities for specific conservation and distribution activities. In many cases, the sharing of such responsibilities among the various institutions concerned can be expected to lead to greater efficiencies than if each collection holder were to work alone. However, such an approach requires that appropriate mechanisms for coordination be established and shared information systems developed.

Such systems would comprise collections that:
- are widely used as a key resource for food security and sustainable development;
- are regionally and internationally important;
- together cover the major part of the genepool of the crops concerned (both cultivated species and their wild relatives);
- are viable and healthy;
- are accessible under the terms of the International Treaty with respect to access and benefit-sharing;
- are maintained by institutions committed to their long-term conservation and availability;
- are managed efficiently and effectively in accordance with appropriate scientific and technical standards;
- are well documented and the information on them is freely and widely available and continues to be developed over time;
- are housed in facilities that are adequate to ensure long-term conservation;
- are duplicated in at least one other location for safety.

Identifying collections and their needs

At least in the early years, the Trust will focus its support on the genetic resources of crops that are included in Annex 1 or referred to in Article 15.1(b) of the International Treaty. Specific strategies for the efficient and effective conservation of individual genepools of these crops are currently under development. These strategies will identify the key genetic resources to be included within the proposed conservation systems and will propose methods for their management, individually and collectively, through the sharing of responsibilities and partnerships. The strategies will identify, both regionally and on a crop basis, those collections most in need of support from the Trust.

The two approaches being followed—regional and crop strategies—are complementary: regional strategies will identify those collections that are of greatest importance to a particular region. The immediate needs of the genebanks that house these collections will be identified and priorities set for support from the Trust for upgrading and capacity building. The crop strategies on the other hand will take a global view and will identify where and how the genepool of a particular crop is distributed around the world. From this, the individual collections that together best represent the total diversity of a crop will be identified and these will be the ones given highest priority for long-term support.

The development of all the regional strategies and several of the crop strategies has already begun and it is aimed to complete all strategies by the end of 2006.

Eligibility principles and criteria for funding

The Trust has adopted, on an interim basis, four basic principles that must be met in order for a collection to be eligible for support:
- *The plant genetic resources are of crops included in Annex 1 or referred to in Article 15.1(b) of the International Treaty*
- *The plant genetic resources are accessible under the internationally agreed terms of access and benefit sharing provided for in the multilateral system as set out in the International Treaty*
- *Each holder of plant genetic resources for food and agriculture commits to its long term conservation and availability*
- *Each recipient of funds from the Trust shall undertake to work in partnership with the aim of developing an efficient and effective global conservation system*

In addition to, or to amplify these principles, the Trust has developed a set of more specific criteria to be met before a collection will be considered for long-term funding. In cases where a collection meets the principles but is currently unable to meet these more specific criteria, the Trust will consider providing support for the necessary upgrading and capacity building.

These criteria and the way in which they are applied will be kept under review and revised as needed. Initially, there are six criteria:

1. The recipient has effective links to users of plant genetic resources.
2. The collection is judged to be important within the context of an agreed regional or crop conservation strategy.

3. The legal status of the collection and holder are such that their ability to meet the eligibility principles with respect to access and benefit-sharing, and their commitment to long-term conservation are assured.
4. The recipient is willing to act in partnership with others to achieve an efficient and effective system for conserving the plant genetic resources in question and for making them available.
5. The recipient has the human resources and management systems needed to maintain the plant genetic resources and can demonstrate conformity with agreed scientific and technical standards of management.
6. The facilities in which the collection is maintained are adequate to ensure long-term conservation.

Priorities for allocating funds

While the Trust aims to develop and support the long-term maintenance of efficient and effective systems for conserving crop diversity, it clearly will be unable to provide funds for each and every collection that goes into making up these systems. The Trust will thus target its funds to helping those most in need of its support. In addition, funds for the provision of specific services and for coordination will be considered on a case-by-case basis.

In determining the extent of such support the Trust will consider:

- the extent, urgency and nature of actual or potential threats to the collection; and
- the availability of alternative funding and other resources to support the work.

The Trust will entertain proposals to cover the following conservation activities:

- collecting (in cases of emergency and only if alternative funds are unavailable)
- storage and maintenance of seed collections, *in vitro* collections and field collections
- regeneration
- documentation
- characterization
- germplasm health
- safety duplication
- distribution

Before finalizing a grant, the recipient must be able to demonstrate that there are adequate mechanisms and procedures in place to ensure sound financial management and accountability.

Governance

The Trust is an independent fund operating under international law. It Constitution was approved in October 2003 and its legal personality was conferred on 21 October 2004 when the required number of countries, from 5 of the 7 FAO regions, signed the *Agreement to Establish the Global Crop Diversity Trust*. To date this agreement has been signed by 12 countries: Cape Verde, Ecuador, Egypt, Ethiopia, Jordan, Mali, Morocco, Samoa, Sweden, Syria, Tonga, and Togo.

The policy framework for the Trust is provided by the International Treaty on Plant Genetic Resources for Food and Agriculture. The Trust serves as an element of the Treaty's funding strategy and operates in accordance with the overall policy guidance provided by the Governing Body of the International Treaty.

The other organs of the Trust are:

- an Executive Board of 11–13 members who will be appointed by key Trust stakeholders:
  - four members appointed by the Governing Body of the International Treaty
  - four members appointed by the Donor Council
  - one non-voting member appointed by the Director General of FAO
  - one non-voting member appointed by the Chair of the Consultative Group on International Agricultural Research
  - the Executive Secretary, *ex officio*, and
  - two additional members to be appointed by the Executive Board as appropriate to ensure membership balance
- A Donor Council comprising donors with the largest contributions in the following categories: developed country governments; developing country governments; corporations; foundations; non-governmental organizations; intergovernmental organizations; and any additional donors elected to ensure membership balance.
- The Executive Secretary.

It is expected that the first meeting of the Executive Board will take place in mid 2005. In the meantime, in order to oversee the establishment of the Trust, the Directors General of FAO and IPGRI, in consultation with all main stakeholder groups, have appointed 11 members to an Interim Panel of Eminent Experts. The Interim Panel has assumed the roles and responsibilities foreseen for the Executive Board in the Establishment Agreement and the Constitution, on an interim basis until the formal establishment of the Trust and the first meeting of the Executive Board.

The Trust is currently headquartered in Rome and operates within the institutional frameworks of FAO and IPGRI. These arrangements will remain in force until such time as a permanent headquarters location has been selected and an appropriate headquarters agreement has been negotiated with the host government.

Funding

The Trust is raising funds for the following three areas:

- a non-wasting endowment with an initial target of US$260 million, the proceeds from which will be used primarily to support the costs of conserving eligible collections over the long term;
- short-term funding for capacity-building and upgrading;
- operating costs of the Trust.

While there is still a long way to go, the Trust can report positive results to date, having secured pledges of about $51 million from developed and developing country governments, foundations, private corporations, a farmers' organization and a private individual. The Trust requires a broad funding base in order to guarantee stability and to demonstrate widespread support. The Trust will therefore continue to seek funding from varied sources and expects that the breakdown of funds received will be approximately:

- 70% from governments;
- 20% from private foundations;
- 5% from private corporations;
- 5% from individual philanthropy and other sources.

Initial grants

At its sixth meeting in October 2004, the Interim Panel of Eminent Experts approved for funding the first five grants for upgrading and capacity building. These grants, the details of which are currently being finalized, will directly or indirectly benefit at least 30 developing countries in all regions of the world.

Conclusions

There is an urgent need for the provision of stable and sustainable funding to support the world's most important collections of crop diversity. Without this support many collections will be unable to survive. With every loss of genetic diversity, humanity looses options for addressing urgent needs and challenges of the future. The Global Crop Diversity Trust is an attempt to provide such funding and it aims to put the world's collections on a sound financial basis so that they can underpin food security long into the future.

Collecting crop genetic resources in the Mediterranean agricultural islands: the Maltese Archipelago

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Summary

Collecting crop genetic resources in the Mediterranean agricultural islands: Maltese Archipelago

This collecting mission in Maltese Archipelago opens a new exploration cycle aimed to safeguard autochthonous crop genetic resources still present in the Mediterranean islands. This new research is the sequel to that carried out in the Italian small islands that ended in 2002. Maltese agriculture is an economic activity of low importance, mainly due to the scarcity of arable land and water resources. In this first and preliminary expedition, 32 valuable landraces were collected of 19 species of pulses, vegetables, cereals, forages, industrial plants, spices and condiments. In general, strong crop genetic erosion was observed, but on the Island of Gozo this phenomenon was less severe. The building of a local genebank would certainly help to stop this trend.

Key words: agricultural biodiversity, germplasm collecting, crop genetic erosion

Résumé

Collecte de ressources phytogénétiques agricoles dans les îles méditerranéennes: l'archipel maltais

Cette mission de collecte dans l'archipel maltais inaugure un nouveau cycle d'exploration destiné à sauvegarder les ressources phytogénétiques indigènes encore disponibles dans les îles méditerranéennes. Ce nouveau programme constitue le prolongement des recherches réalisées dans les petites îles méditerranéennes, achevées en 2002. L'agriculture maltaise est une activité marginale, principalement en raison de la rareté des terres arables et des ressources en eau. Au cours de cette première expédition, 32 races locales utiles ont été collectées, appartenant à 19 espèces de légumineuses, légumes, céréales, plantes fourragères, cultures industrielles, épices et condiments. En général, on constate une importante érosion des ressources phytogénétiques, mais le phénomène est moins accentué sur l'île de Gozo. La constitution d'une banque de gènes locale pourrait certainement contribuer à enrayer cette tendance.

Resumen

Recolección de recursos fitogenéticos en las islas agrícolas del Mediterráneo: el Archipiélago Maltés

Esta misión de recolección en el Archipiélago Maltés abre un nuevo ciclo de exploración orientado a salvaguardar los recursos fitogenéticos autóctonos aún presentes en las islas del Mediterráneo. Esta nueva investigación es la secuela de la efectuada en las pequeñas islas de Italia que finalizó en 2002. La agricultura maltesa es una actividad económica de poca importancia, sobre todo dada la escasez de tierras arables y de recursos hídricos. En esta primera expedición preliminar se recogieron 32 valiosas razas autóctonas de 19 especies de legumbres, hortalizas, cereales, forrajes, plantas de uso industrial, especias y condimentos. En general se observó una fuerte erosión fitogenética, si bien en la isla de Gozo este fenómeno era menos grave. Ciertamente, el establecimiento de un banco fitogenético local ayudaría a detener esta tendencia.

Introduction

In September 2002, a collecting mission was carried out in the Maltese Archipelago. This collecting mission opens a new exploration cycle that follows a previous one, which started in 1994 and ended in 2002, thanks to the collaboration between the Germplasm Institute (IG) of Bari (Italy) and the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) of Gatersleben (Germany) (Laghetti et al. 1996; Perrino et al. 2000). The main aim of that first study was to find out and safeguard autochthonous crop genetic resources still present on Italian minor islands. The principal result of those specific collecting expeditions was that, nowadays, small islands represent the last refuges for landraces and they are also richer in crop genetic resources than other mainland areas (Hammer et al. 2001). So this new research in Mediterranean (but not Italian) islands is the sequel to that carried out on Italian minor islands of agricultural interest and has the same aims.

The Maltese Archipelago (316 km^2) consists of several uninhabited islets and three main inhabited islands: Malta (255 km^2), Gozo (67 km^2) and Comino (3 km^2) (Figure 1). The total population of the Maltese islands is ca. 388 000 with a population density of 1228/km^2: the highest in Europe. The archipelago is located in the central Mediterranean, 96 km from Sicily and 290 km from north Africa. The highest elevation is 253 m asl and, geologically, consists of Oligo–Miocene limestones of marine origin with some Quaternary deposits of terrestrial or riparian origin. The climate is typically Mediterranean with warm summers (20°C minimum and 31°C maximum) and mild winters (10°C minimum and 15°C maximum). The average annual rainfall is ca. 530 mm, but with a high variation from year to year (191–1031 mm) falling mainly between October and March (Chetcuti et al. 1992).

The local wild vegetation is typical of warm, low-lying Central Mediterranean coastal areas with calcareous soils (Lanfranco 1995). With its central position in the Mediterranean, the flora of the Maltese islands is of high interest because of their affinities with other Mediterranean areas. Mediterranean maquis is common and mostly of secondary origin, garigues are

Figure 1. Geographical position of the Maltese archipelago.

typical of the karstlands, while steppes grow on all types of substrate. The Maltese flora comprises ca. 1000 species of vascular plants of which ca. 700 are indigenous and 16 taxa are strictly endemic (Haslam et al. 1977). Some interesting information on local uses of Maltese flora is reported in Baldacchino and Stevens (2000).

The industrial sector is characterized by state shipyards and small and medium concerns producing, mainly, textile manufactured products, clothes and electrical and electronic equipment. On Malta there are also a lot of family businesses specialized in artisanship. Today, the local industry is changing towards exports of highly technological products. Malta is substantially poor of mineral resources whereas tourism is very important (27% of GDP), employing almost one third of the labour force; the problem of how to plan local tourism and its integration with agricultural development is well discussed by France (1997). Fishing is not economically important except for a recent tuna export to Asia. Local agriculture with only 3% of GDP is an economic activity of low importance, mainly because of the small extent of arable land (only 12 000 ha) and scarcity of water resources. Also, the employment rate is low, along with its consequent political importance. Maltese agriculture is self-sufficient for only 40% of its own requirements.

Collecting methods

All the information available regarding Maltese agriculture and results from previous collecting missions were consulted with the specific aim of better planning this exploration. The conclusion was that the present research was the first with regard to the safeguarding of Maltese crop genetic resources.

The main sources of the collected seed samples were farmers' stores and their fields. In a few cases samples were also collected from seed sellers and pedlars, which made it possible to find out and collect old traditional material. At each collecting site a passport data sheet was filled in, using data from a hand-held GPS system. In addition to the data recorded directly by the collecting team, further information on Maltese agriculture was registered from local specialists and through several interviews with the growers. Further details on the sampling methods and exploration strategy used are reported in Hammer et al. (1997, 1999) and Laghetti et al. (1996, 1998, 1999a,b, 2001, 2002).

Results and discussion

During the mission a considerable diminution of agricultural and pastoral activities was detected; this phenomenon has become more marked in the last 30 years. Today the main crops cultivated on the archipelago are wheat, potatoes [e.g. variety 'Malta' very resistant to *Phytophthora infestans* (IPR 1973)], onions (exported to northern Europe), vegetables and grapes. The island of Gozo turned out to be more interesting and richer, from an agricultural point of view, than Malta (Figure 2). Agriculture is more developed here and its main products are: melons, tomatoes, cauliflowers, potatoes and grapes. Animal (e.g. pigs, sheep, goats) farming also flourishes on Gozo, where the local peppered goat's cheese 'Gbejniet' is very delicious and famous. Gozo is also less affected by anthropic influence and some endemics grow on it, such as *Iris pseudopumila* Tineo subsp. *gozoensis* (Service 1999).

All the 32 accessions collected belong to 19 species of pulses, vegetables, cereals, forages, industrial plants, spices and condiments (Table 1). The original samples consist of morphologically different lines and contain admixtures of other crops; after their precise detection and separation, the amount of accessions will be increased considerably.

Figure 2. One of the several vegetable gardens on Gozo; this island is much more devoted to agriculture than Malta.

Table 1. Number of accessions collected on the Maltese archipelago

Species	Accessions (*N*)
Allium cepa	1
Avena sativa	2
Brassica oleracea	1
Brassica oleracea var. *gongylodes*	1
Capparis spinosa	1
Carthamus tinctorius	1
Cicer arietinum	1
Cucumis melo	2
Foeniculum vulgare	1
Hedysarum coronarium	1
Hordeum vulgare	2
Ocimum basilicum	1
Petroselimun crispum	1
Phaseolus coccineus	1
Phaseolus vulgaris	1
Pisum sativum	2
Sorghum bicolor	1
Triticum durum	3
Vicia faba var. *major*	3
Vicia faba var. *minor*	3
Vicia sativa	2
Total	32

Cereals

Two barley landraces (locally named 'xejr' and 'scair') were gathered on Malta and Gozo, but it was not possible to ascertain the genetic relationship between this material and the well-known local variety 'Malta'. This last is a high-yielding, hardy two-rowed winter barley, characterized by a high and stable protein content (Ablakulov and Mukhammedov 1986), suitable for use by brewers and resistant to cold and *Erysiphe graminis* (Linais 1974). Three populations of durum wheat were sampled on Malta and Gozo ('Ammarsli' locality); one of these was found in a traditional seed shop where it has been stored for at least 40 years (Figure 3). Bread is still made from sour dough, left from the previous day's batch, often cooked in wood ovens; the bread is crusty yet spongy in the centre. Two landraces of oat and one of sorghum were also collected.

Pulses

Two rare samples of local peas ('pizelli') were taken, one of which was very ancient and guarded scrupulously by a descendant of a traditional family of farmers. Peas on Malta are used to prepare the typical 'pastizzi', a flaky pastry parcel filled with ricotta or mushed peas, of which each baker and bar have their own version. One population of local common bean, used both dried and fresh, was collected together with two samples of chickpea ('cicri') and runner bean (*Phaseolus coccineus* L.). Three variable accessions of broad beans ('ful', *Vicia faba* L. var. *major* Harz.) were found both on Gozo and Malta; the most common type on the islands has violet seeds and is used both fresh and dried, mainly to prepare the traditional Maltese pate 'Bigilla', a thick pate of broad beans with garlic. It is sometimes sold direct from vans in village squares. Three populations of field bean (*Vicia faba* L. var. *minor* Peterm.) were gathered showing a wide variability of seed colour and shape; in particular an uncommon variety not utilized for forage (e.g. seeds were used pressed in several local recipes) was recorded characterized by spherical and small seeds.

Vegetables

Six accessions of Maltese vegetables were collected (Table 1). In particular two local varieties of melon (*Cucumis melo* L.) were found (Figure 4), of which the one from Gozo, called 'Bettiegh', is a rock melon (*C. melo* var. *cantaloupensis* Naud.) locally much appreciated for its delicious organoleptic quality. The problem of time meant that it was not possible to sample another typical melon variety (i.e. 'Winter Green Maltese');

Figure 3. Collecting in an ancient seed shop that still stored very old samples of local crops.

Figure 4. Typical terraces cultivated with a landrace of melon (Island of Malta).

this last one has given good agronomic results also outside of Malta (Talamba 1972). On Gozo ('Qala' locality) one landrace of onion (named 'Basal') was gathered; this type is especially used to prepare the 'Kapunata' (a Maltese vegetable speciality made from eggplant, zucchini, onions, peppers, tomatoes and garlic) and the local snack 'hobz biz-zejt' (a large thick round of bread dipped in olive oil, rubbed with ripe tomatoes and filled with a mix of tuna, onion, garlic, tomatoes and capers) served in nearly every bar and still the 'packed lunch' of farmers and workmen. In the same area an interesting semi-cultivated population of fennel (called 'busbies') was collected, together with a sample of kohlrabi (*Brassica oleracea* L. var. *gongylodes* L.), very common on the Maltese Archipelago. In another locality of Gozo (i.e. 'Ammarsli') a very old landrace of cabbage ('kabocci', *B. oleracea* L.) was found; this type is mainly used to produce seeds for feeding caged birds (Figure 5).

Traditional Maltese food is rustic and based on the seasons. In the era before refrigeration, this made sense. Families were inventive with the best of the seasonal produce. The housewife would stock up with the seasonal gluts, often preserving or drying produce to make use of them later in the year. Nowadays, Maltese specialities still include a lot of vegetables cultivated on the archipelago, from zucchini, artichokes ('qaqocc'), the giant cabbage, cauliflower ('pastard'), tomatoes ('tadam') and potatoes ('patata'). Stuffed marrows and tomatoes, and thick vegetable soups ('minestra') are frequently on the menu in homes and restaurants. Thanks to this culinary tradition many further landraces merit collection in the next exploration missions.

Spices and condiments

Three accessions of local spices and condiments plants were collected as samples (Table 1). Gozo proved to be particularly rich in this type of crop genetic resources, even if it was possible to collect only two landraces of basil ('habaq') and parsley (called 'Tursin'). This parsley variety is particularly utilized in the preparation of the local dish 'Aljotta' a fish soup laced with garlic, chilli, tomatoes, rice and chopped parsley or marjoram ('merqdux'). The 'honey of Gozo', from wild thyme ('saghtar', *Thymus capitatus* (L.) Hoffmanns. & Link) growing on this island, is well known (since the Greek–Roman age) and also appreciated abroad. One population of caper ('kappar' *Capparis spinosa* L.) was also collected in the environs of Valletta (Maltese island).

Industrial plants

A very old sample of safflower ('ghosfor', *Carthamus tinctorius* L.), formerly cultivated on Malta, was found. During the mission no traces of its recent cultivation were observed but its importance in times past is testified by its image on a Maltese coin (Figure 6).

Fodder plants and their wild relatives

Three accessions of fodder crops were sampled. The two populations of vetch (*Vicia sativa* L.) came from Gozo: one (named "gulbiena"), in particular, was considered by Maltese farmers to be agronomically better (e.g. more vigorous) than modern commercial cultivars; the other one collected, with white seeds, was particularly used for feeding of geese. A highly variable sample of a traditional landrace of sulla ('silla', *Hedysarum coronarium* L.) was found too. Collecting of Maltese forage crop germplasm has to continue with further explorations; in fact several autochthonous ecotypes still exist that merit collection, e.g. the *Trigonella foenum-graecum* L. ('fienu') ecotype 'Maltese' (Martiniello and Ciola 1993).

Some interesting wild relatives of pasture plants were observed on Malta, such as *Melilotus messanensis* (L.) All. ('trew'), *Hedysarum spinosissimum* sensu Coste pro parte, non L. ('silla selvagga'), *Medicago rigidula* (L.) All. ('nefel') and *Vicia tenuissima* (M. Bieb.) Schinz & Thell., while *Festuca fenas* Lag. ('zwien') occurred on Gozo as well, as testified by Kramer et al. (1972).

Figure 5. A small, terraced cultivation of a local variety of cabbage on Gozo. In the foreground are some ornamental palms very common on the Maltese islands (Mifsud 1995).

Figure 6. A Maltese coin with an image of safflower; proof of its former importance as a local crop.

Fruit trees

A lot of fruit trees are cultivated on the archipelago, such as olive groves ('zebbug'), figs ('tin'), vineyards producing excellent wines, sweet oranges ('laring', *Citrus sinensis* (L.) Osbeck) with several local varieties (e.g. 'Barly Maltese', 'Late Maltese', 'Maltese Half Blood', 'Blood Red', 'Pineapple', 'Jafa', 'Valencia Late', etc.), lemons (cv 'Malta'), tangerines ('mandolin', variety 'Wilking', with a good yield and maturing from January onwards; the fruits are tasty and juicy and very resistant to mechanical injury), pomegranates ('rummien', cv 'Malta', a soft-seeded pomegranate variety), prickly pear ('bajtar tax-xewk', *Opuntia ficus-indica* cv 'Malta', used also as livestock feed; Pretorius et al. 1997), peaches ('hawh'), apricots ('berquq') and almonds ('lewz'). 'Helwa tat-Tork' is a typical very sweet sugary mixture of crushed and whole local almonds, usually eaten after dinner.

Conclusions and future perspectives

Agriculture in the Maltese islands is of relatively modest importance; therefore, we would suggest that to safeguard autochthonous crop genetic resources, a strategic plan of *ex situ* conservation and *in vitro* propagation is implemented. As a matter of fact, a state genebank is lacking and many Maltese scientists, interviewed during the expedition, stressed the urgent necessity of building one up. The local scientific skill on biotechnological applications applied to genebank management appeared very high; some international scientific projects on this item are under way in Malta and the prospects for biotechnology application in Maltese agriculture are convincing (Serracino Inglott 1992).

In conclusion, this preliminary collecting mission should be followed by others to complete the investigation started here. In fact several traditional landraces (e.g. pumpkins , cereals, melons, crop wild relatives, etc.) were not gathered, because of problems of time shortage.

Additional data and details about the present collecting mission are reported in the exploration registers stored and available c/o IG.

Availability of germplasm

The material collected is being stored in the genebank of Bari for further classification and characterization. After its multiplication, it will be ready for distribution to the scientists.

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References

Ablakulov AM, Mukhammedov ZhM. 1986. Characteristics of barley varieties of the intensive type (in Russian). Trudy Uzbekskogo Nauchno Issledovatel'skogo Instituta Zerna. Uzbekistan, No. 22.

Baldacchino AE, Stevens DT, editors. 2000. The Maltese flora: uses and importance (in Maltese). Proceedings of the National Congress, Malta, 24 November 1998. Department 'ghall-Harsien ta' l-Ambjent, Malta.

Chetcuti D, Buhagiar A, Schembri PJ, Ventura F. 1992. The climate of the Maltese islands: a review. Msidia, Malta University Press, vi+108pp.

France LA. 1997. The Earthscan reader in sustainable tourism. Earthscan Publications Ltd, London, UK.

Hammer K, Laghetti G, Perrino P. 1997. Proposal to make the island of Linosa (Italy) as a centre for on-farm conservation of plant genetic resources. Genetic Resources and Crop Evolution 44:127–135.

Hammer K, Laghetti G, Perrino P. 1999. A checklist of the cultivated plants of Ustica (Italy). Genetic Resources and Crop Evolution 46:95–106.

Hammer K, Laghetti G, Perrino P. 2001. The small Italian agricultural islands—refuges for plant genetic resources? In: Swiecicki W, Naganowska B, Wolko B, editors. Eucarpia Symposium, Genetic Resources Section, 16–20 May 2001, Poznan, Poland. Institute of Plant Genetics Polish Academy of Sciences, Poznan, pp. 22–32.

Haslam SM, Sell PD, Wolseley PA. 1977. A flora of the Maltese Islands. Malta University Press. Msida, Malta.

IPR (Institute of Phytopathological Research). 1973. Annual report 1973, Wageningen, Netherlands.

Kramer KU, Westra LYT, Kliphuis E, Gadella TWJ. 1972. Floristic and cytotaxonomic notes on the flora of the Maltese Islands. Acta-Botanica-Neerlandica. 21(1):54–66.

Laghetti G, Hammer K, Perrino P. 1996. Plant genetic resources in Pantelleria and Pelagie archipelago, Italy: collecting and conservation of local crop germplasm. FAO/IBPGR Plant Genetic Resources Newsletter, 108:17–25.

Laghetti G, Hammer K, Olita G, Perrino P. 1998. Crop genetic resources from Ustica island (Italy): collecting and safeguarding. Plant Genetic Resources Newsletter 116:12–17.

Laghetti G, Perrino P, Hammer K. 1999a. Collecting landraces and wild relatives in Neapolitan islands, Italy. Plant Genetic Resources Newsletter, 119:14–18.

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

Laghetti G, Perrino P, Olita G, Hammer K. 2001. Multicrop collecting expeditions in Aeolian archipelago (Italy). Plant Genetic Resources Newsletter, 128:26–34.

Laghetti G, Perrino P, Cifarelli S, Spahillari M, Hammer K. 2002. Collecting of landraces and wild relatives of cultivated plants in Ponziane islands and Tuscan archipelago, Italy. Plant Genetic Resources Newsletter, 131:28–34.

Lanfranco E. 1995. The Maltese flora and conservation. Ecologia Mediterranea, XXI(1/2):165–168.

Linais B. 1974. The best varieties of barley and winter barley (in French). Producteur Agricole Francais 50:27, 155.

Martiniello P, Ciola A. 1993. Effect of agronomic factors on annual leguminous forage crop in Mediterranean environments. Journal of Agronomy and Crop Science 170(5):309–321.

Mifsud S. 1995. Palms on the Maltese islands. Principes 39(4): 190–196.

Perrino P, Laghetti G, Hammer K. 2000. Agrobiodiversity in small Italian islands, isolating mechanisms, maintenance and valorization: a case study. In: Proceedings of the International Conference on Science and Technology for Managing Plant Genetic Diversity in the 21st Century. 12–16 June 2000, Kuala Lumpur, Malaysia. IPGRI, pp. 46–47.

Pretorius CC, Rethman NFG, Wessels AB. 1997. Effect of irrigation on total yield and nutritive value of Opuntia ficus-indica cultivars as livestock feed. Applied-Plant-Science 11(2):53–55.

Serracino Inglott F. 1992. Prospects of biotechnology application in Maltese agriculture. Options-Mediterraneennes, 14: 135–136.

Service N. 1999. A new subspecies of *Iris pseudopumila*. New-Plantsman, 6(3):186–188.

Talamba D. 1972. Contributions to the study of melon varieties under the conditions of Dobrogea. Annals of the Institute 'de Cercetari pentru Legumicultura si Floricultura'. 2:69–77. [In Romanian]

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Gestion dynamique de la diversité variétale des ignames cultivées (*Dioscorea cayenensis–D. rotundata*) dans la commune de Sinendé au nord Bénin

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Résumé

Gestion dynamique de la diversité variétale des ignames cultivées (*Dioscorea cayenensis–D. rotundata*) dans la commune de Sinendé au nord Bénin

Cette étude menée dans cinq terroirs de la sous préfecture de Sinendé a permis de recenser 88 variétés dont 14 appartiennent à l'espèce *D. alata* et 72 à l'espèce *D. cayennensis-rotundata*. Ces dernières occupent 97% des superficies cultivées. Chaque exploitation dispose en moyenne de 12 variétés. Il existe des exploitations où on dénombre plus de 20 variétés dans le champ. Par contre d'autres en ont moins. Les variétés cultivées sont groupées en deux classes : variétés à une récolte : « Yassounou » en Bariba ; et variétés à deux récoltes « tan dwe » en Bariba. Entre ces deux classes existe une classe intermédiaire de variétés à récolte mixte. C'est la variété Morokorou qui est la plus produite dans tous les terroirs. Elle se conserve bien et est bien vendue sur le marché. Par ailleurs Morokou est très appréciée sur le plan culinaire et n'est soumise à aucune barrière culturelle. Les paysans veulent maintenir la pureté de chaque groupe variétal dans toutes les pratiques culturales. Pour cela, la plantation se fait par variété en ligne. Lors du stockage, les variétés sont conservées au champ dans des greniers ou en des tas différents. De nos jours, certaines variétés tendent à disparaître. C'est pour cela que l'étude à abouti à une principale recommandation : celle de la conservation in situ des ressources génétiques de l'igname.

Resumen

Manejo dinámico de la diversidad varietal del ñame cultivado (*Dioscorea cayenensis–D. rotundata*) en el distrito de Sinendé, Benin septentrional

Este estudio, emprendido en cinco aldeas de la ciudad de Sinendé, registró 88 variedades de ñame de las cuales 14 eran especies de *Dioscorea alata* y 72 de *D. cayenensis-rotundata*. El 97% de las tierras cultivadas con ñame están ocupadas por *D. cayenensis-rotundata*. En Benin cada hogar posee un promedio de 12 variedades. Hay también familias que cultivan más de 20 variedades locales en su terreno. Las variedades cultivadas se agrupan en tres clases: 1) variedades de una sola cosecha, llamadas *Yassounou* en Bariba; 2) variedades de dos cosechas, llamadas *Tan doua* en Bariba, y 3) una clase intermedia de variedades con cosechas mixtas. En las cinco aldeas la variedad *Morokorou* es la más producida porque se puede conservar por mucho tiempo y se vende bien en el mercado. Además, la variedad *Morokorou* es muy apreciada por sus características culinarias y su sabor, y se la consume en todos los hogares (no está asociada a rituales o prácticas culturales particulares). Los granjeros se esmeran por conservar la pureza de cada grupo de variedad en cada estadio del cultivo. Cada variedad se planta en una hilera diferente y durante el almacenamiento las variedades se conservan en el campo en diferentes heniles o pilas. Recientemente ciertas variedades comenzaron a desaparecer, por lo que formulamos aquí algunas recomendaciones para la conservación *in situ* de los recursos genéticos del ñame.

Summary

Dynamic management of varietal diversity of cultivated yam (*Dioscorea cayenensis–D. rotundata*) in the Sinendé district of north Benin

This study undertaken in five villages of Sinendé town recorded 88 varieties of yam, of which 14 were from *Dioscorea alata* species and 72 from *D. cayennensis-rotundata*. *D. cayennensis-rotundata* occupies 97% of the land cultivated for yam. In Benin each household had an average of 12 varieties. There are also families who cultivate more than 20 local varieties in the field. The cultivated varieties are grouped in three classes: (1) varieties with one harvest, called *Yassounou* in Bariba; (2) varieties with two harvests, called *Tan doua* in Bariba; and (3) an intermediate class of varieties with mixed harvest. The variety *Morokorou* is the most produced in the five villages. It can be conserved for a long time and sells well at market. In addition, *Morokorou* is very appreciated for its culinary traits and taste, and is consumed by all households (it has no particular cultural or ritual associations). The farmers aim to ensure the purity of each variety group during every stage of cultivation. Each variety is planted in a different row and, during storage, varieties are kept in the field in different lofts or heaps. Recently, certain varieties have begun to disappear, hence we formulated here some recommendations for *in situ* conservation of yam genetic resources.

Key words: Benin, *Dioscorea alata*, *Dioscorea cayennensis–rotundata*, genetic erosion, *in situ* conservation, yam

Introduction

Depuis plusieurs années, certaines régions de l'Afrique subsaharienne sont confrontées à des pénuries alimentaires chroniques (Okolie et Onwueme 1986). La situation ne cesse de se détériorer car la production alimentaire par habitant décline constamment dans la plupart de ces pays. Des désastres naturels ont provoqué des mouvements de population et aggravé ce qu'on appelle maintenant « la crise alimentaire » en Afrique. Cette crise est essentiellement due à une carence alimentaire doublée d'une forte poussée démographique. Comment peut-on assurer la sécurité alimentaire pour ces populations à croissance rapide ? Igué (1974) répond que les populations qui se nourrissent des

tubercules, en particulier de l'igname, et qui vivent loin des zones de conflits ne connaissent pas ce problème. L'intérêt de l'igname par rapport aux céréales est surtout dû à sa faible sensibilité aux aléas climatiques.

En dehors du rôle nutritionnel qu'elle joue et de sa large tolérance climatique, l'igname a une signification dans la vie culturelle des peuples d'Afrique Occidentale (Ayensu et Coursey 1972). Elle est l'aliment de choix lors de plusieurs cérémonies et festivités et est parfois indispensable pour les dots dans certaines sociétés africaines (Hahn et al. 1987). Au Bénin, c'est une culture qui a intégré entièrement les mœurs et traditions des populations du Nord Bénin, à tel point qu'on peut parler ici d'une civilisation de l'igname (Gbèdolo 1986).

Malheureusement, malgré ses fonctions économique, alimentaire et culturelle, l'igname a été pendant longtemps, le parent pauvre de la recherche des pays de l'Afrique de l'Ouest dont le Bénin. Cette situation est lourde de conséquences pour cette culture. L'igname continue d'être produite de manière traditionnelle, extensive, sans mécanisation et liée à de nouvelles friches. La sensibilité aux virus, aux champignons et autres maladies augmente expliquant souvent la médiocrité des rendements réalisés. Après la récolte, commencent les difficultés de conservation et de commercialisation. Il importe de trouver des variétés : à bonne productivité et se conservant bien ; n'exigeant ni friche ni tuteurage ; permettant la mécanisation des pratiques culturales ; adaptées aux choix culinaires des consommateurs.

L'igname est une culture à multiplication végétative pour laquelle les créations variétales par la recherche demeurent encore incertaines. Pour cela, il importe de faire l'état des lieux sur les variétés existantes. La présente étude, menée dans la zone Bariba a permis de faire l'inventaire de la diversité variétale, de voir comment cette diversité est gérée et d'envisager la conservation *in situ* des ressources phytogénétiques. Elle a pour but d'analyser la gestion de la diversité variétale existant tout en prenant en compte sa signification écologique, économique et socioculturelle pour les populations locales dans les politiques de conservation des ressources génétiques.

Matériel et méthodes

Zones de production d'igname

Au Bénin, quatre zones de production d'igname sont signalées : Bariba au Borgou et dans l'Atacora, Wama et Yom dans l'Atacora, Nagot au centre et Fon au sud. Cette étude a été menée dans la zone Bariba inféodée au Département du Borgou. Ce département est le premier producteur national d'igname. Compte tenu des limites matérielles, financières et de temps, nous avons réduit l'étude à la sous-préfecture de Sinendé.

Localisation de l'étude

La commune de Sinendé (Figure 1) a été choisie pour plusieurs raisons : c'est l'un des plus grands sites de production d'igname dans le département du Borgou et Sinendé est occupée en majorité par l'ethnie Bariba dont la civilisation repose sur la culture de l'igname.

Le choix des villages a été précédé de deux enquêtes préliminaires. La première, au mois de février 1999 et la deuxième, au mois de juillet 1999. Cette phase exploratoire nous a permis de choisir : Niaro, Guessou bani, Wari, Goro bani, Yarra comme échantillon.

Méthodes de collecte des données

En moyenne, trois entretiens de groupe ont été organisés par village. Cela est dû au fait que dans les villages, les groupes socio-linguistiques ne vivent pas dans les mêmes aires géographiques. Au total, 150 exploitations ont été enquêtées, à raison de 30 par village. Les données et les informations contenues dans ce travail proviennent de plusieurs sources : le récit vie, les études documentaires, l'observation participante, les transects, les entretiens. Les données collectées concernent l'agrodiversité de l'igname (nombre de buttes par espèce cultivée et par variété), la gestion des plantations et des récoltes d'igname, la conduite de la culture, la pureté variétale, le réseau social de circulation des variétés, les synonymes des noms de variétés.

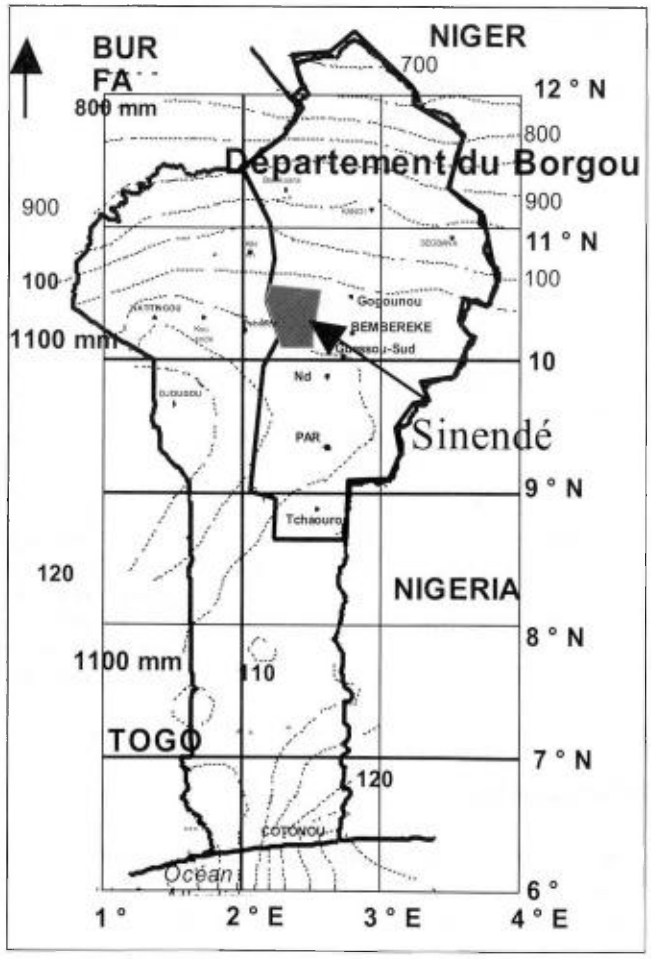

Figure 1. Carte du Bénin montrant la commune de Sinendé.

Tableau 1. Importance relative en nombre de buttes des différentes espèces cultivées

Espèces		Nombre de buttes	Pourcentage (%)
Dioscorea	(1) : une récolte (42 variétés)	618 192	53
cayenensis-	(2) : deux récoltes (20 variétés)	379 018	35
rotundata	(*M*) : récolte mixte (10 variétés)	94 916	9
(« Tassou »)	(1) + (2) + (*M*)	1 092 126	97
Dioscorea alata (« Sangourou »): 14 variétés		38 063	3
Dioscorea dumetorum (« Yanssourérou »): 1 variété		137	–
Dioscorea bulbifera (« Mokorou »): 1 variété		80	–

Tableau 2. Inventaire des surfaces cultivées en igname

Villages	Nombre de buttes (*N*)	Surface totale (*S*) (ha)	Densité *D=N/S*	Surface moyenne d'une butte (m^2)
Niaro	302 427	48	6333	1,58
Guessou bani	208 024	37	5622	1,78
Wari	193 137	34	5681	1,76
Goro bani	179 455	31	5789	1,73
Yarra	214 332	37	5715	1,75
Sinendé	1 097 375	187	5860	1,71

Tableau 3. Agrodiversité de l'igname dans les villages étudiés

Villages	Nombre de variétés	Variété par exploitation			Nombre de paysans ayant	
		moyenne	Maximum	Minimum	plus de 20 variétés	moins de 10 variétés
Niaro	57	14	30	4	4	7
Guessou bani	37	8	16	3	0	23
Wari	53	12	24	4	4	10
Goro bani	54	13	25	5	6	10
Yarra	47	13	24	6	3	8
Sinendé (Total)	88	12	30	3	17 (11%)	58 (39%)

Résultats et discussion

Diversité des ignames cultivées à Sinendé

Pour l'ensemble des terroirs villageois visités et enquêtés, quatre espèces d'ignames regroupant 88 variétés ont été recensées dans les champs. Chacune de ces espèces est représentée par plusieurs ou une seule variété. En considérant les quatre espèces cultivées, *D. cayenensis–rotundata* est la plus rencontrée dans les champs (Tableaux 1 et 2). Le complexe *D. cayenensis–rotundata* est de loin celui qui a la plus grande diversité. Elle est séparée en deux groupes variétaux par le cultivateur Bariba : les *Tandoua* et les *Yassounou*.

Les *Tandoua* réunissent les 20 variétés exploitées en double récolte (fin juin à mi-octobre). Les *Yassounou* recouvrent 42 variétés exploitées en récolte unique (décembre à janvier). Il n'y a pas toujours une limite entre les deux types agronomiques. En effet, 10 variétés peuvent passer d'un groupe à l'autre selon le choix du paysan, les conditions de culture ou selon les techniques culturales mises en œuvre. Ce sont des variétés mixtes.

L'espèce *D. cayenensis–rotundata* occupe environ 97% des superficies cultivées. Ce résultat est conforme à celui obtenu par Dumont (1998) qui rapporte que 90% des surfaces sont consacrées à l'igname dans chacun des différents pays producteurs d'Afrique Occidentale, exception faite toutefois pour la Côte d'Ivoire. L'espèce *D. alata* suit avec environ 3%. Ce pourcentage est faible par rapport à celui de Côte d'Ivoire (60% d'après Dumont et Marti 1997). La production de *D. dumetorum* et *D. bulbifera* demeure encore insignifiante.

Espèce D. cayenensis–rotundata *cultivée à Sinendé*

La richesse variétale de la commune a été évaluée à travers un inventaire des cultivars dans chacun des cinq villages (Tableau 3).

Soixante-douze variétés différentes du complexe *D. cayenensis–D. rotundata* ont été recensées avec une moyenne générale de 12 variétés par paysan. Le nombre de variétés cultivées par exploitation varie de 3 à 30. Le village de Guessou bani a la diversité variétale la plus réduite. Cette situation s'explique par les inondations de la campagne 1998 qui ont causé la perte de plusieurs cultivars dans ce village. Le village de Niaro est celui ayant la plus grande

diversité due à l'introduction de variétés étrangères par des paysans.

Sur les 150 paysans de notre étude, 17 ont plus de 20 variétés dans leur champ. Le fait d'avoir une grande diversité de variétés s'explique par plusieurs raisons :

- La sélection : Les paysans affirment : « on ne sait quelle variété marchera avec soi. » Ils lient ainsi la réussite d'une variété aux chances de l'individu. Pour cela, il faut débuter avec toutes les variétés afin d'éliminer progressivement les moins adaptées.
- La gestion des risques : Les variétés résistent différemment aux perturbations climatiques et aux attaques parasitaires. La détention d'un grand nombre de variétés permet ainsi de maîtriser ces risques. La diversité variétale est un élément de stratégie anti-risque (Emperaire et al. 1998). Sur le plateau adja, Agbo (1991) mentionne aussi que la diversification des cultures et les associations culturales sont des alternatives employées pour gérer le risque et les incertitudes.
- La distinction sociale : la détention de nombreuses variétés confère au paysan un prestige dans le village.
- L'ancien statut social : les paysans qui, dans le passé, avaient été ouvriers agricoles ont reçu plusieurs variétés en contrepartie de leurs efforts.
- L'exode rural : les paysans qui vont à l'aventure ou migrent au Nigeria ou dans d'autres régions du pays ramènent des variétés de leurs voyages. Cela enrichit leur stock disponible.
- La commercialisation : posséder plusieurs variétés permet de vendre des semenceaux (boutures).

58 paysans ont moins de 10 variétés dans leur champ. Comme pour le premier groupe, les raisons sont multiples :

- La satisfaction d'avoir fait la sélection des meilleures variétés.
- L'incapacité (physique et financière) à obtenir de nouvelles variétés, ces dernières pouvant être acquises comme salaire en nature ou par achat auprès d'autres paysans ou au marché.
- L'origine du paysan : Par rapport aux paysans locaux, les paysans venus d'ailleurs (Atacora, Nord Togo...) possèdent peu de variétés.
- Les effets néfastes des facteurs biotiques (parasites) et abiotiques (inondation, fertilité) sur de nombreuses variétés rares.
- Le groupe ethnique : les Peuhl ont un petit nombre de variétés. La grande partie des variétés cultivées se transmet de père en fils. Or les Peuhl ont une tradition récente dans l'agriculture.
- Le genre : toutes les femmes productrices d'igname possèdent moins de 10 variétés en raison de la nouveauté de l'activité débutée en moyenne depuis trois ans.

Importance relative des variétés cultivées de D. cayenensis–rotundata

Pour l'ensemble des variétés recensées, un classement a été fait pour chaque village et pour les cinq villages pour déterminer les ignames les plus produites. Il apparaît que sur les 72 variétés de l'espèce *D. cayenensis–D. rotundata*, plus de la moitié des variétés sont faiblement produites. La diversité constatée mérite donc d'être nuancée : elle s'exprime en nombre de variétés, ne tenant pas compte de l'importance de chaque variété. Seules quelques variétés sont produites à grande échelle (Figure 2).

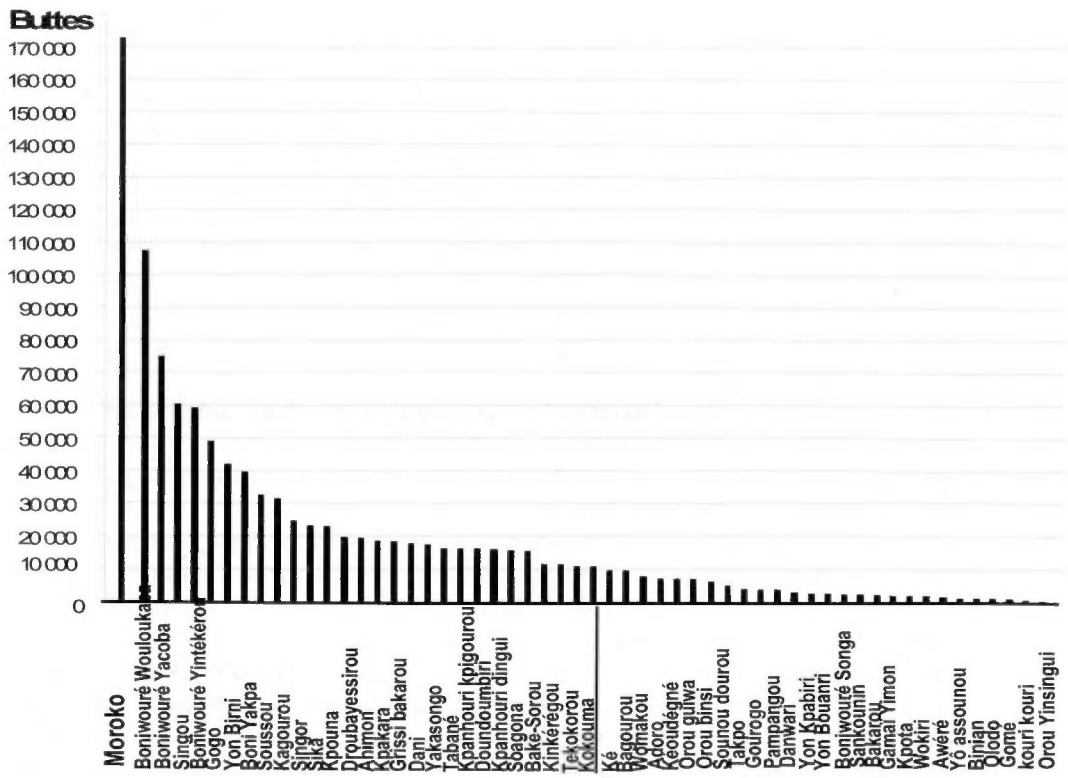

Figure 2. Importance relative des variétés les plus produites dans les cinq terroirs.

La variété 'Morokorou' est la plus produite dans tous les terroirs villageois et, par ricochet, dans toute la commune. Elle occupe 16% des superficies emblavées en igname dans la commune de Sinendé. Ceci pourrait s'expliquer par le fait que cette variété est appréciée sur le plan culinaire et bien vendue dans le commerce. Elle se conserve sur une période assez longue et n'est soumise à aucune barrière socio-culturelle. En ce qui concerne les autres variétés les plus produites, elles sont spécifiques à chaque village. Leur forte production est justifiée par l'un ou l'autre des avantages cités pour 'Morokorou'. Par exemple 'Boniwouré Yakoba', 'Boniwouré Wouloukaba', 'Yon birni', 'Boni Yakpa' qui font partie de cette liste ont une bonne aptitude à la conservation.

En considérant le cycle des variétés, on constate que :

- Pour les variétés à deux récoltes, cinq variétés sur l'ensemble des 20 variétés de ce groupe ('Morokorou', 'Gogo', 'Kpouna', 'Doubayessirou', 'Ahimon') occupent 74% des superficies.
- Sept variétés sur les 42 (soit 17%) que comportent ce groupe occupent 62% des superficies.
- Pour les variétés à récolte mixte, 30% ('Soussou', 'Guirissi bakarou', 'Kpanhouri kpigui') des variétés sont cultivées sur 73% des superficies destinées à ce groupe.
- Quinze variétés sur les 72 (20%) occupent environ 70% des surfaces cultivées alors que les 57 autres occupent les 30% restantes.

Maintien de la pureté variétale

Les multiples variétés dont disposent les paysans sont conduites de manière à garantir à chaque variété une certaine homogénéité. Au cours de la plantation, le chef d'exploitation, censé connaître la systématique des ignames, veille sur la bonne répartition des différentes variétés dans le champ. Cette logique est maintenue jusqu'à la récolte où les moissons sont faites par variété et stockées dans des tas distincts (greniers de champ) par le chef d'exploitation.

Lorsque par mégarde, au cours des manipulations, un mélange des variétés se produit, le paysan procède à un tri avant la plantation. Les ignames douteuses sont mises à l'écart. Elles ne sont reconduites que si le paysan s'assure de la variété à laquelle elles appartiennent. Par ces multiples précautions, la pureté variétale des ignames est entretenue. Toutefois, l'observation que nous avons faite du matériel végétal amène à reconnaître que cette pureté est parfois approximative. D'une manière générale, quelles que soient les variétés considérées, on constate, au sein des cultivars, une diversité morphologique plus ou moins importante concernant le plus souvent la forme du tubercule, la coloration de la chair et/ou la forme des feuilles, dont on ne sait, *a priori*, si elle est attribuable à la plasticité phénotypique naturelle des ignames ou au regroupement de plusieurs clones dans un même cultivar appelé « groupe variétal » (Dansi et al. 1998; Hamon et Lebot 1998).

Les précautions ne sont pas toujours prises pour garantir la pureté variétale dans toutes les exploitations. Au niveau des exploitations concernées, ce sont les plus jeunes qui plantent, récoltent et gèrent les stocks. Or, ils ont une connaissance limitée des variétés. D'un autre côté, la classification sur la base des descripteurs morphologiques n'est pas toujours fiable et il est possible qu'un clone soit mal classé.

Analyse des réseaux d'échange

Les pratiques agricoles de maintien et de renouvellement de la diversité sont couplées à des pratiques sociales qui dépassent le cadre de l'unité de production et favorisent un brassage des variétés et leur dissémination géographique. Elles s'inscrivent dans une dynamique sociale qui tient compte de certains aspects du fonctionnement de la société Bariba. Les variétés d'igname sont obtenues de différentes sources : la famille, les voisins, les achats et l'extérieur. L'apport extérieur reste le plus faible et se produit à l'échelle sous-régionale. Quatorze variétés ont été introduites dans l'agriculture des cinq villages. Les introductions sont faites du Nigeria ou d'autres régions du Bénin (Atacora, Donga). Les variétés circulent toujours de l'est vers l'ouest, c'est-à-dire du Nigeria vers le Bénin et vers le Togo. Les ouvriers venant du Togo n'amènent presque jamais de variétés de chez eux. Par contre, ceux qui reviennent du Nigeria en ramènent.

Quatre cas illustrent la diversité des situations économiques et sociales rencontrées à Sinendé qui amène le paysan à solliciter des cultivars :

- Les impératifs économiques font parfois que certains paysans privilégient les activités non agricoles et donc ne parviennent pas à assurer la reproduction de leur stock variétal ;
- Une absence ou une maladie lors des périodes de stockage peut induire des pertes de semenceaux et donc de variétés ;
- En raison des conditions agro-climatiques, une pénurie collective de semenceaux peut être enregistrée; 1998 est un exemple. La saison très pluvieuse occasionna des inondations et des pertes collectives et généralisées de semenceaux dans le village de Guessou bani ;
- Les ouvriers immigrants sont parfois gratifiés en semenceaux.

L'obtention des semenceaux est régie par une obligation de donner et de recevoir mettant en scène les différentes facettes des échanges. Lorsqu'une variété est sollicitée par un paysan, le propriétaire des semenceaux répond positivement à cette demande. Dans les monts Mandara du Nord Cameroun, Seignobos (1992) remarque qu'on ne remet jamais gratuitement un plant d'igname. Il est échangé dans certains cas contre une chèvre ou contre deux ou trois fers de houe ou contre la promesse d'une alliance matrimoniale. Cette attitude notée chez le paysan Bariba est donc la manifestation d'une solidarité collective limitée à des réseaux de connaissances (famille et voisinage). Les semenceaux peuvent être aussi offerts par le propriétaire à une personne qu'il tient à gratifier ou à qui il veut manifester une reconnaissance. Dans ce cas l'échange a valeur d'offrande et marque des relations d'alliances. Les variétés données sont les variétés rares ('Yon birni', 'Sogoto',...) ou les variétés d'introduction récente

('Assi', 'Ayé',...) ou les variétés particulières ('Kpouna'...). Les échanges se font donc entre paysans bien déterminés sur le plan social suivant des réseaux de relations déjà constitués.

Selon que le paysan est en position de recevoir, de donner ou de demander, la dette sociale qu'il contracte n'a pas la même signification. Un retour différé est en veilleuse. Elle pourra se manifester par une entraide ou un don en retour d'une autre variété que le premier ne possède pas. Dans le cas contraire, le donateur universel renforce sa position sociale alors que, à l'autre extrémité, ceux qui ont tendance à être systématiquement demandeurs entrent dans un processus d'exclusion sociale et de dépendance.

Conservation des ressources génétiques

Malgré la libre circulation des variétés entre terroirs, il existe des cultivars qui sont spécifiques à des villages donnés malgré la prise en compte des synonymies.

Au total, il existe 18 variétés (Tableau 4) qui ne se rencontrent que dans un village. 14 d'entre elles sont faiblement produites mais une est produite en grande quantité : la variété 'Yon birni' à Guessou bani. En ce qui concerne les variétés introduites récemment, la faible représentativité est due au fait que les opérations de multiplication et de propagation prennent du temps (plusieurs campagnes agricoles). Par contre, le cas des 10 variétés anciennes pourrait être lié à un abandon progressif de ces cultivars traditionnels. Une érosion génétique guette à nouveau le stock variétal des ignames dans la sous-préfecture.

Dans le cadre de la conservation des ressources phytogénétiques, deux choix sont envisageables : la conservation *ex situ* et la conservation *in situ*. Généticiens et protecteurs de la nature s'accordent de nos jours à penser que la conservation *in situ* constitue le moyen privilégié de conserver les espèces sauvages et les ressources génétiques des espèces cultivées (Olivier et Chauvet 1992). Dans le même temps, Engelmann (1992) trouve que la conservation *ex situ* est difficile à réaliser, à cause de la taille des échantillons à conserver, pour avoir une bonne représentation de la diversité génétique des espèces. De plus, les coûts d'entretien de telles collections sont élevés. Enfin, le matériel, une fois remis en culture, reste exposé aux pathogènes et aux accidents climatiques. Les paysans ont pratiqué inconsciemment la sélection massale et créent leurs propres banques génétiques de même que des systèmes d'échange très étendus, pour être aptes à acquérir du nouveau matériel génétique (Rhoades 1994). Ce savoir-faire doit être valorisé.

Conclusion

L'étude s'est attachée à la connaissance du matériel végétal tout en examinant ses relations avec le milieu humain et l'agriculture. Le matériel végétal est associé de façon très ancienne à la population qui l'exploite. Il possède une diversité très grande susceptible de garantir la sécurité alimentaire. Au total, 88 différentes variétés ont été dénombrées. Elles sont réparties au sein de quatre espèces à savoir *D. cayenensis–rotundata*, *D. alata*, *D. dumetorum et D. bulbifera*. C'est la première qui est la plus produite. Elle occupe 96% des superficies cultivées.

Chaque exploitation dispose d'une moyenne de 12 variétés d'igname. C'est surtout la gestion et la prévention des risques qui amènent certaines exploitations à détenir plusieurs variétés (plus de 20). Dans le même temps, d'autres

Tableau 4. Variétés de *D. cayenensis–rotundata* spécifiques à chaque village

Villages	Variétés	Production	Introduction
Niaro	'Agarou'	Faible	Récente
	'Assi'	Faible	Récente
	'Binian'	Moyenne	Ancienne
	'Boro boro'	Faible	Ancienne
	'Dikpi'	Faible	Ancienne
	'Koré singui'	Faible	Ancienne
	'Kpota'	Moyenne	Ancienne
Guessou bani	'Ayé'	Faible	Récente
	'Orou yabouri'	Faible	Récente
	'Yon birni'	Importante	Récente
Wari	'Gomè'	Faible	Ancienne
	'Kourotoko kokparérou'	Faible	Ancienne
	'Sogoto'	Faible	Ancienne
	'Yon bouanri'	Moyenne	Récente
Goro bani	'Kéoudèguè'	Faible	Ancienne
	'Kouri kouri'	Faible	Ancienne
	'Orou monbou'	Faible	Ancienne
Yarra	'Yon kpabiri'	Faible	Ancienne

exploitations préfèrent se limiter à un nombre très limité de variétés. Elles expliquent cette option par la satisfaction d'avoir sélectionné les variétés les plus performantes et les plus résistantes. La disponibilité variétale est fonction de la superficie d'igname cultivée et non de la connaissance du chef d'exploitation.

Les échanges de variétés entre les paysans se font sans difficulté et sans barrière. Ils se font au sein d'un même village ou entre villes. Ils reposent sur des affinités entre paysans. La circulation des variétés se fait aussi entre pays. C'est le cas surtout avec les *D. alata* qui sont introduites du Nigeria par les paysans exilés.

La gestion de la diversité est assurée par le chef d'exploitation lui-même ou par les aînés immédiats qui disposent d'une bonne connaissance du matériel cultivé. Ils veillent à garantir la pureté variétale tant au niveau de la plantation qu'au niveau du stockage. Les variétés sont manipulées distinctement, ce qui évite les mélanges qui altèrent leur homogénéité.

Sur la base des connaissances recueillies chez les paysans « domesticateurs », il sera possible de définir de nouvelles stratégies pour la sélection de nouvelles variétés. Ces variétés :

- doivent être saines, exemptes de virus ; résistantes aux agents phytopathogènes et donc avoir une bonne aptitude à la conservation;
- ne doivent pas être exigeantes pour les friches et les tuteurs. Dans ces conditions on pourra préconiser une culture sédentaire de l'igname et, par conséquent, contribuer à la protection de l'environnement;
- doivent donner une bonne aptitude à faire de l'igname pilée.

La diversité ainsi créée doit être préservée. On peut envisager freiner l'érosion génétique des ignames à travers la conservation *in situ* des ressources génétiques.

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Références

Agbo VA. 1991. Civilisation et agricultures paysannes en pays Adja-Mono (Bénin) : rites-production-réduction des risques et gestion de l'incertitude [Thèse de Doctorat d'Etat]. Université de Paris V, France.

Ayensu ES, Coursey DG. 1972. Guinea yams. The botany, ethnobotany, use and possibible future of yam in Western Africa. Économie Botanique 26:301–318.

Dansi A, Mignouna D, Zoudjihèkpon J, Sangaré A, Asiedu R, Quin M. 1998. Variétal identification key of the cultivated yams (*Dioscorea cayenensis/Dioscorea rotundata* complex) of Bénin Republic. Plant Genetic Resources Newsletter 116 : 18–25.

Dumont R. 1998. L'igname dans l'agriculture traditionnelle ouest africaine. In: L'igname plante séculaire et culture d'avenir. Actes du séminaire international CIRAD–INRA–ORSTOM–CORAF, Montpellier, France, pp. 71–76.

Dumont R, Marti A. 1997. Panorama sur l'igname. In: Ressource millénaire et culture d'avenir. CIRAD-CA, Montpellier, France.

Emperaire L, Pinton F, Second G. 1998. Gestion dynamique de la diversité variétale du manioc en Amazonie du nord-ouest. Nature, Sciences et Sociétés 6(2):27–42.

Engelman F. 1992. Les nouvelles méthodes de conservation *ex situ*. In: Actes du colloque en hommage à Jean Pernes, pp. 435–445.

Gbedolo YM. 1986. Factors limiting yam production in Benin. In: Terry ER, Akoroda MO, Arene OB, editors. Tropical root crop and the Africa food crisis. Proceedings of the Third Symposium ISTRC-AB, 15–23 August 1986. Nigeria. IDRC, Ottawa, Canada, pp. 57–60.

Hahn SK, Asiedu SO, Akoroda MO, Otoo JA. 1987. Yam production and its future prospects. Outlook on Agriculture 16: 105–110.

Hamon P, Lebot V. 1998. Les ressources génétiques de l'igname : Caractérisation et classification. In : Berthaud J, Bricas N, Machand JL, éditeurs. L'igname plante séculaire et culture d'avenir. Actes du séminaire international CIRAD–INRA–ORSTOM–CORAF, Montpellier, France, pp. 127–133.

Igué JO. 1974. Le rôle de l'igname dans la civilisation agraire des populations Yorouba.

Okoli O, Onwuemé I. 1986. L'igname et la crise alimentaire en Afrique. In : Terry ER, Akoroda MO, Arene OB, éditeurs. Plantes racines tropicales : les plantes racines et la crise alimentaire en Afrique. Compte-rendu du 3ème symposium triennal de l'ISTRC-AB, IRDC, Ottawa, Canada, pp. 46–52.

Olivier L, Chauvet M. 1992 La conservation in situ de la diversité des espèces végétales. In : Actes du colloque en hommage à Jean Pernes, pp. 455–465.

Rhoades R. 1994. Farmer back to farmer. Ten years later. In participatory on farm research an education for agricultural sustainability. Illinois. Agricultural experiment station, Urban, IL, USA, pp. 17–27.

Seignobos C. 1992 L'igname dans les monts Mandara (nord-Cameroun). Genève-Afrique 30:78–96.

Genetic structure of Guianan wild cocoa (*Theobroma cacao* L.) described using isozyme electrophoresis

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Summary

Genetic structure of Guianan wild cocoa (*Theobroma cacao* L.) described using isozyme electrophoresis

Wild cocoa trees (*Theobroma cacao* L.) from southeast French Guiana, surveyed and collected between 1985 and 1995, represent an original group of germplasm that contributes significantly to the species' genetic structure. Nineteen populations (demes) from the areas around five rivers (Oyapok, Euleupousing, Yaloupi, Camopi and Tanpok) in the main two river basins of French Guiana are represented in collections maintained in French Guiana, in a number of cocoa producing countries and on some quarantine stations. This study describes the genetic structure of the Guianan group and the genetic parameters of the group as a whole (138 clones in total) and of the eight most abundantly represented populations. Isozyme electrophoresis based on six isozyme systems (nine loci) was used. The results revealed a high average number of alleles per locus (1.9), a high percentage of polymorphic loci (77.7) and low average heterozygosity (0.085). The various populations displayed highly variable allelic frequencies. Wright's fixation index, at around 20%, indicated substantial genetic differentiation among populations. The genetic structure of the group showed that populations from the Camopi river area fell into two sub-groups, confirming results obtained earlier with other descriptors, and that the populations from the other areas were significantly different, that of the Kérindioutou differing most from the others. The use of controls from other morpho-geographical groups (Upper and Lower Amazon Forastero, Trinitario) confirmed the uniqueness of the Guianan cocoa trees. The results of the investigations, including the low heterozygosity, suggest the potential for using clones derived from the Guianan genetic group for genetic improvement of the crop, and support arguments for continuing surveys throughout the Guianan shield.

Key words: French Guiana, isozyme electrophoresis, *Theobroma cacao*, wild cocoa trees

Résumé

Description de la structuration génétique des cacaoyers spontanés (*Theobroma cacao* L.) de Guyane Français étudiée par électrophorèse d'iso-enzymes

Les cacaoyers spontanés (*Theobroma cacao* L.) du sud-est de la Guyane Française, prospectés et collectés entre 1985 et 1995, constituent un groupe original et l'un des axes de la structuration génétique de l'espèce. Dix-neuf populations (demes) originaires de cinq rivières (Oyapok, Euleupousing, Yaloupi, Camopi et Tanpok) des deux principaux bassins fluviaux de la Guyane Française sont représentées en collections, en Guyane, dans de nombreux pays producteurs de cacao et en stations de quarantaine. L'étude présentée concerne la structuration génétique de ce groupe et les paramètres de génétique des populations, pour l'ensemble (soit 138 clones étudiés au total) et pour les huit populations les plus richement représentées. La méthode utilisée est l'électrophorèse d'iso-enzymes, à l'aide de six systèmes enzymatiques (neuf locus). Les résultats montrent un nombre moyen d'allèles par locus élevé (1,9), un pourcentage de locus polymorphes élevé (77,7) et une hétérozygotie observée moyenne faible (0,085). Les diverses populations présentent des fréquences alléliques très variables. L'indice de fixation de Wright, d'environ 20 %, indique une grande différentiation génétique entre populations. La structure génétique du groupe montre que les populations de la rivière Camopi se séparent en deux groupes, confirmant des résultats antérieurs obtenus par d'autres descripteurs, et que les populations des autres rivières sont différentes, celle de la Kérindioutou étant la plus éloignée des autres. L'utilisation de témoins appartenant à d'autres groupes morpho-géographiques (Forastero haut et bas Amazoniens, Trinitario) confirme l'originalité des cacaoyers guyanais. Ces résultats, y compris le caractère faiblement hétérozygote, montrent l'intérêt potentiel en amélioration génétique des clones issus de ce groupe et militent pour une poursuite des prospections dans l'ensemble du Plateau des Guyanes.

Resumen

Descripción de la estructura genética de los árboles silvestres de cacao en Guayana (*Theobroma cacao* L.) empleando electroforesis de isozimas

Los árboles silvestres de cacao (*Theobroma cacao* L.) de la zona sudoriental de la Guayana Francesa que fueron recogidos y examinados entre 1985 y 1995, forman un grupo original que contribuye de manera significativa a la estructura genética de la especie. Diecinueve poblaciones (demes) originarias de cinco ríos (Oyapok, Euleupousing, Yaloupi, Camopi y Tanpok) pertenecientes a las dos mayores cuencas fluviales de la Guayana Francesa están representadas en las colecciones mantenidas en dicho país, en varios países productores de cacao y en algunas estaciones de cuarentena. Este estudio describe la estructura genética del grupo guayanés y los parámetros genéticos del grupo entero (es decir 138 clones) y de las ocho poblaciones más abundantemente representadas. Para el análisis se empleó electroforesis de isozimas, con seis sistemas enzimàticos (nueve *loci*). Los resultados revelaron un elevado número promedio de aleles por *locus* (1,9), un alto porcentaje de *loci* polimórficos (77,7) y un bajo promedio de heterocigosidad observado (0,085). Las diversas poblaciones mostraron frecuencias alélicas altamente variables. El índice de fijación de Wright, de alrededor del 20%, indicó una diferenciación genética sustancial entre las poblaciones. La estructura genética del grupo mostró que las poblaciones del río Camopi se dividieron en dos subgrupos, confirmando los resultados obtenidos anteriormente con otros descriptores, y que las poblaciones de los otros ríos fueron significativamente diferentes, siendo la del río Kérindioutou la más diferente respecto de las otras. El empleo de testigos pertenecientes a otros grupos morfogeográficos (Alto y Bajo Amazonas Forastero, Trinitario) confirmaron la originalidad de los árboles de cacao guayaneses. Estos resultados, incluida la baja heterocigosidad, sugieren la posibilidad de usar clones derivados de este grupo genético con fines de mejoramiento genético, y demuestran la necesidad de continuar con los exámenes en todo el escudo guayanés.

Introduction

Cocoa (*Theobroma cacao* L.) is a preferentially allogamous Neotropical tree species of the Sterculiaceae (Cuatrecasas 1964). Cuatrecasas (1964) described two subspecies, *Theobroma cacao* subsp. *cacao* and *Theobroma cacao* subsp. *sphaerocarpum*, corresponding to the two original cultivated types, Criollo (mainly originating from Central America) and Forastero from South America. Within these two major types Cheesman (1944) and Cuatrecasas (1964) identified various sub-sets, dependent on their geographical origins, including, for example, Upper Amazon Forasteros and Lower Amazon Forasteros. A third type, a hybrid between Criollo and Forastero, resulting from human intervention, is known as Trinitario.

Knowledge of the genetic structure of the species has recently been further increased (Lanaud et al. 1999; Motamayor et al. 2002). The existence of two subspecies is challenged in favour of several morpho-geographical groups, though they have yet to be exhaustively specified.

The wild cocoa trees of southeast French Guiana, collected between 1985 and 1995 (Clément 1986; Lachenaud and Sallée 1993; Lachenaud et al. 1997) are considered to represent one of the four axes of the species' genetic structure (Lachenaud 1997; Lanaud et al. 1999). The mother-trees of the Guianan material that currently exist in several collections, or are maintained on a few quarantine stations (Ford et al. 2000), come from 19 populations (demes, according to Hartl and Clark 1997) originating from the areas around five rivers in the two main river basins of French Guiana (Figure 1): the Oyapok (its upper reaches are the Kérindioutou, along with its tributaries, the Euleupousing, Yaloupi and Camopi) and the Maroni (river Tanpok). The material, collected in its wild form as budwood or pods, was planted (as clones or open pollinated progenies) in the reference collection at the CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France) Paracou-Combi station, at Sinnamary (French Guiana), where it was studied and characterized. This stage is a prerequisite for its use in genetic improvement programmes. It is important to determine the diversity of this material and, to this end, the diversity of certain populations has already been described using biochemical, morphological and agronomic descriptors (Lanaud 1987, 1999, 2001). The study described here covered 17 of the 19 known populations using the established and reliable method of isozyme electrophoresis (Sounigo et al. 1999). It also aimed to determine conventional population genetics parameters for the Guianan material as a whole and for the adequately represented populations, and to evaluate genetic differentiation among populations.

Material and methods

Planting material

The planting material comprised 138 clones of Guianan cocoa trees and four control clones belonging to other main morpho-geographical groups.

The Guianan clones belonged to the Oyapok (Borne 7, Ker, Pina, Oya), Camopi (Cam 0, 1, 3, 7, 8, 9, 10, 11, 12, 13), Euleupousing (Elp), Yaloupi (Yal) and Tanpok (Tan) populations. Despite the geographical scatter of the trees on the Oyapok banks downstream of Pina, we considered them as belonging to a single population, termed 'Oya' in the present paper (Figure 1). The numbers of individuals studied per population are given in Table 1. The control clones were

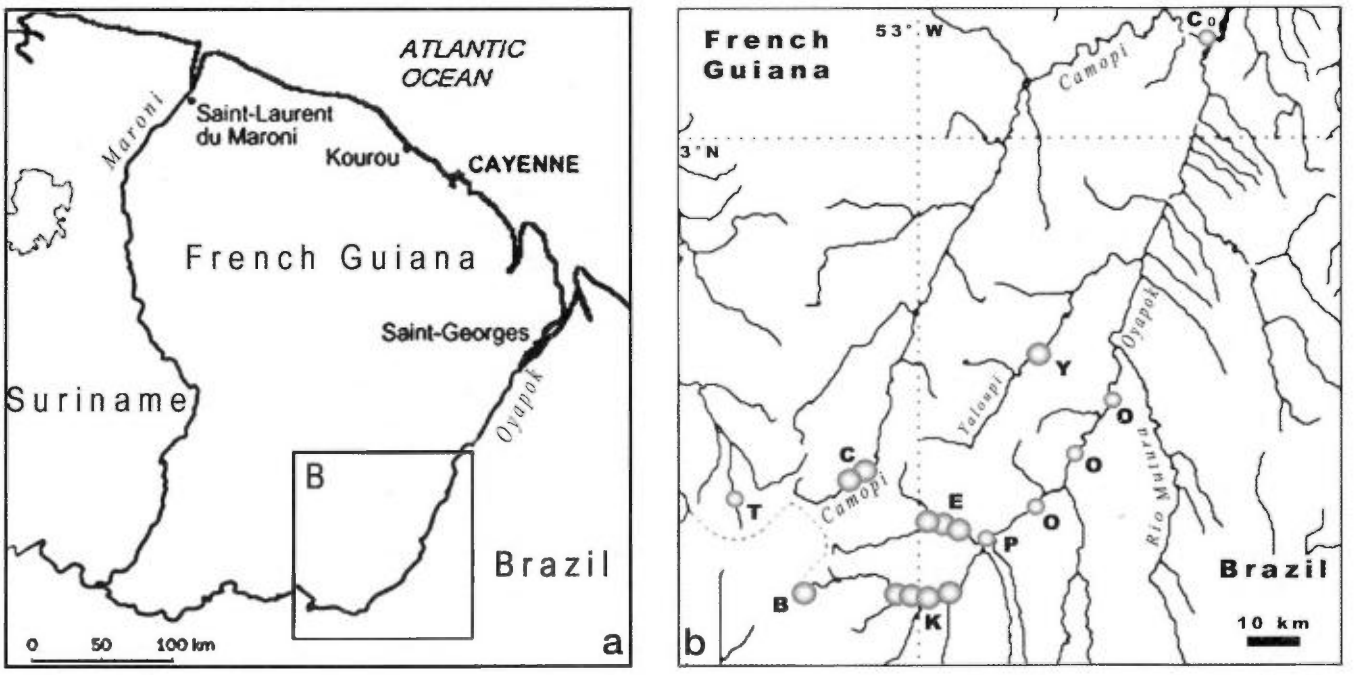

Figure 1. (a) Location of French Guiana and (b) location of the cocoa populations studied (for more precise localization see Lachenaud and Sallée 1993 and Lachenaud et al. 1997), where: B=Borne 7, Co=Cam 0, C=Cam 1 to 13, E=Elp, K=Ker, P=Pina, O=Oya, T=Tan and Y=Yal. The river Oyapok represents the border between French Guiana and Brazil; elsewhere the border is indicated by _ _ _ _

Upper Amazon Forasteros Pa 120 and Pa 121, an Amelonado type Lower Amazon Forastero IFC 1 and the Trinitario ICS 1 (Enriquez and Soria 1967; Ford et al. 2000).

Methods

Isozyme electrophoresis

The method of analysis was isozyme electrophoresis on starch gel (Lanaud 1986a,b). Six isozyme systems were used in the study: glucose phosphate mutase (GPM), glucose-phosphate isomerase (GPI), malate dehydrogenase (MDH), acid phosphatase (ACP), alcohol dehydrogenase (ADH) and isocitrate dehydrogenase (IDH). Some of these systems involved several genes: two for GPM (GPMA and GPMB) and three for MDH (MDHA, MDHB and MDHC). A total of nine loci were therefore considered.

The technique used (grinding, development, buffer solutions) was described by Lanaud (1986a). However, as a portable laboratory was used (Lebrun and Chevellier 1990), a few adaptations were made: the run time was fixed at 8 instead of 18 h and the migration tanks of the portable laboratory were smaller than those used by Lanaud (1986a).

Statistical methods and genetic parameters

Cluster analysis, genetic parameters and distance calculations were performed using Popgene 32 software (Yeh et al. 1999). Nei distances (D_{AB}, Nei 1972) between populations A and B were calculated as follows.

First, at the level of each locus, the Nei similarity index between populations A and B (I_{AB}) was calculated, according to the formula:

$$I_{AB} = \frac{\Sigma_k (p_{kA} . p_{kB})}{\Sigma_k [(p_{kA}^2 . p_{kB}^2)]^{1/2}}$$

where p_k is the frequency of allele k in the population. Then the Nei distance between populations A and B was calculated as:

$$D_{AB} = - \ln \overline{I}_{AB}$$

where $\overline{I}_{AB}$ is the arithmetic mean of the I_{AB} values calculated for each locus.

The genetic parameters calculated were:

1. Shannon's diversity index (Shannon and Weaver 1949): for each locus, this value (H) was calculated as:

$$H_l = -\Sigma_{kl} \ln p(kl)$$

where $p(kl)$ = frequency of allele k of locus l in the population. The mean value of these indices was then calculated for all the loci: $H = \overline{H}_l$

Table 1. Distribution per population of Guianan cocoa clones included in this study

Basin	Sub-basin	Population	Number of analysed clones
Oyapok	Oyapok	Borne 7	6
		Pina	1
		Oya	2
		Ker	8
	Euleupousing	Elp	11
	Yaloupi	Yal	3
	Camopi	0	1
		1	19
		3	15
		7	12
		8	2
		9	35
		10	1
		11	1
		12	5
		13	13
Maroni	Tanpok	Tan	3
Total			138

2. Wright's fixation index (F) is "the reduction in heterozygosity expected with random mating at any one level of a population hierarchy relative to another, more inclusive level of the hierarchy " (Hartl and Clark 1997), i.e. a higher level. In our study, we defined F_{ST} as the fixation index of the populations (demes) relative to the total combined population:

$$F_{ST} = (H_T - H_S) / H_T$$

where H_T is the total (expected) heterozygosity and H_S is the average (expected) heterozygosity among populations. These two H values may be replaced by Shannon's indices if random mating is not verified.

3. Proportion of observed heterozygosity: the proportion of heterozygous genotypes was calculated for each locus and the mean value was calculated for all loci.

4. Mean number of alleles per locus: included all the alleles, irrespective of their frequencies.

5. Number of polymorphic loci included all the loci with more than one allele, irrespective of the frequencies of the alleles.

Factorial Analyses of Correspondences (FAC) were performed using WINSTAT software developed at CIRAD. Cluster analyses were performed using the UPGMA procedure (Unweighted Pair Group procedure with Arithmetic Means, Sneath and Sokal 1973).

Results

Genetic parameters

For the set of 138 clones studied and the eight populations represented by a minimum of six clones in this study, Tables 2 and 3 indicate the numbers of polymorphic loci, the alleles present and their frequencies, the average number of alleles observed per locus, the observed and calculated heterozygosities (assuming panmixia) and Shannon's diversity index values.

Two loci were monomorphic (MDH B and MDH C) and the entire set of material studied displayed allelic frequencies that did not conform to Hardy–Weinberg equilibrium. However, two populations, Cam 1 and Cam 7, appeared to display panmixia.

The average number of alleles per locus ranged between 1.22 for populations Cam 1 and Cam 7 to 1.89 overall. Allelic frequencies differed substantially among populations with, for example, that of allele 3 of the ACP locus ranging from 0.06 in the Ker population to 0.62 in Cam 7, and that of allele 1 of IDH from 0.19 in Ker to 0.88 in Cam 13.

The recorded overall heterozygosity (Nei index) was 8.4% on average, with extremes ranging from 3.7% (Borne 7) to 11.1% (Cam 1); Table 3 indicates an overall expected heterozygosity of 15.2% assuming panmixia. Shannon's diversity index was 0.25 overall, ranging from 0.14 (Cam 1 and Cam 7) to 0.21 in the Elp population.

When Wright's fixation index was calculated from the expected heterozygosities for the 8 main populations and the set they constituted (119 clones), a value of F_{ST}=0.197 was obtained. Using Shannon's indices, the value was 0.227. It can therefore be deduced that around 20% of diversity is among the populations and 80% within.

Tables 2 and 3 also show that the contribution of the populations represented by five or fewer individuals (Tan, Cam 0, Cam 8, Cam 10, Cam 11, Yal, Oya, Pina) was considerable: two additional alleles (involving a further two polymorphic loci, GPMA and GPI), increased the average number of alleles per locus from 1.67 to 1.89 and Shannon's index from 0.22 to 0.25.

Figure 2 is a dendrogram (based on Nei's genetic distances) of the eight most abundantly represented populations. The Camopi populations were grouped into two sub-sets: Cam 1-7 and Cam 3-9-13. The Ker population differed most from the others.

Figure 3 represents the plane defined by the first two axes of the FAC using data for all clones and controls. The first axis accounted for 33% of total diversity, the second for 20%. The graph shows strong grouping of the Cam populations (particularly along the first axis) and the uniqueness of certain representatives of the Ker, Yal, Oya and other populations represented by fewer than five clones (termed 'others' in the legend), such as Cam 0. The Amelonado control (IFC 1) was very different from virtually all the wild Guianan material.

Discussion

When comparing our results with those of Lanaud (1987) on the same loci and on 332 clones representing the different

Table 2. Alleles present and allelic frequencies for the eight loci studied (see text), for the set of Guianan cocoa materials studied (138 clones) and the main eight populations included in this study

Populations	GPMA	GPMB	GPI	MDHA	MDHB	MDHC	ACP	ADH	IDH
Overall	1 (0.02) 2 (0.98)	1 (0.75) 2 (0.25)	1 (0.99) 2 (0.01)	2 (0.98) 3 (0.02)	1	1	2 (0.64) 3 (0.36)	1 (0.91) 2 (0.09)	1 (0.85) 2 (0.14) 4 (0.01)
Borne 7	2	**1 (0.50) 2 (0.50)**	1	2	1	1	2	1 (0.83) 2 (0.17)	1 (0.83) 2 (0.17)
Cam 1	2	**1 (0.71) 2 (0.29)**	1	2	1	1	**2 (0.42) 3 (0.58)**	1	1
Cam 13	2	1 (0.85) 2 (0.15)	1	2	1	1	**2 (0.54) 3 (0.46)**	1	**1 (0.88) 2 (0.12)**
Cam 3	2	1 (0.90) 2 (0.10)	1	2	1	1	**2 (0.50) 3 (0.50)**	1	**1 (0.77) 2 (0.23)**
Cam 7	2	**1 (0.67) 2 (0.33)**	1	2	1	1	**2 (0.38) 3 (0.62)**	1	1
Cam 9	2	1 (0.83) 2 (0.17)	1	2	1	1	**2 (0.74) 3 (0.26)**	1	**1 (0.86) 2 (0.14)**
Elp	2	**1 (0.45) 2 (0.55)**	1	2 (0.91) 3 (0.09)	1	1	2 (0.77) 3 (0.23)	1 (0.86) 2 (0.14)	1
Ker	2	**1 (0.94) 2 (0.06)**	1	2	1	1	**2 (0.94) 3 (0.06)**	1 (0.12) 2 (0.88)	**1 (0.19) 2 (0.62) 4 (0.19)**

Values in bold indicate cases where Hardy–Weinberg equilibrium was verified.

Table 3. Diversity parameters for the main eight cocoa populations and for the whole set of Guianan cocoa material included in this study

Populations	No. polymporphic loci (%)	Average no.alleles observed per locus	Observed heterozygosity	Expected heterozygosity (Nei)	Shannon index
Overall	7 (77.7)	1.89	0.0845	0.1520	0.2508
Borne 7	3 (33.3)	1.33	0.0370	0.1173	0.1771
Cam 1	2 (22.2)	1.22	0.1111	0.0999	0.1425
Cam 13	3 (33.3)	1.33	0.0769	0.1068	0.1641
Cam 3	3 (33.3)	1.33	0.0963	0.1153	0.1735
Cam 7	2 (22.2)	1.22	0.0648	0.1015	0.1442
Cam 9	3 (33.3)	1.33	0.0762	0.1012	0.1598
Elp	4 (44.4)	1.44	0.0808	0.1387	0.2142
Ker	4 (44.4)	1.56	0.0833	0.1102	0.1962
Total 8 pop.	5 (55.5)	1.67	0.0822	0.1387	0.2219

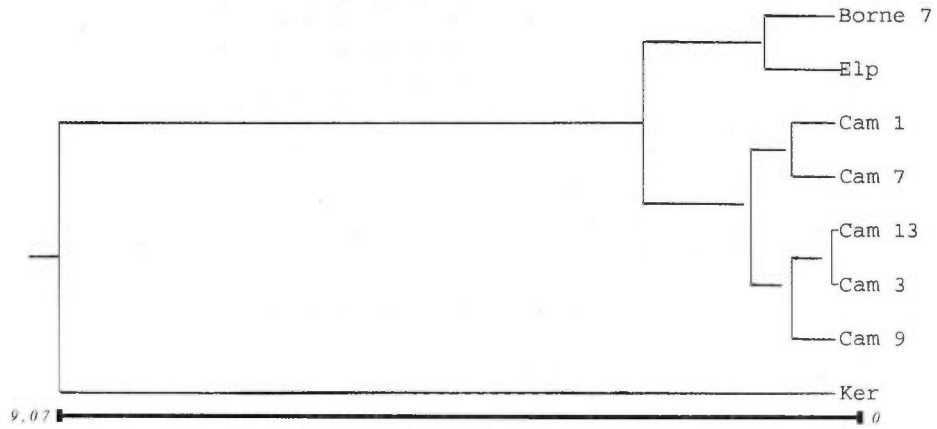

Figure 2. Dendrogram based on Nei's genetic distances (UPGMA).

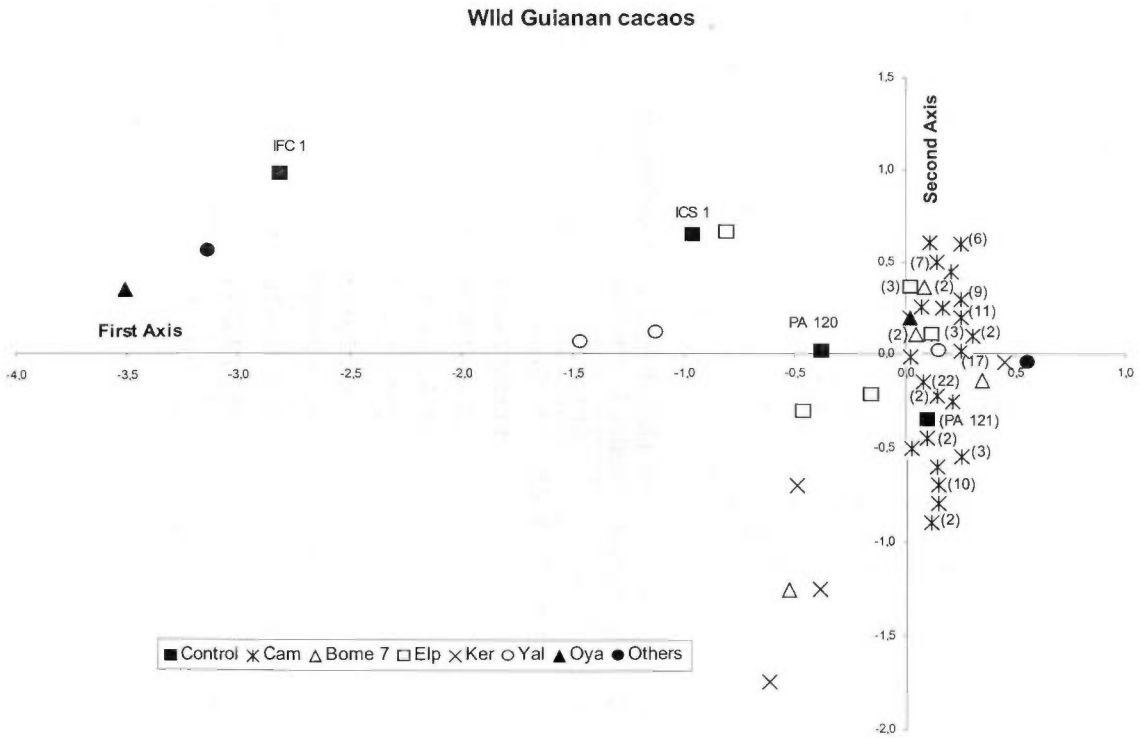

Figure 3. Plane of the first two FAC axes for all the cocoa clones (Guianan and controls). The numbers of clones from the same population sharing the same coordinates on the plane are included in parentheses.

morpho-geographical groups in the species, the average number of alleles per locus for the set of wild Guianan clones (1.9) was high. It was higher for example than that of the hybrid group of Trinitarios cultivated in America (American Trinitarios, 1.7), about the same as that for Trinitarios cultivated in Africa (African Trinitarios, 2.0), but lower than that for Upper Amazon Forasteros (between 2.0 and 2.6). The number of polymorphic loci was high, and the percentage (77.7) equal to or greater than that for all the groups studied by Lanaud (1987), except for certain Upper Amazon Forasteros (EBC and G0). However, the average observed heterozygosity (0.0845) was low, indicating substantial fixation of certain alleles. Wright's fixation index (F_{ST}), at around 20%, indicated considerable genetic differentiation (Hartl and Clark 1997) through a highly substantial reduction in heterozygosity and is in line with the values reported by Ronning and Schnell (1994) and Sounigo et al. (1996). Shannon's genetic diversity index for the whole set (0.25) was typical of allogamous perennial plants (Hamrick et al. 1992). However, a study of individuals grown from seed and maintained in a collection under suitable growing conditions tends to increase the survival of homozygotes and could therefore bias the observed heterozygosity rate (Hamrick et al. 1993).

Among the alleles revealed in our study, the presence of an allele different from the two major alleles IDH 1 and IDH 2 is worth noting. We named this allele IDH 4, but have been unable to verify whether it is original, or is identical to allele IDH 3 described by Lanaud (1987) in certain LCT-EEN clones of Upper Amazon Forasteros from Ecuador (Allen 1983; Allen 1987). This allele IDH 4 was found in the Ker population at a frequency of 0.19, whereas it was rare or absent in the set of populations studied by Lanaud (1987).

Lanaud (1987) used the same method to study cocoa populations from the Camopi (Cam 1, 3, 7, 9, 12, 13) and a comparison with her results shows that the surveys of 1990 and 1995 in three other valleys (Lachenaud and Sallée 1993; Lachenaud et al. 1997) led to considerable enrichment of wild Guianan cocoa tree representation. The average number of alleles per locus increased from 1.3 to 1.9, the percentage of polymorphic loci increased from 25.0 to 77.7 and average heterozygosity from 0.058 to 0.085. This enrichment can clearly be seen in the plane defined by the first two axes of the FAC, where the Oyapok populations (especially Oya and Ker) and the Yaloupi population (Yal) appear very distinct from the Camopi populations (Cam).

Grouping the various Cam populations indicated by the dendrogram in Figure 2 tallies with the results obtained using floral (Lachenaud et al. 1999) and agronomic descriptors (Lachenaud et al. 2001). Populations Cam 1 and 7 are clearly distinct from group 3-9-13, in which Cam 3 and 13 are very close. Likewise, the Ker population appears to be different from population Borne 7, which is located further up the same river. Charters and Wilkinson (2000) published a comparable dendrogram, isolating Ker clones from Cam and Borne 7 clones.

The plane defined by the first two axes of the FAC shows that most of the Guianan clones are very distinct from the Trinitario (ICS 1) and Amelonado (IFC 1) controls. On the other hand, one of the two Upper Amazon controls (PA 121) is observed close to several CAM clones.

Conclusion

This study of clones representing virtually all the cocoa populations surveyed in southeast French Guiana between 1985 and 1995 shows their overall richness (average number of alleles per locus, % of polymorphic loci, rare alleles), diversity (highly variable allelic frequencies) and the fixed nature of most of the alleles (low average heterozygosity, high F_{ST}). The results obtained therefore make it possible to moderate the conclusions obtained earlier on the Cam populations alone (Lanaud 1987; Sounigo et al. 1996) as to the diversity encountered in wild Guianan cocoa trees. The inclusion of six additional populations (Elp, Borne 7, Ker, Pina, Oya, Yal) shows that genetic diversity is substantial, at least in the area considered (around 7000 km^2), when compared with that for Upper Amazon G0 or LCT-EEN (Pound 1938, 1943; Allen 1983; 1987) collected from much larger areas. This observation suggests continuing surveys, both in French Guiana, where there are other zones with wild cocoa tree stands (Lachenaud et al. 1997), and also throughout the Guianan shield (Cuatrecasas 1964).

There is substantial among-population diversity (around 20%), which is typical of allogamous perennial plants that have been subjected to substantial genetic mixing, and particularly for cocoa, which occurs in its wild state in demes, where considerable fixation occurs. The wild cocoa trees of French Guiana display a low overall rate of recorded heterozygosity (0.085), which could be a considerable advantage for their use as parents in genetic improvement programmes, to obtain more uniform progenies and to maximize potential heterosis effects.

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References

Allen JB. 1983. London Cocoa Trade Amazon Project. Final Report Phase I. Cocoa Growers' Bulletin 34:1–71.

Allen JB. 1987. London Cocoa Trade Amazon Project. Final Report Phase II. Cocoa Growers' Bulletin 39:1–95.

Charters YM, Wilkinson MJ. 2000. The use of self-pollinated progenies as 'In groups' for the genetic characterization of cocoa germplasm. Theoretical and Applied Genetics 100:160–166.

Cheesman EE. 1944. Notes on the nomenclature, classification and possible relationships of cacao populations. Tropical Agriculture 22 (8):144–159.

Clément D. 1986. Cacaoyers de Guyane. Prospections. Café Cacao Thé 30 (1):11–36.

Cuatrecasas J. 1964. Cacao and its allies. A taxonomic revision of the genus *Theobroma*. Contributions from the United States

National Herbarium 35 (6). Smithsonian Institution. Washington, DC, USA.
Enriquez GA, Soria JV. 1967. Cacao cultivars register. IICA, Teaching and Research Center, Costa Rica.
Ford CS, Wadworth RM, End MJ, Hadley P. 2000. International Cocoa Germplasm Database 2000. CD version, School of Plant Sciences, University of Reading, Reading, UK.
Hamrick JL, Godt MJW, Sherman-Broyles SL. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6:95–124.
Hamrick JL, Murawsky DA, Nason JD. 1993. The influence of seed dispersal mechanism on the genetic structure of tropical tree population. Vegetatio 107/108:281–297.
Hartl DL, Clark AG. 1997. Principles of population genetics. 3rd edition. Sinauer Associates, Sunderland, MA, USA.
Lachenaud Ph. 1997. Genetic/taxonomic structuring of the *Theobroma cacao* L. species. Fresh hypotheses. Ingenic Newsletter 3:10–11.
Lachenaud Ph, Sallée B. 1993. Les cacaoyers spontanés de Guyane. Localisation, écologie, morphologie. Café Cacao Thé 37 (2):101–114.
Lachenaud Ph, Oliver G. 2001. Variability in various agronomic traits of wild cocoa trees (*Theobroma cacao* L.) from the Camopi and Tanpok basins (French Guiana). Plant Genetic Resources Newsletter 128:35–40.
Lachenaud Ph, Mooleedhar V, Couturier C. 1997. Wild cocoa trees in French Guiana. New surveys. Plantations, Recherche, Développement 4 (1):25–32.
Lachenaud Ph, Bonnot F, Oliver G. 1999. Use of floral descriptors to study variability in wild cocoa trees (*Theobroma cacao* L.) in French Guiana. Genetic Resources and Crop Evolution 46: 491–500.
Lanaud C. 1986a. Utilisation de marqueurs enzymatiques pour l'étude génétique du cacaoyer : *Theobroma cacao* L. I. Contrôle génétique et 'linkage' de neuf marqueurs enzymatiques. Café Cacao Thé 30 (4):259–266.
Lanaud C. 1986b. Utilisation de marqueurs enzymatiques pour l'étude génétique du cacaoyer: *Theobroma cacao* L. II. Etude du polymorphisme de six systèmes enzymatiques. Café Cacao Thé (Paris) 30 (4):271–280.
Lanaud C. 1987. Nouvelles données sur la biologie du cacaoyer (*Theobroma cacao* L.) Thèse de doctorat d'état, Université de Paris XI, Orsay, France.
Lanaud C, Motamayor J-C, Sounigo O. 1999. Le cacaoyer. In: P. Hamon et al., editors. Diversité génétique des plantes tropicales cultivées. Collection Repères, CIRAD, France.
Lebrun P, Chevallier MH, editors. 1990. Starch and polyacrylamide gel electrophoresis of *Hevea brasiliensis*: a laboratory manual. IRCA/CIRAD, France.
Motamayor JC, Risterucci AM, Lopez PA, Ortiz CF, Moreno A, Lanaud C. 2002. Cacao domestication I: the origin of the cacao cultivated by the Mayas. Heredity 89:380-386.
Nei M.1972. Genetic distance between populations. American Naturalist 106:283-292.
Pound FJ. 1938. Cacao and Witchbroom disease (*Marasmius perniciosus*) of South America, with Notes on Other Species of *Theobroma*. Yuille's Printerie, Trinidad and Tobago, 9–49. Reprinted 1982, Archives of Cocoa Research 1:21–64.
Pound FJ. 1943. Cacao and Witches' Broom Disease (*Marasmius perniciosus*). AL Rhodes, Government Printer, Trinidad and Tobago. Reprinted 1982, Archives of Cocoa Research 1:73–91.
Ronning CM, Schnell RJ. 1994. Allozyme diversity in a germplasm collection of *Theobroma cacao* L. The Journal of Heredity 85 (4):291–295.
Shannon CE, Weaver W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, USA.
Sneath PHA, Sokal RR. 1973. Numerical Taxonomy. Freeman, San Francisco, USA.
Sounigo O., Christopher Y, Umaharan R. 1996. Genetic diversity assessment of *Theobroma cacao* L. using iso-enzyme and RAPD analyses. The University of the West Indies, Cocoa Research Unit, Report for 1996, pp. 35–51.
Sounigo O, Ramdahin S, Umaharan R, Christopher Y. 1999. Assessing cacao genetic diversity using IE and RAPD techniques. Annual Report 1998, 25–28. Cocoa Research Unit. The University of the West Indies, St. Augustine, Trinidad and Tobago.
Yeh FC, Yang RC, Boyle T. 1999. Popgene. Microsoft Windows based freeware for population genetic analyses.

Latencia en semillas de lulo (*Solanum quitoense* Lam.) y tomate de árbol (*Cyphomandra betacea* (*Solanum betaceum*) Cav. Sendt) como aspecto básico para la conservación y el monitoreo de viabilidad de las colecciones

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Resumen

Latencia en semillas de lulo (*Solanum quitoense* Lam.) y tomate de árbol (*Cyphomandra betacea* (*Solanum betaceum*) Cav. Sendt) como aspecto básico para la conservación y el monitoreo de viabilidad de las colecciones

La determinación de la latencia y el desarrollo de protocolos de remoción de ésta es fundamental para conocer la viabilidad de las semillas en poblaciones a ser almacenadas en bancos de germoplasma y para el monitoreo de la misma a lo largo del tiempo de conservación. Con base en lo anterior se realizó un estudio con semillas de los frutales andinos lulo *Solanum quitoense* Lam., y tomate de árbol *Cyphomandra betacea* Cav. Sendt. En lulo, no se encontraron diferencias en la germinación de las semillas en frutos con diferente grado de desarrollo de pigmentos carotenoides que, al contrario, fue evidente en tomate de árbol. Con esta especie se logró un incremento sensible en la germinación con las semillas extraídas a partir de bayas con un 100% de desarrollo de pigmentos en la epidermis. Los resultados señalaron que las semillas de ambas especies presentaban fotolatencia, es decir, que son fotoblásticamente positivas, con mayor fotosensibilidad por parte del taxón lulo. Con ambos frutales se logró romper la latencia mediante germinación de las semillas bajo condiciones de luz blanca continua, con temperaturas fluctuantes, 12 horas a 28°C y 12 horas a 24°C, con un incremento sensible en la germinación de las semillas de tomate de árbol al aplicar AG3. Adicionalmente se detectó, en las dos especies, variabilidad entre accesiones como respuesta al protocolo de rompimiento de latencia.

Résumé

Effet de la dormance des graines sur la conservation du matériel génétique et le suivi de la viabilité de la morelle de Quito (*Solanum quitoense* Lam) et du tamarillo (*Cyphomandra betacea* (*Solanum betaceum*) Cav Sendt)

La caractérisation de la dormance des graines et les mécanismes de levée de dormance des graines sont des critères essentiels pour la détermination de la viabilité des graines pour les besoins de la conservation, ainsi que pour le suivi de la viabilité pendant la conservation du matériel génétique. Cette étude a été effectuée pour déterminer la dormance des graines et les mécanismes de levée de la dormance d'espèces fruitières andines, la morelle de Quito (*Solanum quitoense* Lam) et le tamarillo (*Cyphomandra betacea* (*Solanum betaceum*) Cav. Sendt.). Aucune différence dans la germination n'a été observée pour les graines de morelle de Quito obtenues à partir de fruits à différents stades de maturité, déterminés par l'évolution des pigments caroténoïdes du péricarpe. Dans le cas du tamarillo, en revanche, une meilleure germination est obtenue avec les graines extraites de fruits totalement rouges. Les résultats indiquent que les graines des deux espèces manifestent une photodormance, ce qui signifie que la germination des graines est déclenchée par une stimulation lumineuse, la photosensibilité des graines de lulo étant plus élevée que celle de tamarillo. La dormance des graines est levée en les faisant germer sous lumière blanche continue accompagnée d'une fluctuation de régime de températures : 12 h à 28°C et 12 h à 24°C. De plus, le taux de germination des graines de tamarillo augmente sous l'effet d'une application d'acide gibbérellique. Chez les deux taxons, on observe une variabilité de la réponse parmi les accessions en rapport avec la méthode utilisée pour lever la dormance des graines.

Summary

The effect of seed dormancy on germplasm conservation and viability monitoring in lulo (*Solanum quitoense* Lam) and tree tomato (*Cyphomandra betacea* (*Solanum betaceum*) Cav Sendt)

Seed dormancy characterization and the development of dormancy breaking procedures are basic requirements for determining seed viability for conservation purposes, as well as for viability monitoring during germplasm storage. This study was carried out to determine seed dormancy and to develop dormancy-breaking procedures in the Andean fruit species lulo (*Solanum quitoense* Lam) and tree tomato (*Cyphomandra betacea* (*Solanum betaceum*) Cav. Sendt.). No germination differences were found with lulo seeds obtained from fruits at different stages of ripening, monitored by the evolution of rind carotenoid pigments. With tomato tree, however, higher germination was achieved with seed extracted from fruits with 100% red-colour development. The results showed that both species exhibited seed photodormancy, which means that the seeds exhibit positive photoblastic behaviour, with higher photosensibility in lulo than tomato tree seeds. Seed dormancy was broken down by germinating the seeds under continuous white light and a fluctuating temperature regime: 12 h at 28°C and 12 h at 24°C, with an additional increase in tomato tree seed germination by application of gibberellic acid. In both taxa, response variability was found among accessions in relation to the seed photodormancy-breaking procedure applied.

Key words: *Cyphomandra betacea*, germination, lulo, seed dormancy, *Solanum betaceum*, *Solanum quitoense*, tree tomato

Introducción

El lulo y el tomate de árbol son frutales andinos con amplias posibilidades de producción, lo cual, de acuerdo con Lobo (2000) deriva de una serie de aspectos como por ejemplo:

- la presencia de amplia variabilidad genética por ser el área andina el Centro de Diversidad Primaria de estas especies;
- la existencia en la zona de nichos ecológicos apropiados para su siembra;
- la aceptación de las frutas por parte de los consumidores locales y de otras regiones del mundo;
- el déficit en el consumo de frutas por parte de los habitantes del área andina y de América Latina;
- las posibilidades agroindustriales;
- el potencial de producir desarrollo económico a nivel de pequeños productores y generar empleos a nivel de la cadena productiva;
- el ser alternativas para el reemplazo de cultivos ilícitos;
- el escaso grado de competencia, en el caso del lulo, por parte de zonas productoras de otras áreas geográficas del mundos.

El desarrollo de estas especies debe partir de una amplia base genética, aspecto que ha sido considerado crítico en varios cultivos. Al respecto, se ha señalado que, en muchas especies, la cantidad de variabilidad disponible para la selección es limitada (Cooper et al. 2001). Para contar con una base de diversidad que permita cimentar los programas de producción de cultivares con diferente constitución genética, es necesario conformar colecciones con materiales obtenidos en diversas condiciones ecológicas. Para ello son importantes, en especies con poco desarrollo, los materiales de los agricultores, quienes poseen gran variabilidad en conjunto y se caracterizan por una estabilidad amplia y una adaptabilidad estrecha (Lobo 1992).

A partir de una serie de estudios se ha indicado que actualmente persiste una diversidad genética importante a nivel de los agricultores en las áreas que corresponden a los Centros de Diversidad (Brush 1995), lo que se magnifica por el hecho de que las variedades sembradas y desarrolladas por éstos proceden de diferentes fuentes (Brown 2000). En el contexto anterior, es de anotar que la variabilidad de lulo y tomate de árbol para programas de mejoramiento depende casi exclusivamente de este último tipo de material dado el escaso o nulo desarrollo de programas de producción de variedades de dichos frutales en los países de la zona andina. Para ambas especies, con énfasis en lulo, se puede señalar que las mismas no están completamente domesticadas (Lobo 1991, 2000) y que su variabilidad y diversidad no están adecuadamente colectadas y conservadas.

El mantenimiento de las colecciones debe hacerse en forma tal que las mismas sufran cambios mínimos en su composición genética y que a su vez estén disponibles en el momento en que se requieran. Ello implica, en primer lugar, determinar la forma de conservación de los dos frutales. En el caso del lulo y el tomate de árbol, ambos presentan semilla ortodoxa (Hong et al. 1996; Lobo 1988a,b), este tipo de semillas puede secarse, sin sufrir daños, hasta un bajo contenido de humedad y colocarse en almacenamiento a bajas temperaturas. Con lo anterior se logran incrementos sensibles en la longevidad de las unidades de propagación (Hong y Ellis 1996), ello permite la conservación a largo plazo en condiciones ideales que, en el caso de semillas ortodoxas, corresponden a cuartos fríos con temperaturas de –18°C, colocando en éstos el material biológico con 3 a 7% de contenido de humedad (Sackville Hamilton y Chorlton 1997).

Un segundo aspecto fundamental para el almacenamiento de la semilla es la determinación de la viabilidad ya que se requiere conservar un mínimo de 1000 unidades vivas en la colección base, como representación de la variabilidad de cada población (FAO–IPGRI 1994). Ello implica remover latencias para evaluar el estado del germoplasma. Asimismo es importante conocer los cambios que ocurren durante el almacenamiento en el porcentaje de semillas vivas mediante pruebas de geminación. Ello permite determinar el momento en el que se deben realizar los procesos de regeneración para disminuir al máximo los cambios en frecuencias génicas por parte de las poblaciones en conservación.

En lulo y tomate de árbol se presume que existe latencia dados los valores reducidos de germinación que se obtienen con semillas recién extraídas de los dos taxa (Lobo 1988a, 1989, 1991). Con relación a la semilla de lulo, Lobo (1988b) junto a varios colaboradores, acopiaron una serie de resultados obtenidos en procesos de investigación con semillas. A partir de éstos se conoció que en cada fruto hay alrededor de 1000 unidades de propagación. Las investigaciones señalaron que la temperatura óptima para la germinación estaba alrededor de los 21°C. De la misma manera, la reseña indicó que el mejor método para extraer semillas, valorado a través de la germinación y el vigor, fue la fermentación durante 48 h y que la simiente parecía presentar latencia.

El mismo autor reportó investigaciones, con semillas de tomate de árbol, realizadas con diferentes colaboradores (Lobo 1989). Señaló como principales resultados que los frutos de esta especie contienen alrededor de 178 semillas, las que presentan máxima germinación a temperaturas entre 17 y 21°C (Lobo 1989). El autor reportó que el mejor método para extraer semilla de tomate de árbol es el tratamiento con ácido sulfúrico al 30%, por espacio de 30 minutos. Con la especie, Girard y Lobo (1982) encontraron que la madurez fisiológica de la semilla, medida a través de la máxima acumulación de materia seca, se obtenía a las 22 semanas después de la antesis floral. Lo anterior correspondió a simientes extraídas de bayas con 100% de desarrollo de pigmentos carotenoides en la cáscara.

La presente investigación se realizó con base en la posible existencia de latencia en semillas de lulo y tomate de árbol, en la necesidad de conservar y monitorear la viabilidad del germoplasma de estas especies, incluso en los cuartos fríos y en que actualmente se tiene un proceso de conformación de colecciones de estos taxa. La investigación tuvo como objetivos categorizar la latencia exhibida por las semillas de los dos frutales andinos y desarrollar protocolos de remoción del bloqueo de la germinación.

Materiales y métodos

Localización

El trabajo se realizó en el Laboratorio de Semillas del Programa de Recursos Genéticos y Biotecnología Vegetal de la Corporación Colombiana de Investigación Agropecuaria, CORPOICA, localizado en el municipio de Rionegro, Antioquia, Colombia, a 2120 msnm, con una temperatura promedio de 17°C. El sitio donde se encuentra el Centro presenta una precipitación anual de 1700 mm, una humedad relativa del 78% y pertenece a la formación ecológica bosque húmedo montano bajo (Espinal 1977).

Material biológico

Las semillas utilizadas en la investigación se obtuvieron a partir de cultivos de campo de las dos especies establecidos en el C.I. 'La Selva'. Se extrajeron semillas mediante fermentación durante 48 h y secado posterior en una cámara con aire circulante a 25°C durante 24 h, para su utilización inmediata en los estudios realizados en la presente investigación.

Ensayos realizados

Determinación del porcentaje de germinación en semillas extraídas a partir de frutos con diferente grado de maduración

Para la realización del trabajo se obtuvieron semillas a partir de frutos de lulo y tomate de árbol con 25, 50, 75 y 100% de desarrollo de los pigmentos carotenoides en la cáscara. Una vez extraída la semilla, ésta se desinfestó con hipoclorito de sodio al 2% y previcur al 1% durante tres minutos en cada una de las soluciones. Posteriormente, se llevaron a cabo pruebas de germinación en una cámara de crecimiento (Biotronette Mark III, Lab-Line) a 20°C, con un régimen de luz fluorescente durante 8 h y 16 de oscuridad, con remojo de las semillas cada dos días, en agua destilada.

En el estudio se registró la germinación cada dos días a partir del momento en que la misma fue evidente hasta el día 30 del inicio de la evaluación. El trabajo se realizó con un diseño completamente al azar, con 4 repeticiones de unidades experimentales integradas por 100 semillas. Las variables obtenidas a partir de la información fueron:

- germinación total, que corresponde al registro obtenido luego de los 30 días de instalada la prueba;
- velocidad de germinación, que se refiere al tiempo en días transcurrido hasta lograr el 50% de germinación de las semillas en cada unidad experimental;
- índice de vigor, calculado a partir del procedimiento sugerido por Maguire (1962), que es la sumatoria de los porcentajes de germinación obtenidos a través de las diferentes lecturas, divididos por el número de días transcurridos hasta el respectivo recuento.

Para efectos del análisis estadístico, los datos de germinación total se transformaron mediante el procedimiento angular del arcoseno, que se recomienda con este tipo de variables (Gómez 1997). A los promedios se les aplicó pruebas de partición de promedios mediante la metodología de la Diferencia Mínima Significativa (Prueba de Student), con una confiabilidad del 95%.

Tratamientos de remoción de latencia

El trabajo se realizó con dos accesiones por especie. En éste se evaluaron diferentes protocolos para remoción de latencia, que se presentan en la Tabla 1. La germinación se evaluó en una cámara climática (Biotronette Mark III, Lab-Line) en que se tuvo luz fluorescente continua y regímenes de temperatura alternos de 28°C durante 12 h y 24°C por espacio de 12 h.

Para la prueba de germinación las semillas del estudio, obtenidas a partir de frutos con completo desarrollo de color en la epidermis, fueron sometidas al mismo tratamiento de desinfestación utilizado en el ensayo anterior. El estudio se llevó a cabo con un diseño completamente al azar con 4 repeticiones, con unidades experimentales de 100 semillas. La información se registró durante 30 días. Como variables se registraron la germinación total, la velocidad de germinación y el índice de vigor en la forma que se describió para el ensayo precedente.

En la Tabla 1 se incluyen los tratamientos evaluados, con las dos especies, para la liberación de la latencia de las semillas.

Tabla 1. Tratamientos evaluados con semillas de lulo y tomate de árbol para remoción de latencia(s)

Número de tratamiento	Tipo de tratamiento	Procedimiento
T1	Luz continua	Semilla sin tratamiento adicional alguno
T2	Escarificación mecánica	Lija de agua N° 260
T3	Escarificación química	Ácido sulfúrico 0.3%, 5 minutos
T4	Nitrato de potasio	Humedecimiento cada dos días con una solución al 0.2% durante la prueba
T5	Temperaturas bajas	Almacenamiento previo a 5°C durante 15 días
T6	Temperaturas alternas	Almacenamiento previo a temperaturas alternas: 5°C durante 16 h y 28°C durante 8 h
T7	Ácido giberélico	AG3, 1000 ppm durante 24 h
T8	Temperaturas altas	Pretratamiento a 50°C durante 15 h
T9	Testigo sin iluminación	Cajas de petri cubiertas con polietileno negro en la cámara climática

Germinación obtenida con diferentes accesiones de lulo y tomate de árbol en dos ambientes

El experimento se llevó a cabo con semillas obtenidas a partir de frutos con 100% de desarrollo de pigmentos carotenoides en la cáscara. En el mismo se evaluó la germinación de 11 accesiones por especie. El trabajo se condujo en dos regímenes ambientales diferentes: el primero en una cámara climática (Biotronette Mark III, Lab-Line) con luz fluorescente continua y temperatura alterna de 28°C durante 12 h y 24°C durante 12 h y el segundo con luz fluorescente durante 12 h y oscuridad durante 12 h bajo condiciones ambientales de laboratorio, es decir con una temperatura promedio de 20°C durante el período de luz y de 18°C en el de oscuridad.

Las semillas del estudio se sometieron al mismo tratamiento de desinfestación utilizado en los ensayos anteriores. El trabajo se realizó con un diseño completamente al azar con 4 repeticiones. Cada unidad experimental estuvo integrada por 100 semillas. La información se registró durante 30 días. En el estudio se obtuvieron las variables germinación total, velocidad de germinación e índice de vigor, en la forma descrita para los estudios precedentes.

Resultados y discusión

Determinación del porcentaje de germinación en semillas extraídas a partir de frutos de lulo y tomate de árbol con diferente grado de maduración

En el estudio se buscó determinar el momento más apropiado para extraer la semilla de los frutos, lo cual está generalmente relacionado con el máximo desarrollo de color durante la maduración en las bayas. En la Tabla 2 se incluyen, para las dos especies, la germinación, la velocidad de germinación y el índice de vigor con la semilla extraída a partir de frutos con diferentes estados de maduración, de acuerdo con el desarrollo de carotenoides en la epidermis.

Como puede apreciarse en la Tabla 2, con la especie lulo (*Solanum quitoense*) no hubo diferencias para las variables de germinación y vigor entre semillas extraídas de frutos con diferente desarrollo de carotenoides en la cáscara. En contraste, con tomate de árbol (*Cyphomandra betacea*) se pudo apreciar que las semillas procedentes de frutos con el 100% de desarrollo de color en el pericarpio, presentaron una germinación y un índice de vigor sensiblemente superiores a los obtenidos con aquéllas en otros estados de evolución de color externo de los frutos. Con esta especie no se logró registrar la velocidad de germinación ya que no se obtuvieron germinaciones del orden del 50%.

Las diferencias en el patrón de germinación y en el del índice de vigor entre las semillas de lulo y las de tomate de árbol podrían explicarse con base en la presencia de embriones cuyo desarrollo permite la germinación en todas las semillas de lulo que no presentan latencia, extraídas de frutos con más del 25% de desarrollo de carotenoides en la cáscara. En contraste, en las semillas de tomate de árbol sin latencia, los resultados apuntan a que el desarrollo de los embriones se incremente cuando las bayas pasan del 75% al 100% de presencia de carotenoides en la epidermis. Con estas semillas se logra germinación al proveerse condiciones adecuadas para que el evento ocurra. Al respecto, Walck y sus colaboradores (2002) señalaron que las semillas recién maduradas de algunas especies tienen embriones que son muy pequeños respecto al tamaño de la semilla y tienen mucho endosperma y que aún cuando estos embriones tienen cotiledones y radícula distinguibles, deben crecer hasta una longitud crítica antes de que la radícula emerja de la semilla. Raghavan (2002) postuló que la embriogénesis termina con el desarrollo de la semilla dentro del fruto y que luego de la desecación de ésta el embrión entra en un período de quiescencia o de latencia. El autor (Raghavan 2002) agregó que las semillas quiescentes germinan cuando tienen las condiciones apropiadas para retomar el crecimiento del embrión.

En concordancia con el resultado logrado con la semilla de tomate de árbol, en la investigación presente Girard y Lobo (1982), en estudios realizados con la especie, reportaron germinación e índice de vigor máximos con semilla en completa madurez fisiológica, la cual se obtuvo a partir de

Tabla 2. Germinación, velocidad de germinación e índice de vigor obtenido con semillas de lulo y tomate de árbol extraídas de frutos en diferentes estados de maduración, medido por el porcentaje de desarrollo de carotenoides en la cáscara

Especie	Maduración fruto (%)	Germinación total (%) †,‡	Velocidad de germinación en días †	Índice de vigor †
Lulo	25	60.2a	19.3a	4.1a
Lulo	50	53.8a	22.7a	3.3a
Lulo	75	53.0a	21.3a	3.6a
Lulo	100	64.6a	22.7a	3.9a
Tomate de árbol	25	1.4b	– §	0.01b
Tomate de árbol	50	10.9b	– §	0.2b
Tomate de árbol	75	11.3b	– §	0.2b
Tomate de árbol	100	45.2a	– §	1.9a

† Entre promedios marcados con la misma letra, para cada variable y por especie, no hay diferencias estadísticas significativas (Prueba de Student p=0.05).
‡Diferencias basadas en el arcoseno en el caso de los porcentajes de germinación.
§No se alcanzó el 50% de la germinación.

frutos con completo desarrollo de pigmentos carotenoides en la epidermis.

Los valores máximos de germinación, logrados con lulo y tomate de árbol, 64.6 y 45.2% respectivamente, apuntan a que en ambos taxa existan semillas viables germinables y no germinables. En la categoría de las no germinables debe haber semillas latentes dado que las evaluaciones se realizaron con unidades de propagación recién extraídas que se encontraban en madurez fisiológica o próximas a dicho estado. Al respecto se ha indicado que el grado de latencia está monitoreado en cierta medida por la tasa de germinación y el inicio de ésta luego de la imbibición (Schutz 2000). La latencia es un mecanismo de supervivencia de las especies en condiciones naturales, a través de éste se pospone la germinación hasta que se presenten períodos favorables para el crecimiento de las plántulas (Anderson y Milberg 1998). La latencia previene la germinación cuando hay poca probabilidad de que las plántulas sobrevivan (Mathews 1976; Allen y Meyer 1998; Baskin y Baskin 2001). Schutz (2000) postuló que las características de la latencia de una especie o de una población particular, son adaptaciones a un hábitat especial donde la especie o la población se manifiesta (Schutz 2000).

Durante la domesticación de las plantas se ha practicado, en forma generalizada, una selección antrópica negativa de la latencia, que busca máxima germinación en períodos de tiempo reducidos. Ello conduce a que las plantas cultivadas difieran en cuanto a germinación y latencia de sus progenitores silvestres (Bewley y Black 1982). El lulo y el tomate de árbol son plantas no completamente domesticadas que conservan latencia en las semillas. En estas especies no se ha practicado selección alguna para aumentar la germinación de las semillas recién extraídas.

La afirmación de que los dos frutales andinos son taxa en proceso de domesticación, que conservan latencia en su germoplasma, puede sustentarse con base en varios aspectos como por ejemplo:

- el elevado número de unidades de reproducción por fruto en lulo y moderadamente alto en tomate de árbol, 1000 y 178 por baya respectivamente (Lobo 1988, 1989);
- la demanda reducida en cuanto a número de plántulas para siembra por sitio de producción ya que las plantaciones de estas dos especies, en la región andina, se llevan a cabo en áreas de poca extensión, por lo cual las necesidades individuales de semilla se satisfacen con pocas bayas;
- la baja participación del material de siembra en la estructura de costos de producción;
- la oferta nula o escasa de cultivares mejorados por vía de semilla sexual ya que la existencia de programas de mejoramiento generalmente se une a la selección para la eliminación de latencia en las semillas, aspecto de calidad que incide en la demanda del material para siembra por parte de los cultivadores.

Con base en los resultados reseñados se decidió, para la realización de los estudios posteriores, extraer las semillas a partir de frutos con completo desarrollo de pigmentos carotenoides en la epidermis.

Tratamientos de remoción de latencia

Como puede apreciarse en la Tabla 3, con las dos accesiones de lulo evaluadas se obtuvo completa germinación en la cámara climática sin aplicación previa de procedimientos de remoción de latencia. Igualmente, con las semillas de este tratamiento se obtuvo un elevado índice de vigor y una germinación más rápida, evaluada a través de la velocidad de germinación. Las semillas de este frutal, sin tratamiento previo, colocadas a germinar en la cámara en condiciones de oscuridad, exhibieron bajos valores de germinación y no se obtuvo germinación alguna con las simientes incubadas en condiciones de medio ambiente. En el estudio la adición de ácido giberélico produjo una velocidad de germinación igual a la obtenida con las semillas, sin tratamiento previo

Tabla 3. Germinación, velocidad de germinación e índice de vigor obtenido con semillas de dos accesiones de lulo, con aplicación de tratamientos de remoción de latencia

Tratamiento	Accesión 940125			Accesión 940128		
	Germin. (%)	Índice de vigor	Velocidad de germin.	Germin. (%)	Índice de vigor	Velocidad de germin.
CL†, sin tratamiento previo	100.0a	6.5a	14.0a	100.0a	6.5a	14.0a
CL, estratificación papel de lija	98.6a	4.7b	16.0ab	98.7a	5.3ab	19.5bc
CL, escarificación H_2SO_4	95.0a	4.7b	16.0ab	94.1a	4.8bc	17.0ab
CL, nitrato de potasio	27.7c	2.1d	-	42.8b	2.4d	-
CL, bajas temperaturas	77.0b	2.7cd	20.5bcd	95.5a	4.1bc	19.5bc
CL, temperaturas alternas	87.4ab	3.7bc	18.0abc	95.4a	3.9c	18.0ab
CL, AG_3	100.0a	6.7a	14.0a	100.0a	6.3a	14.0a
CL, altas temperaturas	86.6ab	3.9bc	16.5ab	60.5b	4.1bc	17.5ab
CSL‡	9.1c	0.5e	-	4.6c	0.3e	-
Medio ambiente, testigo§	0.0d	0.0e	-	0.0d	0.0e	-

† CL: Cámara climática (28°C/12 h, 24°C/12 h), luz blanca continua.
‡ CSL: Cámara climática (28°C/12 h, 24°C/12 h), sin luz.
§ Luz difusa 20°C/12 h, 19°C oscuridad 12 h.

Tabla 4. Germinación, velocidad de germinación e índice de vigor obtenido con semillas de dos accesiones de tomate de árbol luego de la aplicación de tratamientos de remoción de latencia

Tratamiento	Accesión 285030			Accesión 285007		
	Germin. (%)	Índice de vigor	Velocidad de germin.	Germin. (%)	Índice de vigor	Velocidad de germin.
CL[†], sin tratamiento previo	73.6a	1.7b	22.0b	89.0ab	2.1c	21.0cd
CL, estratificación papel de lija	59.8bc	1.7b	22.5b	84.5[a]b	2.6bc	12.5[a]
CL, escarificación H_2SO_4	68.6ab	2.0ab	20.5b	87.2[a]b	4.3a	15.0ab
CL, nitrato de potasio	21.7d	0.5c	–	54.0d	1.7c	–
CL, bajas temperaturas	54.4bc	1.4b	25.0bc	90.2[a]b	2.7bc	22.0cd
CL, temperaturas alternas	80.0a	2.1ab	21.0b	81.7bc	3.9a	19.5bc
CL, AG_3	78.1a	2.5a	15.0a	95.5a	3.4ab	13.5a
CL, altas temperaturas	47.1c	1.6b	28.0c	72.0c	1.9c	24.0cd
CSL[‡]	5.1d	0.1c	–	2.0e	0.1d	–
Medio ambiente, testigo[§]	9.8d	0.3c	–	49.0d	0.2d	–

[†] CL: Cámara climática (28°C/12h, 24°C/12h), luz blanca continua.
[‡] CSL: Cámara climática (28°C/12h, 24°C/12h), sin luz.
[§] Luz difusa 20°C/12 h, 19°C oscuridad 12 h.

alguno, imbibidas en la cámara climática en condiciones de luz (Tabla 3).

Con el tomate de árbol, y como puede verse en la Tabla 4, los valores máximos de germinación se obtuvieron con la semilla no tratada en forma previa y colocada a incubar en la cámara climática, sin diferencias significativas al respecto, con la aplicación de algunos procedimientos de remoción. Con la especie se pudo apreciar, en ambas accesiones, una mayor velocidad de germinación al tratar las semillas en forma previa con AG3. De la misma manera, con este tratamiento se obtuvo un alto índice de vigor, que fue significativamente superior al logrado en la cámara climática con las semillas sin tratamiento previo. Las semillas de tomate de árbol, sin tratamiento de remoción de latencia, colocadas a germinar en condiciones de oscuridad en la cámara climática, presentaron baja germinación mientras que las incubadas en medio ambiente presentaron una germinación e índice de vigor sensiblemente inferiores a las de los mejores tratamientos. Éstas no alcanzaron el 50% de germinación durante el período de realización de la prueba.

A partir de los resultados obtenidos, es decir, amplias diferencias en la germinación entre las semillas incubadas bajo luz continua y sin luz, se puede inferir que el lulo y el tomate de árbol presentan fotolantencia. Se trata de un mecanismo regulado por la calidad de la luz a través del pigmento fitocromo (Furuya y Scafer 1996; Neff et al. 2000) que ha sido descrito en especies que forman bancos de semillas en los bosques tropicales (Vásquez Yanes y Orozco Segovia 1994). Se ha reportado fotolatencia en especies solanáceas. De esta manera, Kasperbauer (1968) y Leubner-Metzger y Meins (2000) señalaron niveles variables de fotolatencia en lotes de semillas de diferentes variedades de tabaco, fenómeno que también ha sido observado en tomate (Kasperbauer 1968; Leubner-Metzgeer y Meins 2000).

Desde el punto de vista de la germinación, las semillas de los dos frutales andinos son fotoblásticamente positivas, es decir que requieren de luz blanca, rica en el espectro rojo, para su germinación (Vásquez Yanes y Orozco Segovia 1993; Vásquez Yanes et al. 1996). Al respecto Casal y Sánchez (1998) señalaron que la germinación de las semillas en muchas especies depende de la luz lo cual, como se anotó, se ha relacionado con el pigmento fitocromo (Hennig et al. 2002). Éste se sintetiza durante la formación de las semillas y puede estar en la forma activa Pfr o inactiva Pr, ello depende de las propiedades ópticas de las cubiertas de la semilla o del fruto y del grado de sombrío del follaje adyacente, que absorbe la luz roja en forma notoria, en comparación con la infraroja (Casal y Sánchez 1998).

El hecho de que las dos especies sean fotoblásticamente positivas apoya la afirmación de que éstas no están completamente domesticadas. En este sentido Baskin y Baskin (1988) señalaron que la mayoría de las especies cuyas semillas responden a la luz no están domesticadas. Mayor germinación en condiciones tanto de luz como de oscuridad ha sido reportada en un amplio conjunto de especies (Grime et al. 1981; Baskin y Baskin 1985, 1988, 2001; Roberts 1986; Vásquez Yanes et al. 1996; Schutz y Rave 1999).

Las semillas de lulo y tomate de árbol son pequeñas, 2.20 y 6.10 mg por unidad respectivamente (Valencia y García 1977). Se ha encontrado que las especies con semillas pequeñas, como es el caso de las dos solanáceas estudiadas, tienen a menudo mayor sensibilidad a la luz para su germinación, presumiblemente para evitar que ésta ocurra a una profundidad, en semillas enterradas, en la que las plántulas no alcanzarían a emerger (Pons 1992) ya que la luz sólo puede penetrar unos pocos centímetros en el suelo (Egley 1984). De igual manera, la fotosensibilidad de estas semillas pequeñas les permite germinar en sitios en donde se encuentran claros en la vegetación, ya que la calidad y cantidad de luz que alcanza el suelo son afectadas sensiblemente por la densidad del dosel y por los residuos de cultivos que han caído en la superficie del bosque (Dyer 1995).

La acción del fitocromo sobre la germinación de las semillas se ha relacionado con el balance entre la capacidad del embrión para crecer y con el obstáculo que proveen los tejidos circundantes de éste (Bewley y Black 1982). Leubner-Meztger (2001) ha considerado que el proceso es limitante en especies de algunas familias, entre las que se encuentran a las solanáceas. En estas últimas el factor se ha estudiado en tomate, tabaco, pimentón y taxa del género *Datura*, que poseen semillas endospérmicas. El autor (Leubner-Meztger 2001) señala que el fitocromo actúa sobre la capacidad del embrión para crecer y sobre la liberación del impedimento físico de los tejidos circundantes de éste. Al respecto Sánchez y De Miguel (1992) indicaron que en semillas de *Datura ferox*, la forma activa del fitocromo, Pfr, promueve la germinación, incrementa el crecimiento del embrión y reduce los obstáculos impuestos por el endosperma. Con las especies solanáceas reseñadas se indicó que para que ocurra la germinación es necesario que la resistencia del endosperma micropilar sea baja (Leubner-Metzger 2001); ello se alcanza mediante el debilitamiento de la pared celular por acción de hidrolasas específicas (De Miguel y Sánchez 1992; Bewley 1997).

El hecho de que la aplicación de AG3 haya producido una mayor velocidad de germinación en tomate de árbol podría deberse a una acción complementaria de la luz y la hormona. Al respecto se ha señalado, con semillas de tabaco, que la luz y las giberelinas están relacionadas con el mismo patrón, que involucra el fitocromo, a través de una acción antagónica a los efectos del ácido absícico, ABA. Ello es necesario para dos funciones, liberación de la latencia y promoción de la ruptura del endosperma (Leubner-Metzger 2001). Se ha demostrado que la luz roja regula la biosíntesis de giberelinas, las cuales inducen la formación de hidroxilasas en semillas en germinación de lechuga y *Arabidopsis* (Toyomasu et al.1993, 1998; Yagamuchi et al. 1998; Kamiya y García Martínez 1999).

La diferencia en el efecto del ácido giberélico entre el lulo y el tomate de árbol, con relación a la velocidad de germinación, podría atribuirse al hecho de que ambas especies, pese a haber demostrado fotoblastismo positivo (Tablas 3 y 4), requieren diferentes temperaturas para la germinación. Ésta fue aparentemente óptima para lulo, lo cual se desprende de la alta germinación y velocidad de germinación con las semillas de esta especie, incubadas en luz, sin efecto adicional, en las variables con la aplicación de giberelinas. En cambio, en el caso de tomate de árbol, es posible que el régimen de temperatura, 28°C/12 h y 24°C/12 h, no haya sido el mejor conjunto de condiciones para la germinación. Lo anterior se tradujo en un incremento en la velocidad de esta última como respuesta a la aplicación del ácido giberélico. Al respecto se ha señalado que la calidad de la luz y la temperatura parecen afectar a la síntesis y a la sensitividad de las giberelinas antes y durante la germinación (Derkx y Karssen 1993). En concordancia con lo anterior, Lobo (1988, 1989) en estudios previos realizados sin luz artificial, reportó una temperatura óptima menor para la germinación del tomate de árbol con relación a la del lulo.

En el estudio se apreció, con lulo y tomate de árbol, que la germinación y la velocidad de germinación fueron reducidas en forma sensible por la aplicación de nitrato de potasio, lo que contradice resultados obtenidos por otros investigadores con diversas especies fotoblásticas. De esta manera Williams (1983) señaló que las soluciones de nitrato y, en especial, las de nitrato de potasio, estimulaban la germinación y podían liberar la latencia. En igual sentido Taylorson (1969) reportó aumentos en la germinación con aplicación de nitratos, lo que se manifestó especialmente cuando éstos se aplicaron en secuencia o en combinación con la luz. En línea con lo anterior, Hilhorst, Smith y Karssen (1986) señalaron una fuerte interacción de la luz y los nitratos en la germinación de semillas de la especie *Sisymbrium officinales* Scop., siendo indispensable la presencia de ambos factores para que ésta ocurriera. Hilshort (1990) en trabajos realizados con la misma especie, concluyó que el nitrato puede funcionar como un cofactor para la acción del fitocromo y sugirió que las cantidades óptimas del nitrato pueden generar más receptores activos del fitocromo o inhibir la inactivación de dichos receptores. Fleck y su grupo (2001) postularon que la aplicación de fertilizantes nitrogenados podría estimular la germinación de arvenses, facilitando la aplicación de métodos de control de las mismas a nivel de campo.

El resultado obtenido en la investigación presente, al aplicar el nitrato, podría explicarse como un efecto tóxico derivado de la concentración de la solución del químico aplicada, lo que fue sugerido por Mayer y Poljakoff-Mayber (1989) al discutir efectos negativos de los nitratos sobre la germinación de algunas semillas. En igual sentido, Fleck y sus colegas (2001) reportaron disminución en la germinación de semillas de la especie fotosensible *Bidens pilosa* con aplicación de sulfato de amonio y de *Sida rombifolia* con nitrato de amonio, que atribuyeron al efecto tóxico de los nitratos sobre las semillas con las concentraciones mayores, evaluadas en los estudios (0.4%).

Germinación obtenida con diferentes accesiones de lulo y tomate de árbol en dos ambientes

Como se aprecia en la Tabla 5, con lulo se presentó una mayor germinación en las semillas incubadas en la cámara climática con luz continua, bajo condiciones de 28°C durante 12 h y 24°C por 12 h en todas las accesiones, con excepción del material 940150, con el que no se obtuvo germinación alguna en las dos condiciones evaluadas, con variación entre accesiones en los niveles de germinación. Asimismo, con las accesiones con las que se obtuvo emergencia de la radícula a través de la testa en ambos tratamientos, se logró un índice de vigor sensiblemente superior en la cámara climática en comparación con el medio ambiente.

En el caso del tomate de árbol, como se aprecia en la Tabla 6, se obtuvo germinación con todas las accesiones en ambas condiciones ambientales, con variación en los valores tanto a nivel de genotipos como entre condiciones de germinación y con una tendencia de mayor germinación en la cámara climática; ello fue evidente en 9 de los 11 materiales estudiados. Con las dos poblaciones restantes no se pudo apreciar una diferencia significativa al respecto. Con la especie se consiguieron diferencias significativas en el índice de vigor

Tabla 5. Germinación, velocidad de germinación e índice de vigor obtenido con diferentes accesiones de lulo Solanum quitoense Lam. en dos ambientes

Accesión	Cámara climática luz continua 28°C, 12h, 24°C, 12h			Medio ambiente 20°C, luz 12h, oscuridad, 18°C, 12h		
	Germin. (%)	Índice de vigor	Velocidad de germin.§	Germin. (%)	Índice de vigor	Velocidad de germin.
940167	60.6 Ca	3.5 Da	23.0 Fa	0.0 Cb	0.0 Bb	– †
940059	100.0 Aa	7.7 Aa	12.5 Aa	0.0 Cb	0.0 Bb	– †
940120	100.0 Aa	6.6 Ba	15.0 Ba	0.0 Cb	0.0 Bb	– †
940128	100.0 Aa	5.8 Ba	17.0 CDa	0.0 Cb	0.0 Bb	– †
940057	80.0 Ba	5.7 BCa	15.5 BCa	0.0 Cb	0.0 Bb	– †
940127	99.8 Aa	5.9 Ba	18.0 DEa	1.5 BCb	0.1 Bb	– †
940125	53.0 Ca	3.9 Da	– †	0.8 Db	0.0 Bb	– †
940101	91.0 Aa	4.9 Ca	19.5 Ea	0.0 Db	0.0 Bb	– †
940162	100.0 Aa	7.6 Aa	14.0 ABa	42.00 Ab	1.9 Ab	– †
940155	100.0 Aa	4.8 Ca	22.5 Fa	3.8 Bb	0.2 Bb	– †
940150	0.0 Da	0.0 Ea	– †	0.0 Ca	0.0 Ba	– †

† No se alcanzó el 50% de la germinación.
§Diferencias basadas en el arcoseno en el caso de los porcentajes de germinación.
Entre promedios marcados con la misma letra mayúscula, para cada variable y entre promedios marcados con la misma letra minúscula, por variable y accesión, no hay diferencias estadísticas significativas (Prueba de Student p=0.05).

Tabla 6. Germinación, velocidad de germinación e índice de vigor obtenido con diferentes accesiones de tomate de árbol Cyphomandra betacea Cav. Sendt. en dos ambientes

Accesión	Cámara climática luz continua 28°C, 12 h, 24°C, 12 h			Medio ambiente 20°C, luz 8 h, oscuridad 12 h		
	Germin. (%) §	Índice de vigor	Velocidad de germin.	Germin. (%) §	Índice de vigor	Velocidad de germin.
285007	92.0Aa	4.1Aa	20.5Aa	53.7Bb	1.7b	– †
285030	53.7Da	1.4Ca	30.0Ba	6.8Eb	0.2b	– †
285020	57.3Da	1.0Ca	30.0a	31.1Db	0.8[a]	– †
285023	90.0ABa	4.8Aa	20.0Bb	79.1Aa	3.7[a]	14.5Aa
285028	25.4Ea	0.6a	– †	16.1Ea	0.4[a]	– †
285031	78.8BCa	2.5Ba	21.5Aa	57.0Bb	2.6[a]	30.0Bb
285032	85.8ABa	3.8Aa	19.0Aa	70.1Ab	3.6[a]	19.0Aa
285047	72.7Ca	2.4Ba	19.0Aa	49.3BCb	1.1b	– †
285051	70.7Ca	2.3Ba	22.0Aa	54.0Bb	1.8[a]	30.0Ba
285013	80.5ABCa	2.4Ba	19.A0a	55.1Bb	1.9[a]	30.0Bb
285052	88.6ABa	2.7Ba	19.5Aa	37.8CDb	1.0b	– †

† No se alcanzó el 50% de la germinación.
§ Diferencias basadas en el arcoseno en el caso de los porcentajes de germinación.
Entre promedios marcados con la misma letra mayúscula, para cada variable y entre promedios marcados con la misma letra minúscula, por variable y accesión, no hay diferencias estadísticas significativas (Prueba de Student p=0.05).

y la velocidad de germinación entre accesiones y dentro de las accesiones en los dos conjuntos ambientales de germinación. Al respecto, en ninguna ocasión el índice de vigor fue superior con las semillas incubadas en condiciones ambientales con relación a las imbibidas en la cámara climática, con obtención de una velocidad mayor de germinación en una de las accesiones, bajo condiciones de medio ambiente.

Los resultados obtenidos indican una mayor fotosensibilidad en las semillas de lulo en comparación con las de tomate de árbol, ello se colige del hecho de que con el tomate de árbol se obtuvo germinación, con todas las accesiones, en condiciones ambientales con 12 h de iluminación con luz blanca y, en lulo, 8 de los 11 genotipos no germinaron incubados en este conjunto de condiciones. Al respecto se ha señalado que las especies con semillas más pequeñas tienen requerimientos de luz mayores que las de semillas de mayor tamaño (Schutz 2000), presentando semillas más pequeñas el lulo en comparación con las del tomate de árbol (Lobo 1988, 1989).

Otro aspecto que podría influir en lo anterior es que las condiciones de luz y temperatura óptimas para la germinación de las dos especies sean diferentes. Lobo (1988, 1989) reportó una menor temperatura óptima para la germinación de tomate de árbol, sin luz artificial, respecto a la utilizada en la cámara climática que es inferior a la del lulo sin iluminación adicional. Esto, en unión con lo obtenido en la investigación presente, conduce a recomendar la evaluación de diferentes períodos de luz continua y de temperaturas alternas que simulen ciclos diurnos y nocturnos para el tomate de árbol, especie con la que se obtuvieron menores niveles de germinación. En el sentido anterior, Schutz y Milberg (1997), Vleeshowers et al. (1995) y Young et al. (1991) señalaron que a menudo en los estudios de niveles de latencia se emplea un conjunto reducido de ambientes, siendo recomendable evaluar un número amplio de condiciones. Baskin y Baskin (2001), indicaron que los requerimientos de luz pueden variar con la temperatura. Para sustentar los resultados anteriores obtenidos por otros investigadores, los autores incluyeron las especies *Bidens pilosa*, *Cynodon dactilon*, *Deschampsia caespitosa*, *Nicotiana tabacum* y *Typha latifolia* (Evenari 1952 y Morinaga 1926, citados por Baskin y Baskin 2001; Toole et al. 1957; Felippe 1978).

Las diferencias en el grado de latencia, monitoreadas a través de la germinación, tanto en tomate de árbol como en lulo, indican que en las accesiones evaluadas existe polimorfismo para esta característica. Dicha variabilidad ha sido reportada aún a nivel de plantas individuales en poblaciones de varias especies (Strand 1989; Pérez García 1993; Philippi 1993; Mildberg y Anderson 1994; Evans y Cabin 1995; Meyer et al. 1995). Al respecto se ha indicado que el nivel de latencia en las semillas está determinado por otros factores además del origen genético, entre los que se encuentran: el ambiente materno durante la maduración, la edad fisiológica de la planta madre y la posición de la semilla en ésta (Fenner 1991; Baskin y Baskin 2001).

Cabe señalar que cuando se manipula la germinación de un conjunto de poblaciones, como es el caso actual de las colecciones de lulo y tomate de árbol, es de esperarse que haya variabilidad en cuanto a la germinación y la latencia, entre accesiones, por polimorfismo genético entre éstas. Todo ello ha sido comprobado en un número importante de especies y diferencias en las condiciones ambientales requeridas para la germinación entre subpoblaciones de una misma especie (Baskin y Baskin 2001) que están referidas a la temperatura, a la relación luz-oscuridad, a la sensibilidad al fotoperíodo, a la iluminación y a la calidad de luz (diversos investigadores, citados por Baskin y Baskin 2001).

En el sentido anterior, se han reportado diferencias en la velocidad de germinación entre semillas de diferentes poblaciones de una especie (Baskin y Baskin 2001), lo cual está en línea con los resultados obtenidos con esta variable con las semillas de lulo y tomate de árbol (Tablas 5 y 6). En el tomate de árbol se detectaron dos genotipos en los que la velocidad de germinación fue superior tanto en condiciones de medio ambiente como en la cámara climática, lo que no se reflejó en mayor germinación. Los resultados indican un comportamiento diferencial para las dos características.

Las diferencias encontradas, en cuanto a germinación, entre poblaciones de cada una de las especies estudiadas, indican que es importante clasificar, en grados de profundidad, la latencia de las accesiones de ambas colecciones, lo que debe complementarse con la medición de la heredabilidad de la característica. Al respecto, el componente hereditario de este atributo ha sido demostrado en una serie de especies, entre las que se encuentran algunas solanáceas como son el tomate y la papa (Whittington et al. 1965, Simmonds 1964, citados por Baskin y Baskin 2001). La categorización permitiría desarrollar protocolos para cada clase, con lo cual se podrían realizar monitoreos de germinación concordantes con la viabilidad de las semillas en conservación. La evaluación de lo anterior en una sola localidad tiene dos ventajas: (a) eliminación de la interacción genotipo-ambiente ya que el estudio se realiza en un solo sitio, (b) categorización de la latencia en el lugar en donde se tienen las colecciones de campo y en el que se realiza la renovación de la semilla, lo que valida la medida de heredabilidad obtenida para la latencia en la localidad en que se tienen los bancos de germoplasma.

Conclusiones

Con las semillas de lulo extraídas a partir de frutos con diferente grado de evolución de pigmentos carotenoides en la epidermis no hubo diferencias en cuanto a la germinación total, a la velocidad y al vigor de germinación a partir del momento en que las bayas exhibieron un 25% de color en la cáscara.

En tomate de árbol se logró un incremento sensible en la germinación y el vigor de germinación en las semillas extraídas en frutos con el 100% de pigmentos carotenoides en la epidermis.

Las semillas de lulo y tomate de árbol son fotoblásticamente positivas y exhiben fotolatencia.

En la presente investigación se logró rompimiento de la latencia de los dos frutales andinos, al colocar a germinar las semillas en una cámara climática, con luz blanca continua, con un regimen fluctuante de temperaturas: 12 h a 28°C y 12 h a 24°C.

Adicionalmente, con tomate de árbol se logró un incremento en la velocidad y vigor de germinación con aplicación previa de AG3 a la semilla (1000 ppm durante 24 h).

El nitrato de potasio, en la dosis empleada en el estudio, redujo sensiblemente la germinación y la velocidad de germinación en las dos especies.

Tanto en lulo con en tomate de árbol se presentó polimorfismo para la germinación entre accesiones al aplicar el protocolo de luz continua con temperatura fluctuante. Ello señala que es necesario clasificar las subpoblaciones en conservación por profundidad de latencia y estudiar posibles protocolos para cada una de las categorías de ésta.

Referencias

Allen PS, Meyer SE. 1998. Ecological aspects of seed dormancy loss. Seed Science Research 8:183–191.

Andersson L, Milberg P. 1998. Variation in seed dormancy among mother plants, populations and years of seed collection. Seed Science Research 8:29–38.

Baskin CC, Baskin JM. 1988. Germination ecophysiology of herbaceous plant species in a temperate region. American Journal of Botany 75:286–305.

Baskin CC, Baskin JM. 2001. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, California, USA.

Baskin JM, Baskin CC. 1985. The annual dormancy cycle in buried weed seeds: a continuum. Bioscience 35:492–498.

Bewley JD. 1997. Breaking down the walls: a role for endo-ß-mannase in release from seed dormancy? Trends Plant Science 2: 464–469.

Bewley JD, Black M. 1982. Physiology and biochemistry of seeds in relation to germination. Vol 2. Springer Verlag, Berlin, Germany.

Brown AHD. 2000. The genetic structure of crop landraces and the challenge to conserve them *in situ* on farms. In: Brush SB, editor. Genes in the field. IPGRI, IDRC. Lewis Publishers, Boca Raton, FL, USA. pp. 29–48.

Casal JJ, Sánchez RA. 1998. Phytochromes and seed germination. Seed Research 8:317–329.

Cooper HD, Spillane C, Hodgkin T. 2001. Broadening the genetic base of crops: an overview. In: Cooper HD, Spillane C, Hodgkin T, editors. Broadening the genetic base of crop production. IPGRI, FAO. CABI Publishing, Wallingford, UK. pp. 1–23.

De Miguel LC, Sánchez RA. 1992. Phytochrome induced germination, endosperm softening and embryo growth potential in *Datura ferox* seeds: Sensitivity to low water potential and time to escape to FR reversal. Journal of Experimental Botany 43:969–974.

Dyer WE. 1995. Exploiting Weed seed dormancy and germination requirements through agronomic practices. Weed Science 43: 498–503.

Egley GH. 1984. Ethylene, nitrate and nitrite interactions in the promotion of dark germination of common purlane seeds. Annals of Botany 53:833–840.

Espinal S. 1977. Zonas de vida: formaciones vegetales del departamento de Antioquia. Universidad Nacional de Colombia, Medellín, Colombia.

Evans AS, Cabin RJ. 1995. Can dormancy affect the evolution of post germination traits? The case of *Lesquerella fendleri*. Ecology 76:344–356.

FAO, IPGRI. 1994. Normas para bancos de genes. Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), Instituto Internacional de Recursos Fitogenéticos (IPGRI), Roma, Italia.

Fenner M. 1991. The effects of the parent environment on seed germinability. Seed Science Research 1:75–84.

Fleck NG, Agostinetto D, Ribas AV, Junior AM. 2001. Efeitos de fontes nitrogenadas e de luz na germacao de sementes de *Bidens pilosa* e *Sida rhombifolia*. Ciencia Agrotecnica 25: 592–600.

Furuya M, Schafer E. 1996. Photoreception and signalling of induction reactions by different phytochromes. Trends in Plant Science 1:301–307.

Girard E, Lobo M. 1982. Madurez fisiológica de la semilla de tomate de árbol (*Cyphomandra betacea* Cav. Sendt). Revista ICA 17(2):59–62.

Gómez H. 1997. Estadística experimental aplicada a las ciencias agrícolas. Universidad Nacional de Colombia. Facultad de Ciencias Agropecuarias, Medellín, Colombia.

Grime JP, Mason G, Curtiz AV, Rodman J, Band SR, Mowforth MAG et al. 1981. A comparative study of germination characteristics in a local flora. Journal of Ecology 69:1017–1059.

Hennig L, Stoddart WM, Dieterle M, Whitelam C, Schafer E. 2002. Phytochrome E controls light–induced germination of *Arabidopsis*. Plant Physiology 128:194–200.

Hilhorst HWM. 1990. Dose–response analysis of factors involved in germination and secondary dormancy of seeds *Sisymbrium officinales*. I Phytochrome. Plant Physiology 94:1090–1095.

Hilshort HWM, Smitt A, Karssen CM. 1986. Gibberelin–biosynthesis and sensitivity mediated stimulation of seed germination of *Sisymbrium officinale* by red light and nitrate. Physiologia Plantarum 67:285–290.

Hong TD, Ellis RH. 1996. A protocol to determine seed storage behaviour. Engels J, Toll J, editors. IPGRI Technical Bulletin No. 1. IPGRI, Rome, Italy.

Hong TD, Linington S, Ellis RH. 1996. Seed storage behaviour: a compendium. Handbooks for Genebanks No. 4, IPGRI, Rome, Italy.

Kamiya Y, García Martínez JL. 1999. Regulation of gibberellin biosynthesis by light. Current Opinion in Plant Biology 2: 398–403.

Kasperbauer MJ. 1968. Dark–germination of reciprocal hybrid seed from light–requiring and indifferent *Nicotiana tabacum*. Physiologia Plantarum 21:1308–1311.

Leubner–Metzger G. 2001. Brassinosteroids and gibberelins promote tobacco seed germination by different pathways. Planta 213:758–763.

Leubner–Metzger G, Meins F. 2000. Sense transformation reveals a novel role for class I ß–1,3–glucanase in tobacco seed germination. Plant Journal 23:215–221.

Lobo M. 1988a. Investigaciones con semillas de lulo (*Solanum quitoense* Lam). Semillas 13(2):17–20.

Lobo M. 1988b. Recursos genéticos y desarrollo de variedades de frutales andinos. En: Memorias 1° Curso nacional de frutales de clima frío. Técnicas de cultivo. 21–30 de noviembre de 1988, ICA, Palmira, Medellín, Colombia. pp. 57–77.

Lobo M. 1989. Semillas de tomate de árbol. Semillas 14:9–12.

Lobo M. 1991. Perspectivas de la siembra de lulo o naranjilla. Boletín Técnico, Facultad de Ciencias Agropecuarias, Universidad Nacional, Palmira, Colombia 2(2):123–130.

Lobo M. 1992. Los recursos fitogenéticos: evolución, tipos y utilización. En: Memorias Curso internacional sobre recursos fitogenéticos. Vol. 1. 9 de noviembre–4 de diciembre de 1992. Universidad Nacional de Colombia, ICETEX, IBPGRI, OEA, Universidad del Valle. Palmira, Colombia.

Lobo M. 2000. Papel de la variabilidad genética en el desarrollo de los frutales andinos como alternativa productiva. En: Memorias 3° Seminario de frutales de clima frío moderado. Centro de desarrollo tecnológico de frutales, Manizales, Colombia, 15–17 de noviembre de 2000 pp. 27–36.

Maguire JD. 1962. Speed of germination–aid in selection and evaluation for seedling emergence and vigor. Crop Science 2:176–177.

Mayer AM, Poljakoff–Mayber A. 1989. The germination of seeds. 4° Ed., Pergamon Press, Oxford, UK.

Meyer SE, Kitchen SG, Carlson SL. 1995. Seed germination timing patterns in intermountain *Penestemon* (Scrophulariaceae). American Journal of Botany 82:377–389.

Milberg P, Andersson L. 1994. Effect on emergence date on seed production and seed germinability in *Thlaspi arvense*. Swedish Journal of Agricultural Research 24:143–146.

Neff MM, Fankhauser C, Chory J. 2000. Light: an indicator of time and place. Genes & Development 14:257–271.

Nikolaeva MG. 1977. Factors controlling the seed dormancy pattern. In: Khan AA, editor. The physiololgy and biochemistry of seed dormancy and germination. North Holland, Amsterdam, The Netherlands. pp. 54–74.

Pérez García F. 1993. Germination behaviour of *Onopordum acanthium* L. (Asteraceae): effect of the origin of the cypsela. In: Proceedings of the 4th International workshop on seeds: basic and applied aspects of seed biology. Vol. 2. ASFIS, Paris. pp. 417–422.

Philippi T. 1993. Betg–hedging germination of desert annuals: variation among populations and maternal effects in *Lepidium lasiocarpum*. American Naturalist 142:488–507.

Pons TJ. 1992. Seed responses to light. In: Fenner M, editor. Seeds: the ecology of regeneration in plant communities. CAB International, Wallingford, UK, pp. 259–284.

Raghavan V. 2002. Induction of vivipary in *Arabidopsis* by silique culture: implications for seed dormancy and germination. American Journal of Botany 89(5):766–776.

Roberts HA. 1986. Seed persistence in soil and seasonal emergence in plants species with different habitats. Journal of Applied Ecology 23:639–656.

Sackville Hamilton NR, Chorlton KH. 1997. Regeneration of accesions in seed collections: a decision guide. Handbook for Genebanks No. 5. Engels J, editor. SGRP–FAO–IPGRI, Rome, Italy.

Schutz W. 2000. Ecology of seed dormancy and germination in sedges (*Carex*). Perspectives in Plant Ecology, Evolution and Systematics 3(1):67–89.

Schutz W, Milberg P. 1997. Seed dormancy in *Carex canescens* regional differences and ecological consequiences. Oikos 78: 420–428.

Schutz W, Rave G. 1999. The effect of cold stratification and light on the seed germination of temperate sedges (*Carex*) from various habitats and imlications for regenerative strategies. Plant Ecology 144:215–230.

Strand E. 1989. Studies on seed dormancy in samll grain species. II. Wheat. Norwegian Journal of Agricultural Sciences 3:101–115.

Toyomasu T, Tsuji H, Yamane H, Nakayama M, Yamaguchi I, Murofushi N et al. 1993. Ligth effects on endogenous levels of gibberelins in photoblastic lettuce seeds. Journal Plant Growth Regulation 12:85–90.

Toyomasu T, Kawaide H, Mitsuhshi W, Inoue W, Kamiya Y. 1998. Phytochrome regulates gibberelin biosynthesis during germination of photoblastic lettuce seeds. Plant Physiology 118:1517–1523.

Valencia A, García E. 1977. Índices generales, temperatura óptima de germinación y métodos de extracción de semilla de tomate de árbol (*Cyphomandra betacea* Sendt) y lulo (*Solanum quitoense* Lam). Trabajo de investigación. Universidad Nacional de Colombia, Faculta de Agronomía, Medellín, Colombia.

Vásquez Yanes C, Orozco Segovia A. 1993. Patterns of seed longevity and germination in the tropical rainforest. Annual Review of Ecology and Systematics 24:69–87.

Vásquez Yanes C, Orozco Segovia A. 1994. Signals for seeds to sense and respond to gaps. In: Caldwell M, Pearcy R, editors. Exploitation of environmental heterogeneity by plants: ecophysiological processes above and below ground. Academic Press, New York. pp. 261–318.

Vásquez Yanes C, Rojas Arechiga M, Sánchez Coronado ME, Orozco Segovia A. 1996. Comparison of light–regulated seed germination in *Ficus* spp. and *Cecropia obtusifolia:* ecological implications. Tree Physiology 16:871–875.

Vleeshowers LM, Bowmeester HJ, Karssen CM. 1995. Redefining seed dormancy, an attempt to integrate physiology and ecology. Journal of Ecology 83:1031–1037.

Walck JL, Hidayati SN, Okagami N. 2002. Seed germination ecophysiology of the Asian species *Osmorhiza aristata* (Apiaceae): Comparison with its Northamerican congeners and implications for evolution of types of dormancy. American Journal of Botany 89(5):829–835.

Young JA, Palmquist DE, Evans RA. 1991. Temperature profiles for germination of big sage-brush seeds from native stands. Journal of Range Management 44:385–390.

Germination ability of seeds of 23 crop plant species after a decade of storage in the National Gene Bank of China

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Summary

Germination ability of seeds of 23 crop plant species after a decade of storage in the National Gene Bank of China

Seed viability of 18 576 accessions of 23 crops was monitored. After 10–12 years' storage in the National Gene Bank of China, more than 96% of the accessions maintained a high germination percentage (>85%). The germination of 95 accessions, accounting for 0.5% of the total, declined significantly from above 80% to below 70%. For each crop of carrot (*Daucus carota* var. *sativa* DC.), lettuce (*Lactuca sativa* L.), cotton (*Gossypium* sp. L.), flax (*Linum usitatissimum* L.) and castor (*Ricinus communis* L.), the mean monitored germination percentage declined significantly; carrot and lettuce seeds lost viability most rapidly. Seed initial germination and pre-storage environments affect subsequent seed viability in storage.

Key words: genebank, germination, long-term seed storage, seed viability

Résumé

Capacité de germination de graines de 23 espèces cultivées après une dizaine d'années de conservation dans la banque de gènes nationale de Chine

La viabilité de graines de 18 576 accessions de 23 espèces cultivées a été analysée. Après 10 à 12 ans de conservation dans la banque de gènes nationale chinoise (*National Gene Bank of China*, NGBC), le pourcentage de germination (>85%) reste élevé pour plus de 96 % des accessions. La germination de 95 accessions, représentant 0,5 % de l'ensemble, a significativement diminué, passant de plus de 80 % à moins de 70 %. Dans le cas de la carotte (*Daucus carota* var. *sativa* DC.), de la laitue (*Lactuca sativa* L.), du coton (*Gossypium* sp. L.), du lin (*Linum usitatissimum* L.) et du ricin (*Ricinus communis* L.), le pourcentage moyen de germination observé a significativement diminué, la perte de viabilité étant la plus rapide pour les graines de carotte et de laitue. La germination initiale des graines et les conditions avant conservation conditionnent la viabilité des graines en cours de stockage.

Resumen

Capacidad de germinación de semillas de 23 especies de cultivos vegetales después de un decenio de almacenamiento en el Banco Fitogenético Nacional de China

Se efectuó el seguimiento de la viabilidad de las semillas de 18 576 accesiones de 23 cultivos. Después de 10 a 12 años de almacenamiento en el Banco Fitogenético Nacional de China, más del 96% de las accesiones mantenían un alto porcentaje de germinación (>85%). La germinación de 95 accesiones que representan el 0,5% del total, disminuyeron significativamente de más del 80% a menos del 70%. Para los cultivos de zanahoria (*Daucus carota* var. *sativa* DC.), lechuga (*Lactuca sativa* L.), algodón (*Gossypium* sp. L.), lino oleaginoso (*Linum usitatisimum* L.) y ricino (*Ricinus communis* L.), el porcentaje medio de germinación monitoreado disminuyó de manera significativa: las semillas de zanahoria y lechuga perdieron más rápidamente la viabilidad. La germinación original de las semillas y el ambiente anterior al almacenamiento afectaron la subsiguiente viabilidad de las semillas almacenadas.

Introduction

Ex situ seed storage is important for preserving crop genetic resources. Seeds can remain viable for extended periods if kept under conditions of low temperatures and moisture contents. Approximately 90% of the world collection of 6 100 000 seed accessions is stored in more than 1000 low-temperature genebanks (FAO 1996). The number of accessions stored in National Gene Bank of China (NGBC) has grown continuously since its foundation in 1986, and the genebank currently holds about 330 000 accessions of 712 species. Seed deterioration during storage has always been a great problem. For some accessions, seeds had low germinability even after short storage duration (Specht et al. 1997, 1998; Stoyanova 2001). An even more serious risk is that 50% of the accessions stored in genebanks lose viability or encounter genetic drift after regeneration (Singh et al. 1984). The challenge for genebank curators is, therefore, to regenerate seed samples before their viability becomes critically low.

To determine the general variation of seed viability during storage, seed samples of a number of crop species stored in the National Gene Bank of China have been selected since 1991 for monitoring seed viability. The purpose of this work was to study the influence of crop species (types), initial germination rates and multiplication regions on the viability variation of stored seeds, thus to provide scientific bases for seed viability monitoring protocols for the National Gene Bank.

Materials and methods

In general, seed viability was tested before seeds were put into the cold-storage facilities in the Chinese National Gene Bank. The initial germination percentage should be above the lowest viability standard required for each crop (Table 1). Seeds were dried to 5–7% moisture content (8% for soybean) and then hermetically stored at –18°C. The seed drying conditions for cereals, legumes and other crops were 38°C with 8–10% RH, 35°C with 10–12% RH and 20–25°C with 20–30% RH, respectively (Appendix 1 online details seed drying conditions at the National Gene Bank of China).

Monitored seeds included two categories: (1) 18 576 accessions of 23 crops, including rice (*Oryza sativa* L.) and wheat

Table 1. Monitored result of germination percentage for 23 crops stored for 10–12 years

Species	Lowest initial viability standard	No. of accessions monitored	Mean initial germination percentage (%)	Mean monitored germination percentage (%)	No. of accessions with germination rates of				
					<70%	70–74%	75–79%	80–84%	>85%
Oryza sativa	90	4020	98	97	3	1	5	13	3998
Triticum aestivum	85	3000	96	95	10	6	22	68	2894
Hordeum vulgare	85	2010	97	98	–	–	1	–	2009
Setaria italica	85	1080	92	94	6	–	12	27	1035
Sorghum bicolor	85	3300	94	92	18	18	67	214	2983
Zea mays	90	1380	96	98	–	2	1	10	1367
Avena sativa	80	733	97	99		1	–	1	726
Fagopyrum esculentum	80	234	97	97	1	1	5	5	222
Glycine max	85	1020	97	98	5	–	1	1	1013
Food legume‡	80	754	98	97	1	1	5	4	743
Gossypium arboreum	90	200	96	90†	3	3	14	19	161
Linum usitatissimum	90	45	97	93†	–	–	1	2	42
Arachis hypogaea	85	200	99	98	–	–	–	–	200
Helianthus annuus	90	100	95	96	1	–	2	4	93
Ricinus communis	90	200	98	94†	3	4	6	10	177
Allium fisdtulosum	80	100	98	94	5	4	2	4	85
Lactuca sativa	80	100	97	85†	13	2	5	19	61
Daucus carota	80	100	95	81†	21	4	15	14	46
Total		18576			95	47	164	415	17855
Percentage					0.51	0.25	0.88	2.23	96.12

†Significant decline; ‡Food legume includes cowpea (*Vigna unguiculata*), pea (*Pisum sativum* Linn.), mung bean (*Vigna rabiata* (Linn.) Wilczek), adsuki bean (*Vigna angularis* (Willd.) Ohwi), rice bean (*Vigna umbellate* (Thunb) Ohwiet Ohashi) and lentil (*Lens esculenta* Moench.).

(*Triticum aestivum*), which had been stored in the NGBC for 10–12 years. These were tested to determine the general viability variation of seeds stored in the genebank and the influence of different ecological multiplication regions on viability variation. (2) Seventy-eight accessions of rice seeds, 85 accessions of wheat seeds and 40 accessions of peanut (*Arachis hypogae* L.) seeds, with different initial germination percentage levels, which were monitored for five consecutive years. These were tested to find out the influence of different initial germination percentage levels on viability dynamics of stored seeds.

Testing methods

Germination tests were carried out referring to the International Board for Plant Genetic Resources preferred seed viability monitoring test methods (IBPGR 1985) and sequential tests method (Ellis et al. 1980) for genebanks. With the consideration that stored seeds are invaluable resources and to avoid wasting these, and to avoid the depletion of accessions in storage (seed numbers in a seedbank are severely restricted) the monitored amounts of each accession and each replicate were reduced to half the standards.

Threshold for claiming a significant viability decline

To allow for testing errors, tolerances indicated in Table 5c of ISTA compatibility tests (Anonymous 1985) were used as thresholds to determine whether seed viability declined significantly. A difference between the monitored value and the initial value exceeding the allowed tolerance indicated a significant viability decline.

Results and analysis

General variation of seed viability after 10–12 years storage

The seed viability of the 18 576 accessions of 23 crops monitored is shown in Table 1. Generally, a high level of seed viability remained after 10–12 years storage, with 96.1%

Table 2. Decline of seed viability grouped according to the multiplication locations

Species	Seed multiplier	No. of accessions monitored	Mean initial germination percentage	Mean monitored germination percentage	Significance
Oryza sativa	AAS of Guizhou	742	99	99	–
	IAR of Xinyang, Henan	93	98	98	–
	AAS of Hunan	2823	97	96	–
	AAS of Sichuan	362	99	99	–
Sorghum vulgare Pers.	IAR of Suxian, Anhui	46	93	93	–
	IAR of Pingliang, Gansu	90	91	89	–
	IAR of Tangshan, Hebei	584	94	92	–
	AAS of Henan	1011	94	92	–
	AAS of Hubei	25	90	82	+
	AAS of Jilin	465	91	91	–
	IAR of Xuzhou, Jiangsu	128	96	94	–
	AAS of Liaoning	168	91	89	–
	AAS of Shandong	555	94	94	–
	IAR of Baoji, Shuanxi	15	93	89	–
	ICGR, CAAS	313	96	95	–
Ricinus communis L.	AAS of Shanxi	50	99	96	–
	IAR of Zhemen, Inner Mongolia	73	98	97	–
	IOC, CAAS	77	96	90	+
Gossypium L.	AAS of Hubei	71	92	90	–
	IAR of Bazhou, Xinjing	126	97	90	+
	IC, CAAS	3	93	91	–
Lactuca sativa	IVR of Fuzhou, Fujian	20	95	86	+
	AAS of Hebei	16	96	81	+
	AAS of Guangdong	11	96	83	+
	AAS of Inner Mongolian	10	99	95	+
	IAR of Chengdu, Sizhuan	9	96	83	+
	IVR, CAAS	8	98	87	+
	IVR of Jilin	9	99	87	+
	AAS of Guangxi	3	99	89	+
	AAS of Liaoning	3	99	88	+
	IVR of Nanchang, Jiangxi	2	99	80	+
	AAS of Hubei	2	98	79	+
	IVR of Heilongjiang	1	96	84	+
	AAS of Guizhou	1	99	80	+
	AAS of Jiangsu	1	96	81	+
	AAS of Hunan	2	99	87	+
	AAS of Henan	2	98	54	+

Note: +: significant decline; –: insignificant decline; AAS: Academy of Agricultural Sciences; CAAS: Chinese Academy of Agricultural Sciences; IAR: Institute of Agricultural Research; ICGR: Institute of Crop Germplasm Resources; IC: Institute of Cotton; IOC: Institute of Oil Crops; IVR: Institute of Vegetable Research.

accessions having germination percentages of 85% or higher. However, seed viability of 0.5% of the accessions tested (95 accessions) declined significantly, with a germination percentage of 70% or below. For three rice varieties, the germination percentages declined from the initial values of 94%, 92% and 95% to 68%, 46% and 15%, respectively. The mean germination percentages of five crops declined significantly: carrot (*Daucus carota* var. *sativa* DC.), lettuce (*Lactuca sativa*), cotton (*Gossypium* sp. Linn.), flax (*Linum usitatissimum* L.) and castor (*Ricinus communis* L.). The decline was especially severe for carrot and lettuce.

Differences of seed viability declining among seeds multiplied in different locations

Five crops—rice, sorghum (*Sorghum vulgare*), castor plant, cotton and lettuce—were grouped according to the locations in which the accessions were multiplied (Table 2). Sorghum seeds supplied by Hubei Academy of Agricultural Sciences showed significant declines in the mean germination percentage while seeds from another 10 suppliers did not. Cotton and castor seeds supplied by Bazhou Agricultural Research Institute of Xinjiang Province and the Institute of Oil Crops of the Chinese Academy of Agricultural Sciences located in Wuchang, Hubei Province, showed significant declines in the mean germination percentage while the others did not (Table 3). The results indicate that differences in seed viability decline among the same crop multiplied in different locations might be related to the different conditions of production before addition to the genebank. Adverse climate at the stages of seed ripening and harvesting, as well as damage caused by seed-extraction, drying and transportation after harvest could affect the rate of seed viability decline during storage (Sai Babu et al. 1983).

Differences of seed viability decline among seeds with different initial germination levels

Influences of the initial germination rate on the decline of seed viability after storage are shown in Table 3. After 5 years of storage, viability of Japonica rice and peanut (*Arachis hypogaea*) seeds that had lower initial germination percentages declined more rapidly than accessions with higher initial germination percentages of the same species (types). However, for *Indica* rice and wheat, there were no significant differences of seed viability decline between accessions with lower and higher initial germination percentages. Significant differences were also observed between two peanut groups, which had the same initial germination percentage but different multiplication locations. This also indicates that pre-storage environmental factors could have important influences on seed viability.

Discussion

Of the 18 576 accessions monitored in the present study, 96% had germination percentages of 85% or higher. This indicated that most of the seeds stored in the National Gene Bank maintained high viability after 10 years' storage. The germination percentage of 95 accessions declined significantly from above 80% to below 70%. The viability decline of a portion of the accessions might be due to several reasons, of which genetic characteristics of species and pre-storage environments are the main factors. For example, seeds of carrot and lettuce are short-lived, and the storability of the two crops was poorer under low temperature conditions. Different genotypes within a same species might also have different longevity, which would result in a faster loss of viability for certain varieties (Priestley et al. 1985). Adverse climate conditions before harvest (such as harsh climate

Table 3. Influence of initial germination rate on the decline of seed viability after storage

Species	Initial germination percentage levels (%)	Monitored accessions	Mean germination percentage in 1985 (%)	Tolerated range (%)	Decline in percentage compared with 1985 (%)				
					1991	1992	1993	1994	1995
Indica rice	>99	12	100	2	–1.67	–0.75	–1.17	–0.75	–1.00
	95–96	19	95.79	3	–0.05	–0.79	–0.90	–0.47	–0.32
	90–91	13	90.39	5	–0.54	–1.31	–1.62	–0.46	–1.62
Japonica rice	>99	12	100	2	–2.33	–3.58	–4.08	–2.92	–2.75
	95–96	15	95.80	3	–0.33	–0.60	–0.73	+0.27	–0.13
	90–91	7	90.43	5	–7.72	–9.29	–10.57	–7.72	–7.43
Triticum aestivum	>98	30	98.2	2	–0.8	–2.0	–1.8	–1.4	–1.6
	90	25	90	5	+2.7	–1.5	–2.9	–1.2	–3.1
	85	30	85	5	+0.9	–2.7	–3.2	–1.1	–2.6
Arachis hypogaea L.	95–96	18	94.5	3	+3.0	+2.4	+2.0	+1.3	+1.6
	85–86	5†	85.4	5	+3.9	–1.1	–3.0	–3.0	–1.7
	85–86	7†	85.9	7	–19.0	–13.2	–13.5	–19.0	–21.0

† Accessions from different suppliers.

during ripening and harvest) are other important factors influencing seed viability. The unsatisfactory results of storage for sorghum seeds is probably because of threshing carried out in extremely dry weather in 1981, which might have affected some accessions (Stoyanova 2001). There may also be additional factors affections seed viability, such as insufficient understanding of seed storage characteristics. It was reported that the optimal moisture content for tabasco pepper (*Capsicum frutescens*) was 10.3% when seeds are stored at 2°C (Sundstrom 1990), therefore, whether or not –18°C storage temperature and 5%–8% moisture content is optimal for all crops in long-term storage needs further study and verification. In addition, drying at high temperatures before addition to the genebank might be a potential factor affecting seed viability.

The results of this study showed that there were significant differences in loss of seed viability among crops, varieties and multiplication localities. Furthermore, a significant decline in viability of seed accessions with low initial germination ability and vigour is easily detected within five years of storage. Therefore, seed vigour should also be tested in seed viability monitoring. Further, it is important to study warning indexes of seed viability decline (Lu et al. 2002), and to develop non-destructive monitoring methods of seed viability and vigour, so as to ensure the long-term safety of resources stored in the National Gene Bank.

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References

Ellis RH, Roberts EH, Whitehead J. 1980. A new, more economic and accurate approach to monitoring the viability of accessions during storage in seed banks. Plant Genetic Resources Newsletter 41:3–18.

FAO. 1996. Report on the state of the worlds plants genetic resources. International Technical Conference on Plant Genetic Resources, Leipzig, Germany. FAO, Rome, Italy.

IBPGR. 1985. IBPGR advisory committee on seed storage report of the third meeting, IBPGR, Rome, Italy.

ISTA. 1985 International Rules for Seed Testing. Seed Science and Technology 15:299–355.

Lu Xinxiong, Chen Xiaoling. Characteristics and warning indices of rice seeds viability loss during storage at 45°C constant temperature. Agricultural Sciences in China 2002(8):911–915.

Priestley DA, Cullinan VI, Wolfe J. 1985. Differences in seed longevity at the species level. Plant Cell and Environment 8:557–562.

Sai Babu KG, Husaiai SH, Muralimonan RB. 1983. Effect of moisture and container on the storability of paddy seed under ambient conditions of Hyderabad. Seed Research 11(1):71–73.

Singh RB, Williams JT. 1984. Maintenance and multiplication of plant genetic resources. In: Crop Genetic Resources: Conservation and Evaluation. Allen and Unwin, St Leonards, Australia, pp. 120–130

Specht CE, Keller ERJ, Freytag U. 1997. Survey of seed germinability after long-term storage in the Gatersleben genebank. Plant Genetic Resources Newsletter 111:64–68.

Specht CE, Keller ERJ, Freytag U. 1998. Survey of seed germinability after long-term storage in the Gatersleben genebank. Part 2. Plant Genetic Resources Newsletter 115:39–43.

Stoyanova SD. 2001. *Ex situ* conservation in the Bulgarian genebank: 1. Effect of storage. Plant Genetic Resources Newsletter 128:68–76.

Sundstrom FJ. 1990. Seed moisture influences on tabasco pepper seed viability, vigour and dormancy during storage. Seed Science and Technology 18:179–185.

Appendix 1 — Seed drying conditions at the National Gene Bank of China — is available online: www.ipgri.cgiar.org/pgrnewsletter

Genetic diversity in some *Aegilops* species in Jordan revealed using RAPD

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Summary
Genetic diversity in some *Aegilops* species in Jordan as revealed by RAPDs
Genetic diversity among 31 *Aegilops* accessions collected from 18 locations in Jordan was estimated using random amplified polymorphic DNA (RAPD). Using a set of five random decamer primers, 581 data points were scored over all accessions. The data points corresponded to a total of 47 loci for which 52 markers were polymorphic. A genetic similarity matrix based on Dice coefficients was constructed using the RAPD data to assess the genetic relatedness. The mean similarity indices associated with the 31 accessions ranged from 0.24 to 0.94 for all accessions with a mean of 0.55. The resulting phenograms indicated that clusters of the same species have common genomes, but clusters were not associated with collection site. The clustering trend was also associated with ploidy level. There was an average of 95% reproducibility of results using two to three replications of five primers. Under stringent reaction conditions RAPD patterns were highly reproducible. There was agreement between classical classification and that based on RAPD analysis.

Key words: *Aegilops*, genetic diversity, Jordan, RAPD

Résumé
Diversité génétique au sein de certaines espèces jordaniennes d'*Aegilops* mise en évidence par RAPD
La diversité génétique parmi 31 accessions d'*Aegilops* collectées dans 18 localités de Jordanie a été estimée par amplification aléatoire d'ADN polymorphe (RAPD). En utilisant un ensemble de cinq amorces décamères, 581 marqueurs ont été identifiés dans l'ensemble des accessions correspondant à 47 loci ; 52 d'entre eux révèlent un polymorphisme entre accessions. La capacité moyenne de détection du polymorphisme est de 47,3 % par amorce. Une matrice de proximité génétique basée sur les coefficients de Dice a été construite en utilisant les données RAPD pour évaluer la proximité génétique. Les indices moyens de similitude pour l'ensemble des 31 accessions vont de 0,24 à 0,94 avec une moyenne de 0,55. Les phénogrammes résultants font apparaître un regroupement des mêmes espèces ou des accessions qui ont des génomes communs mais ne révèlent pas de regroupements en fonction du site de récolte. Le regroupement tend également à être associé au degré de ploïdie. Parmi les 31 accessions utilisées, la reproductibilité est de l'ordre de 95 % en effectuant deux ou trois réplications avec les cinq amorces. Donc, dans des conditions de réaction stringentes, nous pensons que les profils RAPD sont très reproductibles. La classification obtenue par nos analyses RAPD concorde bien avec la classification classique.

Resumen
Diversidad genética de algunas especies de *Aegilops* en Jordania, como surge del análisis APAAD
Se evaluó la diversidad genética entre 31 accesiones de *Aegilops* recogidas en 18 lugares de Jordania, empleando análisis APAAD. Aplicando un conjunto de cinco decámeros primos al azar se registraron 581 puntos de datos en todas las accesiones. Los puntos de datos registrados corresponden a 47 *loci* de los cuales 52 marcadores eran polimórficos, con un porcentaje del 47,3%. Se elaboró una matriz de similitud genética basada en los coeficientes Dice, empleando los datos APAAD para establecer el parentesco genético. Los índices medios de similitud presentados por las 31 accesiones iban de 0,24 a 0,94 para todas ellas, con un promedio de 0,55. Los fenogramas resultantes indican agrupamiento, en las mismas especies, de las accesiones que tienen genomas comunes, si bien los agrupamientos no se relacionan con el lugar de recolección. También se relacionó la tendencia al agrupamiento con el nivel ploideo. De las 31 accesiones estudiadas aplicando dos o tres réplicas con cinco primos resultó una reproducibilidad promedio del 95%. Por ello, creemos que las pautas APAAD son altamente reproducibles bajo rigurosas condiciones de reacción. Hubo acuerdo entre la clasificación clásica y la obtenida empleando el análisis APAAD.

Introduction
Aegilops germplasm is used in wheat breeding as a source of several economically important traits, particularly those associated with disease resistance. However, other useful traits in *Aegilops* could be identified and used in wheat breeding. Nine *Aegilops* species have been identified in Jordan, including *A. bicornis*, *A. longissima* and *A. vavilovi* from the drier parts of Jordan, and *A. kotschyi* , *A. peregrina* and *A. searsii* from the semiarid parts. However, *A. biuncialis* is mainly found in the high rainfall regions of Jordan and its distribution extends to the semiarid and arid areas (Van Slageren et al. 1989).

The genetic variation found in the wild progenitors of crops is very important. Safeguarding and maintaining germplasm is essential for improving crop production. The value of wild species as a genetic resource for crop improvement depends on the amount of genetic variability they represent relative to cultivated crops. A large number of methods are available for the assessment of genetic variability, diversity and relatedness among germplasms as well as for molecular fingerprinting. Morphological and biochemical markers (protein-based techniques) are influenced by the environment, but DNA based techniques represent reliable tools and obviate many of the standard problems associated with other techniques.They allow also a high throughput of material for DNA typing.

Several PCR-based systems are available that differ in complexity, reliability and information generating capacity.

These include RAPD, SSR, AFLP, among others. Each system has its advantages and disadvantages. The introduction of RAPD (random amplified polymorphic DNA) Williams et al. (1990) or AP-PCR arbitrarily primed–PCR (Welsh and McClelland 1990), allowed DNA analysis using the polymerase chain reaction (PCR) in the absence of specific information on nucleotide sequences. It proved valuable in the characterization and evaluation of genetic diversity within and among species and populations. RAPD markers were used to analyze genetic variability and relationships among species, populations and accessions of *Aegilops* (Monte et al. 1999) who found that *A. ventricosa* (DDNN) segregated from the other species, probably owing to the influence of the D genome. *A. biuncialis* (UUMM), and *A. ovata* (UUMM), were clearly separated. In addition, correlation was found between RAPD markers and ecogeographical factors. The results of Kong et al. (1999) showed that the ABD and S genomes of *Triticum* were most consanguineously grouped, genomes C and U had a relatively close relationship, and genome D was distant from all other genomes.

The diversity among 18 *A. geniculata* populations from different floristic regions of Bulgaria was structured on the basis of ecogeographical criteria (Zaharieva et al. 1999). They concluded that neutral markers can provide information on the evolutionary history and migration of a species and help in identifying original accessions and eliminating redundancies, thus facilitating choices of accessions to be conserved in germplasm collections. Genetic distance analysis of 29 accessions of two *A. tauschii* subspecies from different regions was assayed using RAPD. Two evident clusters based on the 297 RAPD bands revealed divergence between the two subspecies that was greater than that within one subspecies from different geographical regions: China, Iran and former USSR (Kong et al. 1998). RAPD analysis of 112 accessions of several *Aegilops* species divided them into two major groups corresponding to the D and U genomes. Associations between altitude and variability among RAPD markers were not established (Okuno et al. 1998). Genetic diversity among 18 Chinese accessions of *A. geniculata* was assessed using RAPD. The phenogram showed that RAPD data are useful in the measurement of genetic variation among and similarity within a species (Zhang et al. 1996).

This study aimed at assessing genetic diversity in some *Aegilops* accessions belonging to several species collected from different parts of Jordan using RAPD.

Materials and methods

Plant materials

Field collections of *Aegilops* populations were carried out in March–June 2000. The collecting sites were selected according to bibliographic information and herbarium samples at the National Center for Agricultural Research and Technology Transfer (NCARTT). A mission visited locations from the south to the north of Jordan. The collection sites ranged in altitude from 804 m to 1512 m. The exact site location and altitude were determined using the Geographical Positioning System (GPS) MAGELLAN (model NAV 5000 DX) and a Digital Barometer–Altimeter (model AIR-HB-1L). The data are given in Table 1. The description of material collected was based on morphological studies of spikes. This was compared with published descriptions (Feinbrun 1986; Kimber and Feldman 1987; Van Slageren et al. 1994). The material was examined at the Herbarium of the Department of Biology at the University of Jordan and at the NCARTT Plant Genetic Resource Unit. It was also confirmed at the Genetic Resources Unit of ICARDA.

Genomic DNA isolation and polymerase chain reaction (PCR)

Genomic DNA was extracted from seedlings germinated in a growth chamber maintained at 21°C. A Promega Wizard genomic DNA purification kit was used according to instructions provided by the manufacturer. Genomic DNA quality and relative quantity were examined using 0.7% agarose gel electrophoresis detected under UV-light and photographed using a Polaroid camera with black and white 667 film. The DNA concentration was determined by spectrophotometry using a GeneQuant II, RNA/DNA calculator (Pharmacia Biotech).

The standard RAPD protocol recommended by Williams et al. (1990) was performed. A total reaction volume of 25 µl of solution at pH 8.3 containing 2.5 µl of 10x ready prepared PCR buffer; 100 mM Tris-Cl, , 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin (Promega,). 2.5 µl of dNTPs stock, 5 picomoles of a single 10-base primer (Operon Technolgies, Inc.) 0.2 µl of (5u/µl) *Taq* DNA polymerase (Promega) and 1 µl (75–100 ng) of genomic DNA template. Double distilled sterile water was added to make up the final volume to 25 µl.

DNA amplification was carried out with a MJ Research model PTC-100 DNA thermocycler. The PCR program was set for: 2 min at 95°C (initial denaturing step), 40 cycles of: 1 min at 94°C (denaturing), 1 min at 36°C (annealing), 2 min at 72°C (extension, ramp time 2 min, 0.3°C s^{-1}) and 2.5 min at 72°C (final extension step). After amplification the tubes were stored at 4°C overnight before gel electrophoresis.

Gel electrophoresis and data analysis

Electrophoresis on 1.4% agarose gel was performed in 0.5xTBE buffer and the gel was stained using ethidium bromide. Molecular sizes of the amplification products were estimated using a 1kb DNA ladder (from Promega). The gel tank was connected to a power supply of a constant 100 V for 1.5 hours, after which the gel was placed on a UV transilluminator (254 nm) and photographed using a black and white type 667 Polaroid film.

Data were analysed on the basis of the presence (1) or absence (0) of the amplified products. Pair-wise comparisons of accessions based on presence/absence of unique and shared polymorphic products were used to generate Dice similarity coefficients. The equation used was $(2N_{ab})/(N_a+N_b)$, where N_{ab}, N_a and N_b are the number of shared bands between sample A and B, and total number of bands detected in sample A

Table 1. Genome constitution, longitude, latitude and altitude of the collection sites of 31 *Aegilops* accessions included in this study

Location	Acc. no.	Genome	°North	°East	Altitude (m)
Khalda (5)/Amman	1	US	32:00.2	35:50.8	990
Khalda(3)/Amman	2	MU	32:00.2	35:50.8	990
Khalda(2)/Amman	3	MU	32:00.2	35:50.8	990
Qadesia/Tafila	4	MU	30:39.6	35:37.1	1512
Qadesia/Tafila	5	MU	30:39.6	35:37.1	1512
Khalda (4)/Amman	6	US	32:00.2	35:50.8	990
Suwilh-Wadi Sair/Amman	7	MU	n.d.	n.d.	n.d.
Khalda(1)/Amman	8	MU	32:00.2	35:50.8	990
Khalda (6)/Amman	9	MU	32:00.2	35:50.8	990
Daboq/Amman	10	MU	n.d.	n.d.	n.d.
Lahda/Tafila	11	MU	30:43.2	35:39.5	1432
Rashadia Cement factory/ Tafila	12	MU	30:40.1	35:38.0	1497
Rashadia Cement factory/ Tafila	13	MU	30:40.1	35:38.0	1497
Rashadia Cement factory/ Tafila	14	MU	30:40.1	35:38.0	1497
Rashadia Cement factory/ Tafila	15	MU	30:40.1	35:38.0	1497
Rashadia Cement factory/ Tafila	16	MU	30:40.1	35:38.0	1497
Rashadia Cement factory/ Tafila	17	MU	30:40.1	35:38.0	1497
Remone/Jerash	18	MU	32:17.5	35:51.6	950
Um-Jlood/Ajlon	19	MU	32:17.8	35:47.2	1023
Alrabad Castle / Ajlon	20	MU	32:19.8	35:43.6	878
Ras-Monif/Ajlon	21	MU	32:23.3	35:47.4	1046
Erhaba/Ajlon	22	MU	32:24.6	35:48.9	1016
Eshtafina/Ajlon	23	US	32:20.0	35:46.0	1029
Zobia/Ajlon	24	US	32:26.7	35:45.0	814
Afna/Ajlon	25	MU	32:38.0	36:32.0	1000
Baoon/Ajlon	26	MU	32:22.2	35:44.2	847
Sakeb/Jerash	27	S	32:16.0	35:47.2	950
Ein-Jana/Ajlon	28	S	32:20.9	35:45.9	865
Samta/Ajlon	29	DMS	32:22.9	35:50.1	1107
Anjara/Ajlon	30	MU	32:18.2	35:44.3	803
Ebin/Ajlon	31	UC	32:20.6	35:49.4	1024

n.d.: not determined

and sample B, respectively (Okuno et al. 1998). The similarity coefficients were then used to construct a phenogram using SPSS-10.0 PC software.

Results and discussion

A total of 59 random decamer primers were evaluated for their ability to prime PCR amplification of DNA from six *Aegilops* accessions that were randomly selected. Five primers (OPA-13, OPN-14, OPN-16, OPT-01 and OPT-07) that showed consistently reproducible polymorphisms were selected and used to analyze DNA of all the 31 *Aegilops* accessions included in this study.

A summary of the RAPD marker data for five primers and distribution across the *Aegilops* accessions is given in Table 2. The primers generated a total of 47 different sized fragments (loci), of which 52 markers were polymorphic over all accessions. An example of typical RAPD variation for a single primer is shown in Figure 1. In total 581 data points were scored with an average of 116 markers per primer across the accessions. The capability of different primers to generate RAPD markers ranged from 90 to 149 over all accessions. The polymorphism percentage for the primers ranged from 31.7% for (OPT-07) to 74% for (OPT-01) with an overall average of 47.3%.

Based on the Dice coefficients (Okuno et al. 1998) the mean similarity indices for the 31 accessions ranged from 0.24 to 0.94 (mean of 0.55), indicating that the accessions had on average 55% of their RAPD fragments in common (Table 3). This wide range of similarity indices indicated that a high

Table 2. RAPD markers scored for the 31 *Aegilops* accessions using five decamer primers

Primer	Total no. loci analysed	Total no. bands	Mean no. bands/ accession	Mean no. polymorphic bands	Polymorphism %
OPA-13	10	90	2.9	11	38.1
OPN-14	9	140	4.5	10	43.5
OPN-16	9	149	4.8	10	49.3
OPT-01	11	130	4.2	12	74.0
OPT-07	8	72	2.3	9	31.7
Total	47	581		52	
Average	9.4	116.2		10.4	47.3

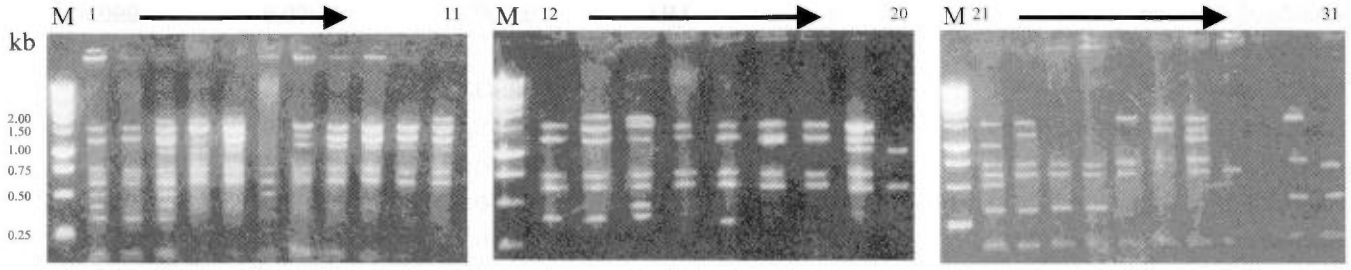

Figure 1. RAPD pattern using OPN-14 primer for the *Aegilops* accessions. M=Molecular weight marker (1 kb DNA ladder). Lanes 1, 6, 23 and 24 are *A. peregrina*, Lanes 2, 3, 16, 19, 21 and 22 are *A. ovata*, Lanes 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 20, 25, 26 and 30 are *A. biuncialis*, Lanes 27 and 28 are *A. searsii*, Lane 29 is *A. vavilovi* and Lane 31 is *A. triuncialis*.

level of polymorphism at the DNA level among accessions. There is therefore a large amount of genetic variation among the accessions. High levels of polymorphism were recorded in Tibetan wheat (Sun et al. 1996), and *Hordeum* species (Gonzalez and Ferrer 1993). These results showed that accessions from the same locality tended to constitute the same sub-cluster, whereas accessions of the same species and those with common genomes tended to form the same cluster.

The 31 *Aegilops* accessions were grouped into two main clusters; the first consisting of a single species, *A. vavilovi*, which has the DMS genome and was the only hexaploid in the collection. The second cluster comprised eight main sub-clusters of accessions with the S genome (*A. searsii*) and the U genome, including *A. peregrina*, *A. triuncialis*, *A. ovata* and *A. biuncialis*. Accessions of *A. ovata* and *A. biuncialis*, both with the UM genome, were closest to each other in the sub-clusters with high similarity coefficients. Our results indicated that clustering sometimes correlated with genome and sometimes with collection site. The findings are in agreement with those of Gonzalez and Ferrer (1993) where all American barley (*Hordeum*) species with the H genome clustered together. Okuno et al. (1998), found that *Aegilops* accessions clustered according to their genomes but not to altitude of collecting site. However, based on RAPD data, Joshi and Nguyen (1993), Weining and Henry (1995) and Vierling and Nguyen (1992) showed that accessions from the same locality tended to cluster together.

Our results also showed that clustering could be associated with ploidy level. All tetraploids (MU, UC and US) were separate from diploid and hexaploid species. The one hexaploid accession, *A. vavilovi*, formed a distinct cluster. The remaining tetraploid accessions formed the largest cluster. Such a clustering pattern could be the result of genome sizes affecting primer annealing during DNA incubation and could consequently affect amplification products.

The high number of sub-clusters formed in this study indicated high genetic variability associated with collection site. The similarity coefficients indicated that the similarity among accessions within the same cluster was high compared with the values between accessions from different clusters. The similarity between accessions from different clusters ranged from 0.24 to 0.72, while it reached 0.94 within the same cluster. The high similarity values among accessions from the same region indicated that the accessions had similar adaptive traits or were of the same origin. These findings were in agreement with those of Jaradat (1992), who reported that the highest levels of variation among landraces were found in material from among different sites within the same collecting regions.

RAPD-PCR was useful for studying genetic variability among *Aegilops* species. Cluster analysis was been used to study the genetic diversity of Central Asian and North Caucasian *Aegilops* species (Okuno et al. 1998), *A. geniculata* (Zhang et al. 1996), durum wheat landraces collected from Jordan (Al-Ajlouni and Jaradat 1997), wild emmer and durum wheat (Malkawi et al. 1998), wheat (Vierling and Nguyen 1992; Joshi and Nguyen 1993; Sun et al. 1996) and *Hordeum* (Gonzalez and Ferrer 1993; Selbach and Cavalli-Molina 2000).

There is a concern expressed in the literature regarding the reproducibility of RAPD patterns from one experiment to another. In this study reproducibility of these results

Table 3. Similarity matrix for 31 *Aegilops* accessions using Dice coefficients

Acc. no.	Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	*A. peregrina*																															
2	*A. ovata*	0,53																														
3	*A. ovata*	0,62	0,59																													
4	*A. biuncialis*	0,49	0,63	0,73																												
5	*A. biuncialis*	0,47	0,61	0,77	0,82																											
6	*A. peregrina*	0,62	0,53	0,54	0,44	0,47																										
7	*A. biuncialis*	0,49	0,68	0,76	0,81	0,89	0,53																									
8	*A. biuncialis*	0,51	0,69	0,65	0,74	0,75	0,46	0,83																								
9	*A. biuncialis*	0,53	0,65	0,75	0,71	0,76	0,54	0,88	0,86																							
10	*A. biuncialis*	0,47	0,67	0,76	0,76	0,85	0,47	0,92	0,86	0,87																						
11	*A. biuncialis*	0,49	0,60	0,71	0,79	0,68	0,44	0,71	0,76	0,82	0,70																					
12	*A. biuncialis*	0,39	0,64	0,52	0,51	0,54	0,31	0,56	0,67	0,63	0,60	0,57																				
13	*A. biuncialis*	0,53	0,65	0,71	0,75	0,72	0,39	0,71	0,62	0,68	0,65	0,73	0,63																			
14	*A. biuncialis*	0,58	0,65	0,74	0,75	0,72	0,46	0,71	0,62	0,72	0,65	0,77	0,58	0,94																		
15	*A. biuncialis*	0,35	0,69	0,45	0,54	0,51	0,33	0,54	0,58	0,55	0,51	0,55	0,83	0,67	0,61																	
16	*A. ovata*	0,47	0,80	0,55	0,59	0,51	0,47	0,59	0,63	0,60	0,56	0,65	0,71	0,76	0,70	0,77																
17	*A. biuncialis*	0,53	0,60	0,59	0,67	0,76	0,39	0,71	0,62	0,68	0,70	0,64	0,57	0,82	0,77	0,67	0,70															
18	*A. biuncialis*	0,46	0,61	0,68	0,68	0,78	0,40	0,72	0,63	0,65	0,76	0,65	0,65	0,74	0,70	0,69	0,72	0,84														
19	*A. ovata*	0,56	0,69	0,65	0,70	0,75	0,46	0,74	0,65	0,71	0,68	0,62	0,67	0,76	0,71	0,65	0,69	0,76	0,78													
20	*A. biuncialis*	0,32	0,48	0,47	0,60	0,52	0,30	0,60	0,53	0,56	0,53	0,61	0,59	0,67	0,62	0,64	0,62	0,61	0,63	0,59												
21	*A. ovata*	0,54	0,61	0,60	0,55	0,65	0,60	0,59	0,58	0,60	0,57	0,50	0,58	0,70	0,70	0,55	0,67	0,70	0,62	0,68	0,50											
22	*A. ovata*	0,56	0,63	0,61	0,61	0,71	0,51	0,65	0,60	0,67	0,64	0,62	0,55	0,71	0,71	0,58	0,63	0,76	0,68	0,70	0,47	0,79										
23	*A. peregrina*	0,51	0,52	0,49	0,43	0,50	0,46	0,48	0,56	0,58	0,50	0,53	0,55	0,53	0,59	0,52	0,52	0,53	0,49	0,50	0,40	0,65	0,72									
24	*A. peregrina*	0,58	0,50	0,59	0,47	0,53	0,77	0,55	0,53	0,64	0,53	0,55	0,47	0,51	0,56	0,39	0,50	0,51	0,48	0,53	0,36	0,65	0,67	0,73								
25	*A. biuncialis*	0,41	0,65	0,58	0,57	0,68	0,51	0,67	0,72	0,68	0,70	0,58	0,62	0,53	0,54	0,59	0,58	0,58	0,60	0,67	0,40	0,65	0,61	0,63	0,63							
26	*A. biuncialis*	0,41	0,58	0,58	0,57	0,64	0,40	0,67	0,72	0,74	0,70	0,63	0,62	0,58	0,59	0,59	0,58	0,58	0,65	0,72	0,47	0,59	0,67	0,56	0,59	0,81						
27	*A. searsii*	0,47	0,64	0,39	0,42	0,40	0,39	0,42	0,52	0,48	0,45	0,48	0,50	0,48	0,44	0,56	0,64	0,48	0,50	0,52	0,29	0,48	0,52	0,35	0,31	0,52	0,61					
28	*A. searsii*	0,47	0,46	0,39	0,42	0,40	0,46	0,42	0,44	0,48	0,39	0,48	0,20	0,41	0,44	0,22	0,36	0,41	0,36	0,44	0,38	0,48	0,44	0,44	0,44	0,44	0,44	0,57				
29	*A. vavilovi*	0,31	0,25	0,32	0,29	0,27	0,29	0,29	0,28	0,39	0,24	0,45	0,27	0,32	0,35	0,30	0,33	0,39	0,33	0,35	0,44	0,37	0,41	0,40	0,47	0,32	0,40	0,13	0,38			
30	*A. biuncialis*	0,41	0,58	0,62	0,52	0,59	0,57	0,62	0,61	0,63	0,60	0,58	0,55	0,53	0,54	0,44	0,58	0,47	0,54	0,67	0,33	0,59	0,56	0,50	0,63	0,75	0,69	0,44	0,44	0,32		
31	*A. triuncialis*	0,39	0,50	0,43	0,41	0,44	0,38	0,41	0,49	0,46	0,43	0,46	0,46	0,51	0,47	0,42	0,50	0,51	0,47	0,55	0,37	0,52	0,55	0,55	0,63	0,62	0,62	0,40	0,50	0,36	0,55	
	Mean	0,48	0,60	0,59	0,60	0,62	0,45	0,62	0,61	0,63	0,58	0,58	0,56	0,62	0,59	0,54	0,57	0,58	0,55	0,58	0,40	0,58	0,57	0,52	0,53	0,58	0,55	0,38	0,44	0,34	0,55	
	Overall mean	0,55																														

was evaluated by replicating the RAPD analysis done on all the accessions two to three times with five primers. Reproducibility averaged 95%. Thus under stringent reaction conditions RAPD patterns were highly reproducible.

These results demonstrated also that RAPD-PCR analysis is useful to assess the extent of genetic diversity among *Aegilops* accessions and can provide practical information for the management of genetic resources collection and identification.

References

Al-Ajlouni MM, Jaradat AA.1997. Diversity in durum wheat landraces collected from Jordan. I. Quantitative traits. Cereal Research Communications 25:17 23.

Feinbrun N. 1986. Flora Palestina. The Israeli Academy of Science and Humanities, Jerusalem.

Gonzalez JM, Ferrer E. 1993. Random amplified polymorphic DNA analysis in *Hordeum* species. Genome 36:1029–1031.

Jaradat AA. 1992. Breeding potential of durum wheat landraces from Jordan. II Differential response to drought. Hereditas 116:305–309.

Joshi CP and Nguyen HT. 1993. Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats. Genome 36:602–609.

Kimber G, Feldman, M. 1987. Wild wheat. An introduction. University of Missouri-Columbia.

Kong L, Dong Y, Jia J, Kong LR, Dong YC and Jia JZ. 1998. Random amplified polymorphic DNA analysis in *Aegilops tauschii*. Acta Botanica Sinica 40:223 227.

Kong XY, Ge CM, Jia JZ, Dong YS. 1999. RAPD analysis of phylogeny for five basic genomes in *Aegilops*. Acta Botanica Sinica 41(4):393-397.

Malkawi HI, Ajlouni MM, Jaradat AA. 1998. Analysis of genetic diversity of wild emmer and durum wheats by RAPDs. In: Jaradat AA, editor. TriticeA, pp. 165–170.

Monte JV, Casanova C, Soler C. 1999. Genetic variation in Spanish populations of the genus *Aegilops* revealed by RAPDs. Agronomie 19:419–427.

Okuno K, Ebana K, Noov B, Yoshida H. 1998. Genetic diversity of Central Asian and North Caucasian *Aegilops* species as revealed by RAPD markers. Genetic Resources and Crop Evolution 45:389–394.

Selbach A., Cavalli-Molina S. 2000. RAPD characterization of Brazilian barley (*Hordeum vulgare* ssp. *vulgare*) varieties. Euphytica 111:127–135.

Sun Q, Huang TC, .Ni ZE, Procunier JD. 1996. Study on wheat heterotic group. I. Genetic diversity revealed by random amplified polymorphic DNA (RAPD) in elite wheat cultivars. Journal of Agricultural Biotechnology 4:103–10.

Van Slageren M.W, Elings A, Holly L, Humeid B, Jaradat AA, Obari Kh. 1989. Cereals, food legumes and their wild relatives in Syria and Jordan. Plant Genetic Resources Newsletter 80: 5–14.

Van Slageren M.W. 1994. Wild Wheats: a monograph of *Aegilops* L. and *Amblypyrum* (Jaub. and Spach) Eig (Poaceae). Wageningen Agricultural University, Netherlands.

Vierling RA, Nguyen H. 1992. Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theoretical and Applied Genetics 84:835–838.

Weining S, Henry RJ. 1995. Molecular analysis of the DNA polymorphism of wild barley (*Hordeum spontaneum*) germplasm using the polymerase chain reaction. Genetic Resources and Crop Evolution 42:273–281.

Welsh J, McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213–7218.

Williams JGK, Kubelik AR, Livar KJ, Rafalskt JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6535.

Zaharieva M, David J, This D, Monneveux P. 1999. Analysis of *Aegilops geniculata* Roth genetic diversity in Bulgaria. Cahiers Agricultures 8 (3):181 188.

Zhang XY, Wang RRC, Dong YS. 1996. RAPD polymorphisms in *Aegilops geniculata* Roth (*A. ovata* auct. non L.). Genetic Resources and Crop Evolution 43:429–433.

Seed science and technology needs of SAFORGEN trees for conservation and sustainable use

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Summary

Seed science and technology needs for SAFORGEN trees for conservation and sustainable use

Currently, 27 sub-Saharan African countries have identified 59 African-tree species, from 22 families, as a priority for conservation, management and sustainable use. Three genera—*Combretum, Entandrophragma* and *Terminalia*—are also cited for urgent action. Of these species, 32% are IUCN (the World Conservation Union) or CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) listed, suggesting an immediate need for underpinning science and technology programmes to protect the species. Whilst seed handling forms an essential part of such programmes, detailed information (hard-copy and electronic) on seed storage and germination appears to be lacking for about half those on the list. Details of seed development and dispersal times are also inadequate. The need for collaborative seed biology and biodiversity studies across the SAFORGEN region is discussed.

Key words: tree seeds, germination, germplasm storage, Darwin Initiative, SAFORGEN, Sub-Saharan Africa

Résumé

Besoins en science et technologie des semences d'espèces d'arbres SAFORGEN pour la conservation et leur utilisation durable

Actuellement, 27 pays Africains au Sud du Sahara ont identifié 59 espèces ligneuses appartenant à 22 familles différentes, comme prioritaires pour leur conservation, l'aménagement et leur utilisation durable. En outre, trois genres (*Combretum, Entandrophragma* et *Terminalia*) sont également cités pour des actions immédiates. Trente deux pourcent (32 %) de ces espèces sont sur les listes IUCN et/ou CITES (CITES, 2000); ce qui suggère un besoin immédiat pour étayer les programmes de science et technologie afin de protéger ces espèces. Bien que la manutention des semences constitue une part essentielle de tels programmes, des informations détaillées (documents et électronique) sur la conservation des semences et leur germination n'existent que pour près de la moitié des espèces listées. Des détails sur le développement des graines et leurs périodes de dispersion semblent également insuffisants. La nécessité d'étudier en collaboration, la biologie de ces semences et la biodiversité à travers toute la région SAFORGEN est mise en évidence dans ce papier.

Resumen

Exigencias de ciencia y tecnología sobre semillas para la conservación y uso sostenible de los árboles de los SAFORGEN

En la actualidad, 27 países africanos subsaharianos han otorgado prioridad de conservación, manejo y uso sostenible respecto de 59 especies arbóreas de 22 familias. También se ha requerido acción urgente para tres géneros, *Combretum, Entandrophragma* y *Terminalia*. De estas especies, el 32% figuran en la lista de la UICN (Unión Internacional para la Conservación de la Naturaleza) o la de CITES (Convención sobre Comercio Internacional de Especies de Fauna y Flora en Peligro de Extinción), lo que sugiere la necesidad inmediata de apuntalar programas de ciencia y tecnología destinados a proteger las especies.En tanto la manipulación de semillas forma una parte esencial de dichos programas, respecto de casi la mitad de la lista parece faltar información detallada (en material impreso o soporte electrónico) acerca del almacenamiento y germinación de las semillas. Tampocon son adecuados los detalles sobre el desarrollo de las semillas y sus períodos de dispersión. Se debate la necesidad de emprender estudios en colaboración sobre biología y biodiversidad en toda la región de los SAFORGEN.

Introduction

Forests in sub-Saharan Africa are still disappearing at a rate of about 1% a year (FAO 1997), despite the many reforestation and conservation programmes. As a consequence, the threat to trees in the region is growing fast. For example, it is estimated that 113 tree species are 'critically endangered', 307 'endangered', 1329 'vulnerable', 27 at 'lower risk, but conservation-dependent' and 328 at 'low risk, but near threatened' (Oldfield et al. 1998; UNEP-WCMC 2001; IUCN 2002). This gives a total of >2000 species that require relatively urgent attention to protect them from loss from communities in sub-Saharan Africa, where they are most needed to support reforestation, to combat desertification, to safeguard the environment and to conserve biodiversity.

Raising trees and preserving their seeds are potentially important actions to counteract the threats expounded above. In recent decades, this need has been met through the cultivation of plantation forests using only a handful of genera and families (Schmidt 2000; FAO 2001). Examples are Fabaceae (Leguminosae), Meliaceae, Myrtaceae and Verbenaceae, for which seed handling and storage are generally not major constraints—the seeds of many of these species are tolerant to desiccation, can be stored dry for periods of time and are easy to germinate. However, this general reliance on exotic, fast growing tree species has resulted in the lower use of well-adapted natural resources and the associated loss of local knowledge with respect to husbandry, including seed handling techniques. Consequently, there is a great need to better understand the biology of indigenous species so that their adaptive advantage for use in local conditions can be promoted and sustained.

Species selection

Recent developments in the planning of efforts for the conservation and sustainable use of sub-Saharan African

trees have seen the identification of a list of species of fundamental value to local communities on the basis of their socioeconomic and ecological value (PRONASEF 1997; Eyog Matig and Ouédraogo 1999; Ouédraogo and Boffa 1999). Scientists from many African countries met in Dakar in 1997 (PRONASEF 1997) and in Ouagadougou, Burkina Faso in 1998 (Ouédraogo and Boffa 1999) to identify highly important tree species with multiple uses. This initial list consisted of 302 species. Ultimately, a species was selected if it was mentioned by at least 10 countries (Eyog Matig and Ouédraogo 1999), and these formed the basis of the SAFORGEN list for priority action. A second regional group, of mainly the South African Development Community Tree Seed Centre Network, met in Arusha, Tanzania in June 2000 for a sub-regional workshop on forest and tree genetic resources. Priority tree species and common issues amenable to regional co-operation on conservation activities were identified by nine countries. Ten important native species to this region were acknowledged as a top priority for consideration by at least two of these countries (Sigaud and Luhanga 2000). In rationalizing and consolidating these two regional programme lists, 59 species were identified for urgent attention for *in situ* and *ex situ* conservation efforts (Table 1). In addition, three genera (*Combretum*, *Entandrophragma* and *Terminalia*) were also cited as requiring immediate actions.

The species on the list fall into four key groups, based mainly on socioeconomic criteria:

- producing edible fruits;
- forage value;
- use as timber and for amenities; and
- craft use and other non-wood products.

The majority of species fit in more than one category of use and, reflecting their potential (over)use, 13 species are CITES controlled, as are all species in *Entandrophragma* and *Terminalia* (CITES 2000). The vast majority of these (i.e. 12 species) are also listed by IUCN as potentially threatened or vulnerable (Table 1). In addition, six more species are IUCN-listed, making 31% of the SAFORGEN-listed species of conservation concern.

These species form the backbone of the sub-Saharan African Forest Genetic resources network, i.e. the SAFORGEN programmes. The SAFORGEN programmes aim to identify efficient methods for sustainable use, *in situ* and *ex situ* conservation of forest genetic resources, and regional

Table 1. SAFORGEN priority list of forest tree species identified by more than 10 countries as the highest priority for management and conservation actions in 27 sub-Saharan countries

	Species	Family	Main uses	Conservation status: IUCN (threatened)/ CITES (concern)
1	*Acacia nilotica* (L.) Willd. ex Delile	Fabaceae	Non-wood	
2	*Acacia raddiana* Savi.	Fabaceae	Forage	
3	*Acacia senegal* (L.) Willd.	Fabaceae	Forage; non-wood	
4	*Adansonia digitata* L.	Malvaceae	Fruit; forage; timber; non-wood	
5	*Afzelia africana* Sm.	Fabaceae	Forage; timber	IUCN+CITES
6	*Afzelia quanzensis* Welw.	Fabaceae	Timber; non-wood	
7	*Aningeria altissima* (A. Chev.) Aubrév. & Pellegr.	Sapotaceae	Timber	
8	*Anogeissus leiocarpus* (DC.) G. & Perr.	Combretaceae	Timber	
9	*Aucoumea klaineana* Pierre	Burseraceae	Timber	IUCN+CITES
10	*Baikiaea plurijuga* Harms	Fabaceae	Fruit; forage; timber	IUCN+CITES
11	*Balanites aegyptiaca* (L.) Del.	Zygophyllaceae	Fruit; forage	
12	*Bauhinia rufescens* Lam.	Fabaceae	Forage	
13	*Borassus aethiopum* Mart.	Arecaceae	Fruit; non-wood	
14	*Borassus flabellifer* L.	Arecaceae	Fruit	
15	*Carapa procera* DC.	Meliaceae	Fruit	
16	*Cola nitida* (Vent.) Sch. & Endl.	Malvaceae	Fruit	
17	*Colophospermum mopane* (J. Kirk ex Benth.) J. Léonard	Fabaceae	Timber; non-wood	
18	*Combretum aculeatum* Vent.	Combretaceae	Forage; timber	
	Combretum sp.	Combretaceae	Timber	IUCN
19	*Commiphora africana* (A. Rich.) Engl.	Burseraceae	Forage	
20	*Dacryodes edulis* (G. Don) H. J. Lam.	Burseraceae	Fruit	
21	*Dalbergia melanoxylon* Guill. & Perr.	Fabaceae	Fruit; timber	IUCN

Table 1. (cont.)

	Species	Family	Main uses	Conservation status: IUCN (threatened)/ CITES (concern)
22	*Daniellia oliveri* (R.) Hutch. & Dalz.	Fabaceae	Timber	
23	*Detarium microcarpum* G. & Perr.	Fabaceae	Fruit; timber	
24	*Diospyros mespiliformis* Hochst. ex A. DC.	Ebenaceae	Fruit; timber	
	Entandrophragma sp.	Meliaceae	Timber	IUCN+CITES (7 sp.)
25	*Faidherbia albida* (Del.) A. Chev.	Fabaceae	Forage; non-wood	
26	*Garcinia afzelii* Engl.	Clusiaceae	Non-wood	IUCN
27	*Garcinia epunctata* Stapf	Clusiaceae	Non-wood	IUCN
28	*Garcinia kola* Heckel.	Clusiaceae	Non-wood	IUCN
29	*Gnetum africanum* Welw.	Gnetaceae	Non-wood	
30	*Grewia bicolor* Juss.	Malvaceae	Forage	
31	*Irvingia gabonensis* (Aubr.) Baill.	Simaroubaceae	Fruit; non-wood	IUCN+CITES
32	*Isoberlinia doka* Craib & Stapf	Fabaceae	Timber	
33	*Khaya anthotheca* (Welw.) C. DC.	Meliaceae	Timber	IUCN+CITES
34	*Khaya ivorensis* A. Chevalier	Meliaceae	Timber	IUCN+CITES
35	*Khaya senegalensis* (Desr.) A. Juss.	Meliaceae	Forage; timber	IUCN+CITES
36	*Lannea microcarpa* Engl. & Kr.	Anacardiaceae	Fruit	
37	*Lophira alata* Banks ex C. F. Gaertn.	Ochnaceae	Timber	IUCN+CITES
38	*Maerua crassifolia* Forsk.	Brassicaceae	Forage	
39	*Milicia excelsa* (Welw.) C.C. Berg	Moraceae	Timber	IUCN+CITES
40	*Nauclea latifolia* Blanco	Rubiaceae	Non-wood	
41	*Parinari curatellifolia* Planch.	Chrysobalanaceae	Non-wood	
42	*Parkia biglobosa* (Jacq.) R. Br. ex G. Don	Fabaceae	Fruit	
43	*Pausinystalia johimbe* (K. Schum.) Pierre ex Beille	Rubiaceae	Non-wood	
44	*Pentadesma butyracea* Sabine	Clusiaceae	Fruit	
45	*Prosopis africana* (G. & Perr.) Taub.	Fabaceae	Forage	
46	*Pterocarpus angolensis* DC.	Fabaceae	Forage; timber	IUCN+CITES
47	*Pterocarpus erinaceus* Poir.	Fabaceae	Forage; timber	
48	*Pterocarpus lucens* Lepr.	Fabaceae	Forage	
49	*Ricinodendron heudelotii* (Baill.) Pierre.	Euphorbiaceae	Fruit; non-wood	IUCN
50	*Sclerocarya birrea* (A. Rich.) Hochst.	Anacardiaceae	Fruit; forage; non-wood	
51	*Spondias mombin* L.	Anacardiaceae	Fruit	
52	*Sterculia setigera* Del.	Malvaceae	Non-wood	
53	*Tamarindus indica* L.	Fabaceae	Fruit	
	Terminalia sp.	Combretaceae	Timber	IUCN+CITES (2 sp.)
54	*Trichilia emetica* Vahl.	Meliaceae	Non-wood	
55	*Triplochiton scleroxylon* K. Schum.	Malvaceae	Timber	CITES
56	*Vitellaria paradoxa* Gaertn.	Sapotaceae	Fruit; non-wood	IUCN+CITES
57	*Warburgia salutaris* (Bertol. F.) Chiov.	Canellaceae	Fruit; non-wood	IUCN+CITES
58	*Ximenia americana* L.	Olacaceae	Fruit	
59	*Zizyphus mauritiana* Lam.	Rhamnaceae	Fruit; non-wood	

Species produce edible fruits (Fruit) and timber (Timber). Some are important forage species (Forage), and amenity and fuel-wood species (non-wood products). Species name authorities come from TROPICOS (http://mobot.mobot.org) or IPNI (http://www.ipni.org) and family names from the Angiosperm Phylogeny Group (APT; http://www.systbot.uu.se/classification/APGclassification.html). The conservation status is from IUCN (2002) and CITES (2000).

exchange of scientific and technical information [Eyog Matig and Ouédraogo 1999; Ouédraogo and Boffa 1999; Sigaud and Luhanga 2000; Proceedings Ouagadougou Workshop 2002 (in press); L. Ouédraogo pers. comm.]. The *ex situ* conservation programme includes the collection, storage and treatment of seeds, and also studies on the physiology and other alternative methods of storage such as *in vitro* gene banking and seed cryopreservation. However, for many of the species listed, their sustainable use is substantially hindered by a shortage of seed biology information.

Seed biology

Seed storage behaviour has been defined into three categories that are commonly used today, based on seed tolerance to desiccation (Roberts 1973; Ellis et al. 1990). Recalcitrant (or desiccation intolerant) seeds, e.g. *Vitellaria paradoxa* (shea butter tree) and *Mangifera indica* (mango), die after relatively little drying. In contrast, orthodox (or desiccation tolerant) seeds survive desiccation to very low (<5%) moisture contents, e.g. *Acacia nilotica* (Egyptian thorn tree) and *Oryza sativa* (rice), and store well in the dry state for long periods of time. Finally, intermediate seeds are able to survive drying to relatively low water contents, between those of orthodox and recalcitrant seeds, but are sensitive to –20°C storage (Ellis et al. 1990). It has been suggested recently that this seed classification system be modified in relation to the seeds' tolerance of dehydration across water sorption regions and appropriate temperatures for storage (Pritchard 2004). Type I (orthodox) seeds tolerate drying to moisture contents at the interface between water sorption zone I (high affinity sorption sites) and II (weak affinity sorption sites), i.e. about 15–20% relative humidity (RH). The longevity of such seeds in conventional seeds banks (c. –18°C) is predicted to be in the region of hundreds of years for many species (see Hong et al. 1998). Type II seeds variably tolerate dehydration into sorption zone II, where mainly weak affinity water sorption sites are occupied, and can be stored at some, but not all, sub-zero temperatures. Seed longevity may be as short as a few months to years if an appropriate sub-zero temperature is not used. Type III (recalcitrant) seeds exhibit desiccation stress during removal of multi-molecular, 'free' water, approximating to sorption zone III of the isotherm (i.e. above about 85% RH). Careful manipulation of seed tissue water and cryopreservation provide the main (but challenging) long-term conservation option for such seeds, otherwise seed longevity is restricted to weeks or months.

During the last two decades, several symposia/workshops have been held on tropical forest seed problems by the International Union for Forest Resources Organisations (IUFRO) (see Kamra and Ayling 1987; Turnbull 1990; Some and De Kam 1993; Olesen 1996; Marzalina et al. 1999). Concurrently, technical books on tropical forest seeds have been produced (Albrecht 1993; IPGRI-DFSC 1996; Tompsett and Kemp 1996; Poulsen et al. 1998; Schmidt 2000). However, the coverage of species in the literature is still exceptionally narrow with respect to indigenous species. For example, in a compendium of information on the seed storage behaviour of c. 7000 species only 0.2% of those listed are tropical trees (Hong et al. 1998).

The seed biology of a small number of species on the list has been investigated in detail since 1996 as part of a project on "the handling and storage of recalcitrant and intermediate tropical forest tree seeds" [e.g. IPGRI-Danida Forest Seed Centre (DFSC) 1996, 2002; Ouédraogo et al. 1996, 1999; Sacande et al. 2004]. The species are: *Khaya anthotheca, K. senegalensis, Lannea microcarpa, Pentadesma butyracea, Sclerocarya birrea, Trichilia emetica, Vitellaria paradoxa, Warburgia salutaris* and *Ximenia americana.*

In addition, there is data on seed storage in the literature and at the African tree seed centres. Such information reveals that about 60% of the species on the SAFORGEN list have confirmed desiccation tolerant, Type I seeds (Figure 1; Hong et al. 1998; RBG Kew and CNSF Seed Banks pers. comm.). However, their long-term storage potential needs to be assessed by analysis of storage records from stores across

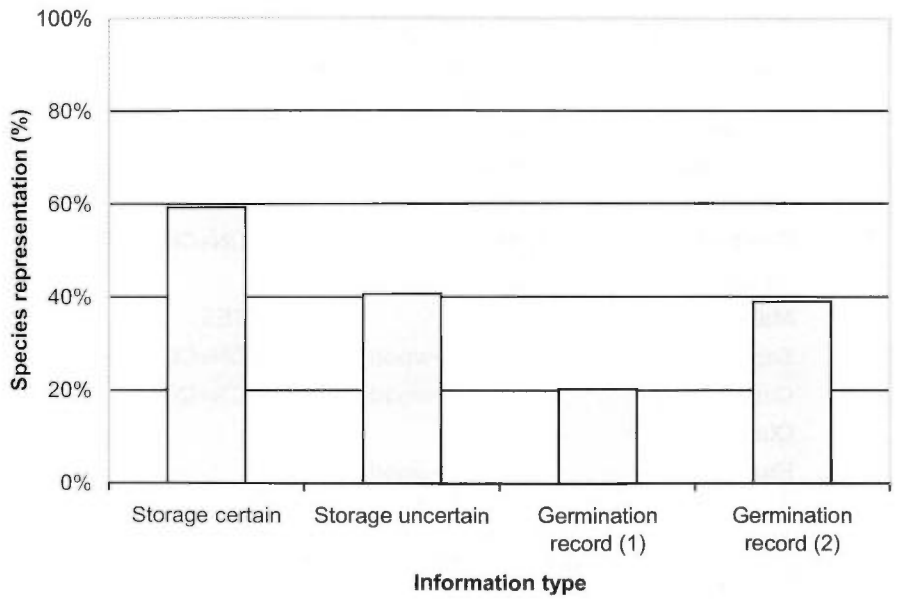

Figure 1. Seed physiology information coverage for 59 SAFORGEN-listed trees. Storage data are analyzed from the combined data sets of Hong et al. (1998) and Tweddle et al. (2003) and signify species for which seeds are either known to tolerate dry storage (storage certain) or not (storage uncertain). The latter category includes species for which data are lacking, are unclear or suggests seed desiccation sensitivity. Germination data are from Baskin and Baskin (1998) (germination record 1) and Tweddle et al. (2003) (germination record 2), the latter including testing data from the seed bank of the Royal Botanic Gardens, Kew.

Africa and, for some species, through seed ageing studies. Also, even for species with more comprehensive data sets (Hong et al. 1998; references in Poulsen et al. 1998), there is still the need to modify/improve seed handling/storage, leading to better practical use in the implementation of *ex situ* and *in situ* conservation activities.

For the other species listed, there is either insufficient information to be certain about the dry storage options for the seeds or they are reputed to produce desiccation sensitive, Type III seeds. To address this shortage of information, it is desirable to subject these target species to desiccation trials, for example using the protocol developed by IPGRI/DFSC (see IPGRI-DFSC 1996; Ouédraogo et al. 1999). This protocol involves the use of thousands of seeds, and requires considerable human resources and consumables. In the case of endangered species, however, even for trees that often have high fecundity, collecting such a quantity of seeds may compromise its sustainability at the local population level. An alternative, lower seed number approach to desiccation tolerance testing has been applied recently to palm seeds (Pritchard et al. 2004). These sorts of seed conservation biology assessments need to be made for about 40% of the SAFORGEN-listed species (Figure 1).

The germination of seeds is crucial to planting and storage programmes. However, there is a lack of detailed data in the literature (hard-copy and electronic) on suitable conditions for germination of tropical tree seeds, in general (see Baskin and Baskin 1998), and for the SAFORGEN species, in particular (Figure 1). The need for pre-treatments, including dormancy breaking, and the optimum temperatures for germination are usually unknown for the SAFORGEN-listed species. In addition, optimization of viability tests (ISTA 2004), such as cutting, conductivity and tetrazolium chloride (TZ) vital staining, is not well established for this group of species. However, because of their importance, it is highly conceivable that some germination testing information is contained within institutional and student reports. The consolidation and release of such information is of utmost importance.

Both germination ability and the desiccation tolerance of seeds increase during development as natural dispersal approaches (Poulsen et al. 1998; CNSF 2000; Hay and Smith 2003). It is therefore important to identify the optimum time for seed harvesting if maximum storability of these seeds is to be achieved. Fruit colour can be, but is not always, a precise marker of physiological maturity of the seed and embryos. For example, embryos of neem (*Azadirachta indica*) seeds from mature-green, yellow and brown fruits showed no significant differences in their initial germinability, but the storage longevity of those from yellow fruits was greatly superior (Sacandé 2000). This indicates that physiological maturity of embryos is not only characterized by the attainment of maximum germination, but also by the seed's ability to withstand stresses. In this context, better characterization of seed quality at dispersal could also provide an insight to the contribution that seeds of these species make to their natural regeneration in the field. Of particular interest is the possibility of comparing seed development and dispersal times for relatively cosmopolitan species between countries and sometimes across the sub-Saharan region.

In conclusion, a better understanding of the seed biology of SAFORGEN-listed species will substantially enhance opportunities for their *ex-situ* conservation and sustainable use across the African continent.

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References

Albrecht J. 1993. Tree seed handbook of Kenya. GTZ Forestry Seed Centre, Muguga. Kenya Forest Research Institute, GTZ/KEFRI.

Baskin CC, Baskin JM. 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, USA.

Centre National de Semences Forestières (CNSF). 2000. Catalogue de Semences Forestières–2000. Ouagadougou, Burkina Faso.

CITES. 2000. Controls as adopted by the Conference of the Parties, valid from 19 July 2000 (see http://www.cites.org).

Ellis RH, Hong TD, Roberts EH. 1990. An intermediate category of seed storage behaviour? I. Coffee. Journal of Experimental Botany 41:1167-1174.

Eyog Matig O, Ouédraogo AS. 1999. State of forest genetic resources in the Sahelian and North-Sudanian zone of Africa. FAO, Forest Genetic Resources, Rome, Italy 27:27-33.

FAO (Food and Agricultural Organization). 1997. State of the World's Forests. FAO, Rome, Italy.

FAO (Food and Agricultural Organization). 2001. Global Data on Forest Plantations Resources Vol. 29. FAO, Forest Genetic Resources, Rome, Italy, pp. 34–39.

Hay FR, Smith RD. 2003. Seed maturity: knowing when to collect seeds from wild plant species. In: Smith RD, Dickie JB, Linington SH, Pritchard HW, Probert RJ, editors. Seed Conservation: Turning Science into Practice. Royal Botanic Gardens, Kew, Richmond, UK, pp. 97–133.

Hong TD, Linington S, Ellis RH. 1998. Compendium of information on seed storage behaviour. International Plant Genetic Resources Institute, Rome, Italy. [Updated version is also searchable at http://www.rbgkew.org.uk/data/sid]

International Seed Testing Association (ISTA). 2004. International rules for seed testing. Edition 2004. ISTA, Bassersdorf, Switzerland.

IPGRI–DFSC. 1996. The project on handling and storage of recalcitrant and intermediate tropical forest tree seeds. Danida Forest Seed Centre, Humlebaek, Denmark. Newsletter No. 1, July 1996, 30pp.

IPGRI–DFSC. 2002. The project on handling and storage of recalcitrant and intermediate tropical forest tree seeds. Danida Forest Seed Centre, Humlebaek, Denmark. Newsletter No. 10, September 2002. [Also searchable at http://www.dfsc.dk/IPGRIProject.htm]

IUCN (the World Conservation Union). 2002. IUCN red list of threatened species. World Conservation Press. Searchable database at http://www.unep-wcmc.org/trees/background/africa.htm [accessed June 2003].

Kamra SK, Ayling RD. 1987. Proceedings of the International Symposium on Forest Seed Problems in Africa, Harare, Zimbabwe, August 23–September 2, 1987. Swedish University of Agricultural Sciences, Umeå, Sweden.

Marzalina M, Khoo KC, Jayanthi N, Tsan FY, Krishnapillay B. 1999. Seed Symposium 1998. IUFRO Conference Proceedings on Recalcitrant Seeds. FRIM, Kuala Lumpur, Malaysia, 451pp.

Oldfield S, Lusty C, MacKinven A. 1998. The world list of threatened trees. World Conservation Press, Cambridge, UK.

Olesen K. 1996. Innovations in tropical tree seed technology. Proceedings of the IUFRO Symposium of the Project Group P.2.04.00, 'Seed Problems'. Arusha, Tanzania, 1995. Danida Forest Seed Centre, Humlebaek, Denmark.

Ouédraogo AS, Boffa JM. 1999. Vers une approche régionale des ressources génétiques forestières en Afrique sub-saharienne. Actes du premier atelier régional de formation sur la conservation et l'utilisation durable des ressources génétiques forestières en Afrique de l'Ouest, Afrique Centrale et Madagascar, 16-27 Mars 1998, Centre National de Semences Forestières (CNSF), Ouagadougou, Burkina Faso. IPGRI, Rome, Italy.

Ouédraogo AS, Poulsen K, Stubsgaard F. 1996. Intermediate and recalcitrant tropical forest tree seeds. Proceedings of a Workshop on 'Improved Methods for Handling and Storage of Intermediate and Recalcitrant Tropical Forest Tree Seeds', 8-10 June 1995, Humlebaek, Denmark. IPGRI, Rome, Italy and Danida Forest Seed Centre, Humlebaek, Denmark.

Ouédraogo AS, Thomsen K, Engels JMM, Engelmann F. 1999. Challenges and opportunities for enhanced use of recalcitrant and intermediate tropical forest tree seeds through improved handling and storage. In: Marzalina M, Khoo KC, Jayanthi N, Tsan FY, Krishnapillay B, editors. IUFRO Seed Symposium 1998. Conference Proceedings on Recalcitrant Seeds. FRIM, Kuala Lumpur, Malaysia, pp. 227–234.

Poulsen KM, Parrat MJ, Gosling PG. 1998. Tropical and sub-tropical tree and shrub seed handbook (1st ed.). International Seed Testing Association, Zurich, Switzerland.

Pritchard HW. 2004. Classification of seed storage 'types' for *ex-situ* conservation in relation to temperature and moisture. In: Guerrant EO, Jr, Havens K, Maunder M, editors. *Ex situ* Plant Conservation: Supporting species survival in the wild. Island Press, Covelo, CA, USA, pp. 139–161.

Pritchard HW, Wood CB, Hodges S, Vautier HJ. 2004. 100-seed test for desiccation tolerance and germination: a case study on eight tropical palm species. Seed Science and Technology 32, 393–403.

Proceeding Ouagadougou Workshop. 2002. Rapport sur la quatrième rencontre des Centres et Programmes Nationaux de Semence Forestières d'Afrique. CNSF, Ouagadougou, Burkina Faso.

Projet National de Semences Forestières (PRONASEF). 1997. Rapport sur la troisieme rencontre des Centres et Programmes Nationaux de Semences Forestieres d'Afrique. Dakar, Senegal.

Roberts EH. 1973. Predicting the storage life of seeds. Seed Science and Technology 1:499-514.

Sacandé M. 2000. Stress, storage and survival of neem seed. PhD thesis. Wageningen University, Netherlands.

Schmidt L. 2000. Guide to handling of tropical and sub-tropical forest seed. Danida Forest Seed Centre, Humlebaek, Denmark.

Sigaud P, Luhanga J. 2000. SADC sub-regional workshop on forest and tree genetic resources. FAO, Forest Genetic Resources, Rome, Italy 28:41-44.

Some LM, de Kam M. 1993. Tree seed problems, with special reference to Africa. Proceedings of the IUFRO Symposium of the Project Group P.2.04.00, 'Seed Problems'. Ouagadougou, Burkina Faso, 1992. Backhuys Publisher.

Tompsett PB, Kemp R. 1996. Database on tropical tree seed research (DABATTS). Database contents. Royal Botanic Gardens Kew, Richmond, UK.

Tweddle JC, Turner RM, Dickie JB. 2003. Seed information database (release 4.0, Jan. 2003) http://www.rbgkew.org.uk/data/sid [accessed June 2003].

Turnbull JW. 1990. Tropical tree seed research. Proceedings of an International Workshop Help at the Forestry Training Centre, Gympie, Queensland, Australia, 1989. ACIAR Proceedings No. 28.

UNEP-WCMC. 2001. http://www.unep-wcmc.org/species/tree_study/1.pdf

Collecting fonio (*Digitaria exilis* Kipp. Stapf, *D. iburua* Stapf) landraces in Togo

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Summary
Collecting fonio (*Digitaria exilis* Kipp. Stapf, *D. iburua* Stapf) landraces in Togo
Fonio (*Digitaria exilis* Kipp. Stapf and *Digitaria iburua* Stapf) is a minor cereal crop cultivated throughout West Africa. In order to preserve its genetic diversity ex situ in Togo for sustainable use by present and future generations, a collecting mission was carried out during November 2001 in the two producing zones of the country. In these two zones, covering three administrative regions (Plateaux, Kara and Savanes), 95 accessions representing 34 landraces were collected from seven ethnic groups in 46 villages. The collected germplasm was duplicated: a part is conserved at the University of Lomé (Togo) and the other at Niaouli Agricultural Research Centre (National Agricultural Research Institute) in Benin. The landraces collected are specific to their producing zones. From our findings, the region of Kara has the most important landrace diversity, and Lamba and Akposso tribes are the most important curators of fonio diversity in Togo. The crop is being abandoned to maize and its production is declining because of the need for tedious harvesting and post-harvest processing. Fonio is under threat from genetic erosion and concerted actions are needed for the sustainable conservation of the diversity of this indigenous crop in Togo.

Key words: *Digitaria exilis*, *Digitaria iburua*, fonio, germplasm collecting, landraces, genetic erosion, Togo

Résumé
Collection de variétés locales du fonio (*Digitaria exilis* Kipp. Stapf et *D. iburua* Stapf) au Togo
Le fonio (*Digitaria exilis* Kipp. Stapf et *Digitaria iburua* Stapf) est une céréale mineure cultivée en Afrique de l'Ouest. Dans le but de préserver ex-situ sa diversité génétique au Togo pour une utilisation durable par les générations présentes et futures, une mission de collecte a été organisée en novembre 2001 dans les deux zones de production du pays. Dans ces zones qui couvrent trois régions administratives (Plateaux, Kara et Savanes), un total de 95 accessions représentant 34 variétés locales ont été collectées dans 46 villages à travers sept groupes ethniques. Le germoplasme collecté est dupliqué: une partie est conservée à l'Université de Lomé (Togo) et l'autre partie est conservée à la Station de recherche agricole de Niaouli (Institut National de Recherche Agricole) au Bénin. Les variétés collectées sont spécifiques à leurs zones de culture. La région de la Kara apparaît avoir la plus importante diversité variétale alors que les *Lamba* et les *Akposso* constituent les plus grands conservateurs de la diversité génétique de fonio au Togo. La production du fonio est en déclin essentiellement à cause des difficultés liées à la récolte et des contraintes de transformation post-récolte et sa culture est entrain de régresser au profit du maïs. L'érosion génétique est croissante et des actions spécifiques sont nécessaires pour la conservation durable de cette culture traditionnelle au Togo.

Resumen
Recolección de razas autóctonas de fonio (*Digitaria exilis* Kipp. Stapf, *D. iburua* Stapf) en Togo
El fonio (*Digitaria exilis* Kipp. Stapf, *D. iburua* Stapf) es un cereal secundario cultivado en toda Africa occidental. En noviembre de 2001 se llevó a cabo una misión de recolección en las dos zonas productivas de Togo a fin de preservar la diversidad genética *ex situ* y de que las generaciones actuales y futuras puedan usarlo de manera sostenible. En estas dos zonas que cubren tres regiones administrativas (Plateaux, Kara y Savanes), se recogieron 95 accesiones que representan 34 razas locales en siete grupos étnicos de 46 aldeas. El germoplasma recogido fue duplicado: una parte se conserva en la Universidad de Lomé (Togo) y la otra en el Centro de Investigación Agrícola de Niaouli (Instituto Nacional de Investigación Agrícola) en Benin. Las razas locales recogidas son específicas de su zona de producción. Con arreglo a nuestros hallazgos, la más importante diversidad de razas locales está en la región de Kara, y las tribus Lamba y Akposso son las principales conservadoras de la diversidad de fonio en Togo. El cultivo se está abandonando en favor del maíz y su producción está disminuyendo porque su cosecha es tediosa y requiere un procesamiento poscosecha. El fonio se encuentra amenazado por la erosión genética y se necesitan acciones concertadas para lograr la conservación sostenible de la diversidad de estos cultivos autóctonos de Togo.

Introduction
Fonio millet (*Digitaria exilis* Kipp. Stapf and *Digitaria iburua* Stapf) is a minor cereal in many countries of West Africa where it is a staple food crop for several millions of tribal people (Vietmeyer et al. 1996). Fonio grows under conditions of low-rainfall and low fertility and is adapted to marginal land farming. Apart from its nutritional importance, fonio is also a crop of high social and cultural value for the local communities traditionally associated with its production.

According to Portères (1976) Togo is one of the centres of diversity of fonio millet in West Africa. Therefore, important landrace diversity is expected to be found there. A regional germplasm collecting mission was jointly organised in 1977 by IPGRI (International Plant Genetic Resources Institute) and IRD (Institut de Recherche pour le Developpement/ex ORSTOM) to conserve the diversity of fonio *ex situ* in West Africa (Clement and Leblanc 1984). The duplicate germplasm collection from Togo, conserved by the National Agricultural Research Institute (ITRA), disappeared in its entirety. In many countries of West Africa, including Togo, fonio has been neglected by the scientific research and agricultural

development practitioners. Consequently, the extent of genetic diversity of the crop in Togo remains unknown and the difficulties related to its cultivation and post-harvest processing reported previously (Portères 1976) still persist and many varieties have disappeared (Dantsey 1998; Anonymous 1999). It is therefore necessary and urgent to rebuild, through an exhaustive germplasm collection, a national collection of fonio landraces for conservation and sustainable use (production and breeding) following Williams (1987) and Kwon-Ndung et al. (1998). This paper reports on the fonio germplasm collecting mission undertaken in November 2001 in different production zones in Togo.

Materials and methods

Production zone of fonio millet in Togo

Fonio millet is cultivated in three administrative regions in Togo; Kara, Savanes and Plateaux. Kara and Savanes are located in the north and Plateaux in the south. These three regions represent two production zones that are located between 0°31'36" and 1°53'37" east and 7°00'00" and 10°20'00" north (Figure 1). The southern zone has a subequatorial climate with annual rainfall of 1300 mm to 1600 mm and elevations ranging from 400 m to 1000 m above sea level. The northern zone receives less than 1000 mm annual rainfall and has more than five months dry season; elevations are rarely over 800 m asl. Both zones are mountainous and soils are mostly poor (sandy, rocky and encrusted or shallow). Not all ethnic groups produce fonio in Togo. Cultivating the crop is the prerogative of the Akposso and Akébou (Plateaux), Lamba, Losso Nawda, Kabyè, Tamberma (Kara), Tchokossi and Gangan (Savanes).

Collecting sites and sampling strategies

Forty-six (46) villages (25 in the south and 21 in the north) were randomly selected in the two production zones (Figure 1). In each village farmers were first asked to list and group the landraces they actually grew and those that they knew during their youth or heard about from their parents and give a short description of each of them. Secondly, farmers' perceptions of the evolution of fonio millet diversity and the factors affecting that diversity were recorded. Seeds of the diverse landraces available in the village were collected directly from farmers' granaries or fields. Seed samples of each landrace were independently collected from two to three randomly selected farmers. Accessions were collected in the field from panicles of at least 50 individuals at many sites (15–20 steps difference) following Brown and Marshall (1995). Each sample was numbered and put in a paper bag.

Results and discussion

A total of 95 accessions, representing 34 landraces, were collected from 81 households. Of the 95 accessions, 50 were collected in Plateaux region, 40 in Kara region and 5 in Savanes region. The accessions were duplicated and conserved *ex situ* at the Faculty of Sciences (University of Lomé) and in the Genebank of Niaouli Agricultural Research Center of INRAB in Benin. The list of the collected landraces, their geographical distribution and description (farmers' descriptions) are summarized in Table 1.

In Togo, as in many countries of West Africa, two species of *Digitaria* are cultivated as fonio: *D. exilis* (white fonio) and *D. iburua* (black fonio). According to Portères (1976), Zeven and de Wet (1982) and Haq and Dania Ogbe (1995), both species exist in the northern production zone, but white fonio is the most widely cultivated in the country. Black fonio is cultivated

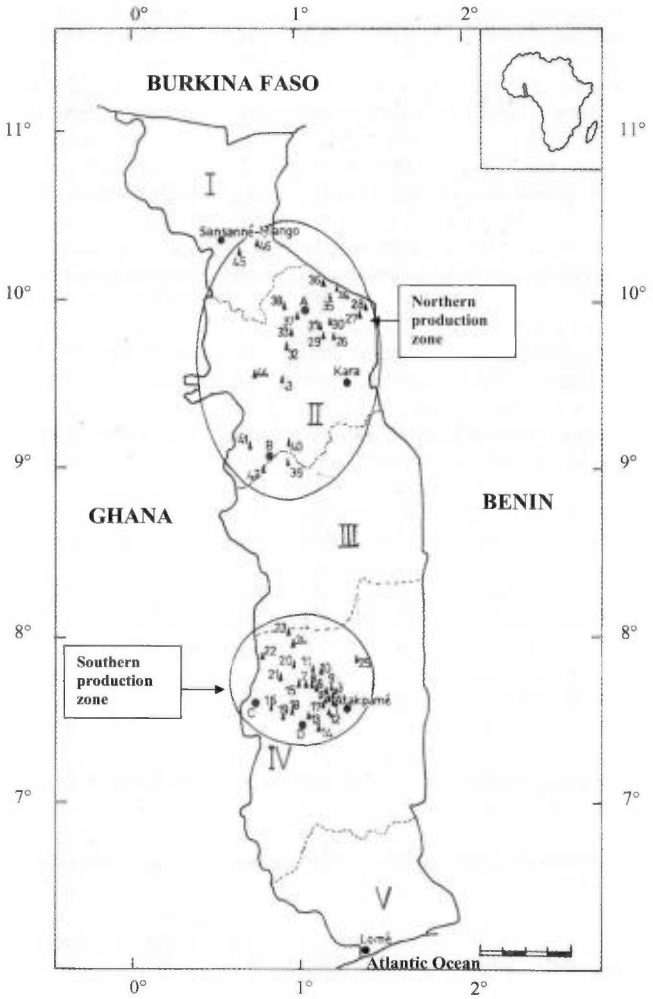

Figure 1. Collecting sites in the fonio production zones of Togo. A: Kantè; B: Bassar; C: Badou; D: Amlamé --------- Limit of administrative regions
I: Savanes region; II: Kara region; III: Central region; IV: Plateaux region; V: Seaside region
Collecting sites: 1. Etchécopé; 2. Dotchécopé; 3. Togblé Aféyé; 4. Kodjo-Aza; 5. Ougbo-Ali; 6. Iwa; 7. Oga; 8. Mouna; 9. Edoko; 10. Ouanibè; 11. Eliko; 12. Yaocopé; 13. Ezimè; 14. Amoutsi; 15. Klabé Akpéganmè; 16. Adossou; 17. Ounabè; 18. Ekéto; 19. Gbadi Gaodo; 20. Kotora; 21. Vhé Nkognan; 22. Yalla; 23. Kabanyi; 24. Wadanyi; 25. Anié; 26. Koka; 27. Koré; 28. Massédéna; 29. Baga; 30. Paha; 31. Défalé; 32. Broukou; 33. Kadjala; 34. Warango; 35. Bassamba ; 36. Nadoba; 37. Adjéidé; 38. Ataloté; 39. Kamagbandè; 40. Kassou; 41. Inaba; 42. Saboundi; 43. Koundoum; 44. Didoudikpre ; 45. Okparobôsso; 46. Gando Djèbouri

Table 1. Local names, species, location, number of accessions collected and farmers' description of the diverse fonio landraces collected in Togo

No.	Vernacular name	Ethnic groups	Species	Production Zone	NA	Farmers' description
1	Ova	Akposso	*D. exilis*	South	27	Late maturing landrace (4–5 months)
2	Afiohoun	Lamba	*D. exilis*	North	2	Early maturing (3 months), white seeds
3	Ipibim	Lamba	*D. exilis*	North	1	Late maturing landrace (4 months)
4	Sémbre	Lamba	*D. exilis*	North	7	Intermediate to late maturing landrace (3.5 –4 months) big and reddish grains
5	Vitchi	Akposso	*D. exilis*	South	4	Early maturing landrace (3 months), dwarf and reddish grains
6	Trikpa	Akposso	*D. iburua*	South	2	Late maturing landrace (4 months) with big and black grains, hard bran and difficult to husk
7	Ezio	Akposso	*D. exilis*	South	1	Early maturing landrace (3 months), white seeds
8	Vafoo	Akposso	*D. exilis*	South	3	Early maturing landrace (2.5 months), easy cultivation
9	Egniva	Akposso	*D. exilis*	South	3	Intermediate (3.5 months)
10	Avècasho	Akébou	*D. exilis*	South	2	Early maturing landrace (3 months),
11	Ougniva	Akposso	*D. exilis*	South	1	Intermediate (3.5 months), big grains
12	Gninminbi	Akébou	*D. exilis*	South	5	Early maturing landrace (3 months) tiny and easy husking grains
13	Oufapôh	Akébou	*D. exilis*	South	1	Late maturing landrace (4 months)
14	Fig'm	Nawda	*D. exilis*	North	3	Early maturing landrace (3 months)
15	Namba	Nawda	*D. exilis*	North	1	Early maturing landrace (3 months)
16	Djibiga	Nawda	*D. exilis*	North	1	Late maturing landrace (4 to 5 months)
17	Lamfig'm	Nawda	*D. exilis*	North	2	Late maturing landrace (4 months), reddish grains
18	Tchabigô	Lamba	*D. exilis*	North	1	Late maturing landrace (4 months)
19	Tchibam	Lamba	*D. iburua* ?	North	2	Late maturing landrace (4 months), black grains, difficult to husk
20	Ayôrô	Lamba	*D. exilis*	North	3	Extra-precocious (2.5 months), white seeds, mature in raining period and difficult to harvest
21	Kopordagou	Tamberma	*D. exilis*	North	2	Late maturing landrace (4 months), rounded and reddish grains
22	Itamali	Tamberma	*D. exilis*	North	2	Intermediate (3.5 months), tiny, rounded and reddish grains
23	Iporlepiah	Tamberma	*D. exilis*	North	2	Early maturing landrace (3 months), white grains
24	Kiwo	Lamba	*D. exilis*	North	3	Early maturing landrace (3 months), rounded, hairy and reddish grains
25	Folom	Lamba	*D. exilis*	North	1	Late maturing landrace (4 months), tiny, rounded and white grains
26	Yolum	Lamba	*D. exilis*	North	2	Intermediate (3.5 months)
27	Ounvonikpa	Tchokossi	*D. exilis*	North	2	Late maturing landrace (5 months)
28	Ounfissa	Gangam	*D. exilis*	North	1	Early maturing landrace (3 months), reddish grains
29	Iponi	M'berme	*D. exilis*	North	1	Late maturing landrace (4 months)
30	Ipoaga	Otamari	*D. exilis*	North	1	Early maturing landrace (3 months)?
31	Kayara	Lamba	*D. exilis*	North	2	Late maturing landrace (4 months)
32	Tchapionga	Nawda	*D. exilis*	North	2	Late maturing landrace (4 months)
33	Naman	Otamari	*D. exilis*	North	1	Intermediate (3.5 months)
34	Ipoeda	Otamari	*D. exilis*	North	1	Late maturing landrace (4 months)

NA=number of accessions.

by few farmers and has hard bran and is very difficult to husk (Ndoye and Nwasike 1993). Landraces Trikpa and Tchibam, collected during the survey, were reported by farmers as having black seeds and being difficult to husk. We believe that these landraces are probably *D. iburua*. Future detailed morphological and molecular characterization is however needed for clarification.

According to farmers, landraces actually grown in the southern zone (Plateaux region) are different from those cultivated in the northern zone: each zone has its specific landraces. Landraces from the northern production zone are better adapted to dry conditions than those cultivated in the south, which are adapted to a relatively wet climate. No formal seed exchange exists between farmers of the two zones because of geographical distance and ecological and cultural factors. Farmers from the south scornfully consider the northern landraces as 'birds' fonio' due to the small grain size. If this is true, landraces cultivated in these two zones could be considered as ecotypes. In depth agronomic, morphological and genetic studies are needed for verification of this hypothesis.

With regard to the amount of diversity maintained at the village and region level, Kara in the north appeared to have the most important diversity (29 landraces inventoried, but 19 were collected) followed by Plateaux in the south with 10 landraces (all collected) and Savanes in the north (4 landraces, 2 collected). At the ethnic group level, the most important landrace diversity is maintained by the Lamba followed by the Akposso, Losso-Nawda and Tamberma (Table 1). The least numbers of landraces were associated with the ethnic groups Akébou, Tchokossi and Gangan.

Eight landraces were reported lost by the farmers (Table 2). Some of them, including Kpéntiki, Waareh and Yaarang, were collected in 1977 during the first fonio collecting survey in Togo (Clement and Leblanc 1984). As reported by farmers, the main reason for fonio genetic erosion lies in the neglect of fonio cultivation due to difficulties in harvesting and post-harvest processing. During our survey, abandonment of fonio cultivation was recorded at both village (some Akébou villages in Wawa district) and district (Dankpen and Binah districts in Kara region) levels. Many of the landraces still in cultivation are under threat. For example, Ezio and Djibiga were found with only one nostalgic farmer. On the one hand, the shortened rainy season represents climate change that is leading many farmers to abandon cultivation of the late-maturing landraces. On the other hand, many farmers abandon the extra early maturing fonio landraces (Vafoo and Ayôrô) for maize because of its higher yield, easier cultivation, harvesting and processing. Erosion of fonio genetic resources is evident in Togo. Specific action is needed to halt loss of landraces and to improve fonio production and its sustainable conservation in Togo.

Conclusion

The survey allowed collection of substantial fonio millet diversity present in Togo and showed that genetic erosion is evident and increasing in the two production zones. Complementary strategies (farmers' awareness, on-farm alternatives) have to be developed for sustainable conservation and use of fonio genetic resources in Togo. Morphological and genetic characterization of the collected germplasm is on-going in close collaboration with IPGRI, IPK (Institut für Pflanzengenetik und Kulturpflanzenforschung) and the Department of Plant Breeding (University of Giessen) in Germany. This investigation will provide some scientific information required for genetic improvement and it will help create modern varieties for the promotion of this indigenous cereal in Togo and beyond.

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Table 2. List of fonio landraces reported as disappeared by the farmers in northern production zone of Togo

Landraces	Ethnic group	Description reported
Adjougouri	Lamba	Produce many tillers
Angim	Lamba	Late maturing variety (4 months)
Awèrô	Lamba	Early maturing landrace
Foukmum	Nawda	Produce many tillers, late maturing variety (4 months)
Hobi	Lamba	Late maturing variety (4 months)
Kpéntiki	Tchokossi	Early maturing variety
Yaarang	Nawda	Early maturing variety (3 months) rounded, hairy and reddish grains
Waareh or Waaroh	Lamba	Difficult to husk, late maturing variety (4 months), big, black grains, difficult cultivation

References

Anonymous. 1999. Statistiques de production agricole. Direction des Statistiques agricoles, de l'Information et de la Documentation. Lomé, Togo.

Brown AHD, Marshall DR. 1995. A basic sampling strategy: theory and practice. In: Guarino L, Ramanatha Rao V, Reid R, editors. Collecting Plant Genetic Diversity, Technical Guidelines. CABI, Wallingford, UK, pp. 75-91.

Clément J, Leblanc JM. 1984. Prospection des *Digitaria exilis* (Fonio) en Afrique de l'Ouest. Collecte IBPGR-ORSTOM de 1977 au Togo. Catalogue ORSTOM 1984:3-7.

Dantsey H. 1998. Situation du fonio (*Digitaria exilis*) au Togo. In: Vodouhè SR, Zannou A, Achigan Dako E, éditeurs. Actes du premier atelier sur la diversité génétique du fonio (*Digitaria exilis* Stapf.) en Afrique de l'Ouest,. Conakry, Guinée, du 04 au 06 Août 1998. IPGRI, Rome, Italy, pp. 27–28.

Haq N, Dania Ogbe F. 1995. Fonio (*Digitaria exilis* and *Digitaria iburua*). Chapter 5, Cereals and Pseudocereals. Chapman and Hall, London, UK.

Kwon-Ndung E H, Misari SM, Dachi SN. 1998. Collecting germplasm of acha, *Digitaria exilis* (Kipp. Stapf.), accessions in Nigeria. Plant Genetic Resources Newsletter 116:30–31.

Ndoye M, Nwasike CC. 1993. Fonio millet (*Digitaria exilis* Stapf) in West Africa. In: Riley KW, Gupta SC, Seetharam A, Mushonga JN, editors. Advances in Small Millets. Oxford and IBH, New Delhi, India, pp. 85–94.

Portères R. 1976. African cereals, *Eleusine*, fonio, black fonio, *Brachiaria*, *Paspalum*, *Pennisetum*, and African Rice. In: Harlan JR de Wet, JMJ, Stemler ABL, editors. Origins of African Plant Domestications. Mouton, The Hague, Netherlands, pp. 409–452.

Vietmeyer ND, Borlaugh NE, Axtell J, Burton GW, Harlan JR, Rachie KO. 1996. Fonio (Acha). In: Vietmeyer ND, editor. Lost Crops of Africa. BOSTID, National Academic Press, Washington, DC, USA.

Williams WM. 1987. Adaptive variation. In: Baker MJ, Williams WM, editors. White Clover. CAB International, Wallingford, UK, pp. 300–317.

Zeven AC, de Wet JMJ. 1982. Dictionary of cultivated plants and their regions of diversity. Excluding most ornamentals, forest trees and lower plants. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands.

News and Notes

International congress on *Musa*: harnessing research to improve livelihoods

Between 6 and 9 July 2004, some 250 delegates converged on Penang, Malaysia, to attend the First International Congress on *Musa* organized by International Network for the Improvement of Bananas and Plantains (INIBAP) and the Malaysian Agricultural Research and Development Institute (MARDI). The theme 'Harnessing research to improve livelihoods' was chosen to illustrate INIBAP's commitment to the building of knowledge across disciplines and regions that, in due course, should translate into improved livelihoods for the world's banana farmers and communities. One such example is the promotion in Micronesia of the locally grown Fe'i bananas to alleviate vitamin A deficiency, which is associated with increased vulnerability to infections, such as diarrhoea, skin diseases and respiratory infections. The data, presented to great interest at the congress and picked up by the media, show that a common variety, the Karat, contains up to 25 times the levels of vitamin A precursors found in Cavendish bananas.

In other sessions, researchers presented the latest findings on the *Musa* genome. Members of the Global *Musa* Genomics Consortium, created in 2001, have made progress in decoding the banana's genetic instructions by teaming up with their counterparts who work on the much-better-known genome of rice. Delegates also turned their attention to the enemies of bananas: the fungi, nematodes and viruses that keep down yields in many parts of the tropics and a session on sustaining the natural resource base reminded everyone that there are great gains to be made by synthesizing and exchanging knowledge of ecologically based agriculture between cropping systems. Finally, enterprise development, an often overlooked perspective at scientific meetings, was discussed since establishing successful banana-based businesses provides farmers with the means and incentive to invest in soil fertility and improved crop protection.

Abstracts of the congress presentations are available from: http://www.inibap.org/index.php?page=home->meeting.

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 the International Plant Genetic Resources Institute (IPGRI) 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
In the first instance papers may be submitted in typescript form or as an Email message. The final version may be submitted as an Email file or as a Windows-readable file on diskette. Manuscripts submitted for publication and other communications on editorial matters should be addressed to IPGRI's Communications Services.

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 l'Institut international des ressources phytogénétiques (IPGRI) 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 intêrêt 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
En premier lieu, les documents doivent être soumis dactylographiés ou par courrier électronique. La version définitive doit être présentée en fichier de courrier électronique ou sur disquettes compatibles Windows. Prière d'adresser les manuscrits présentés pour être publiés et d'autres communications sur des questions de rédaction au Bureau de rédaction de l'IPGRI.

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 Instituto Internacional de Recursos Fitogenéticos 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
Los documentos deben entregarse, incialmente, en forma de texto mecanografiado o a través del correo electrónico. La versión final debe presentarse como un archivo de correo electrónico o en disquete compatible con el sistema operativo Windows. Los manuscritos para publicar y otras comunicaciones sobre asuntos relativos a la redacción deberán dirigirse a la Oficina de Redacción del IPGRI.

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