ISSN 1020-3362

Plant Genetic Resources Newsletter

Bulletin de Ressources Phytogénétiques

Noticiario de Recursos Fitogenéticos

No. 143, 2005

Food and Agriculture Organization of the United Nations and the International Plant Genetic Resources Institute
Organisation des Nations Unies pour l'alimentation et l'agriculture et l'institut international des ressources phytogénétiques
Organización de las Naciones Unidas para la Agricultura y la Alimentación y el Instituto Internacional de Recursos Fitogenéticos

Editorial Office

Managing Editor
Plant Genetic Resources Newsletter
IPGRI
Via dei Tre Denari, 472/a
00057 Maccarese
Rome, Italy
Tel.: (+39)0661181
Email: p.neate@cgiar.org
Fax: (+39)0661979661
Web: http://www.ipgri.cgiar.org

Bureau de rédaction

Manuscript submission and queries
PGRNManuscripts@cgiar.org

Oficina de Redacción

Cover: A true wheat landrace relic from the Pietralba countryside with bread made from its flour. This landrace was widely cultivated on the terraced farms of Corsica prior to the Second World War; today it is almost extinct. This wheat landrace is just one of the crops discussed by Bullitta et al. (pp. 27-34). Photo: S. Cifarelli

Couverture : Véritable variété de blé « relique », cultivée dans les environs de Pietralba et pain préparé avec la farine de celle-ci. Cette variété était largement répandue dans les cultures en terrasses en Corse avant la seconde guerre mondiale ; aujourd'hui elle a pratiquement disparu. Cette variété de blé n'est qu'un exemple des cultures décrites dans l'article de Bullitta et al. (pp. 27-34). Photo: S. Cifarelli

Portada: Una verdadera reliquia de raza local de trigo de la campiña de Pietralba y el pan hecho con su harina. Antes de la Segunda Guerra Mundial esta raza local era cultivada extensamente en las fincas en terrazas de Córcega, pero en la actualidad está prácticamente extinguida. Esta raza local de trigo es precisamente uno de los cultivos de los que trata el artículo de Bullitta et al. (pág. 27-34). Foto: S. Cifarelli

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

Identificación de variedades de arroz (*Oryza sativa* L.) cultivadas en Argentina mediante marcadores bioquímicos: su utilidad potencial para el registro de cultivares

R.D. Medina, ✉ **M.M. Faloci, M.A. Marassi y L.A. Mroginski**
Instituto de Botánica del Nordeste (IBONE), Facultad de Ciencias Agrarias (UNNE), CC 209, 3400 Corrientes, Argentina.
Tel: +543783427589; Fax: +543783427131; E-mail: ricardomedina@agr.unne.edu.ar

Resumen

Identificación de variedades de arroz (*Oryza sativa* L.) cultivadas en Argentina mediante marcadores bioquímicos: su utilidad potencial para el registro de cultivares

Cuarenta y cinco variedades de arroz cultivadas en Argentina fueron caracterizadas por electroforesis de isoenzimas, utilizando geles discontinuos de poliacrilamida no desnaturalizantes (PAGE). Los sistemas isoenzimáticos analizados fueron: Fosfatasa ácida (ACP), Diaforasa (DIA), Esterasas (EST), Malato deshidrogenasa (MDH) y Siquimato deshidrogenasa (SDH). El sistema SDH mostró la mayor variabilidad formando 21 grupos, mientras que MDH evidenció el menor polimorfismo generando sólo 9. La identificación de todas las variedades se logró evaluando los cinco sistemas. Ello muestra que los marcadores bioquímicos pueden constituir un complemento o una alternativa a ser utilizada para caracterizar, identificar y registrar las variedades de arroz en Argentina.

Résumé

Identification de variétés de riz (*Oryza sativa* L.) cultivées en Argentine en utilisant des marqueurs biochimiques : utilité potentielle pour le classement de cultivars

Quarante-cinq variétés de riz cultivées en Argentine ont été étudiées par une analyse d'isozymes en utilisant l'électrophorèse sur gels de polyacrylamide (PAGE) discontinus natifs. Les isozymes polymorphes utilisés pour distinguer les cultivars sont : la phosphatase acide (ACP), la diaphorase (DIA), des estérases (EST), la malate déshydrogénase (MDH) et la shikimate déshydrogénase (SDH). Le système SDH présente le degré de variabilité le plus élevé dans le profil des bandes—formation de 21 groupes—comparativement au système MDH qui présente le moins de polymorphisme isozymique—formation de 9 groupes seulement. Les variétés testées peuvent être identifiées sans ambiguïté lorsque les cinq isozymes sont évalués. Ces résultats montrent que des marqueurs biochimiques peuvent être utilisés comme méthode complémentaire ou alternative pour la caractérisation, l'identification et le classement des variétés de riz en Argentine.

Summary

Identification of rice varieties (*Oryza sativa* L.) cultivated in Argentina using biochemical markers: their potential utility for the registration of cultivars

Forty-five rice varieties grown in Argentina were analyzed through isozyme analysis using discontinuous native polyacrylamide gel electrophoresis (PAGE). Polymorphic isozymes used to distinguish among the cultivars were: acid phosphatase (ACP), diaphorase (DIA), esterases (EST), malate dehydrogenase (MDH) and shikimate dehydrogenase (SDH). The SDH system showed the highest degree of variability in the banding patterns—21 groups were formed—compared with the MDH system which showed the least isozyme polymorphism—only 9 groups were formed. The tested varieties could be uniquely identified when all five isozymes were evaluated. These results show that biochemical markers could complement or be an alternative method of characterization, identification and registration for rice varieties in Argentina.

Key words: isozyme analysis, varietal identification, *Oryza sativa*

Introducción

Desde los comienzos del siglo XX la necesidad de protección de las creaciones fitogenéticas hizo que los fitomejoradores buscaran los medios legales por los que se les conceda un dominio temporal sobre su obtención, logrando en 1930 en EE.UU. la aprobación de la Ley de Patentes de Plantas (Jadue Díaz 1999). Treinta años después se creó la Unión Internacional para la Protección de las Obtenciones Vegetales (UPOV) que, desde entonces, se ha encargado de armonizar las normas jurídicas para la protección de variedades en los estados miembros. De común acuerdo las naciones participantes reconocen los exámenes de distinción varietal como un prerrequisito para su protección (Bayle 1983).

La Ley Argentina de Semillas y Creaciones Fitogenéticas N° 20.247 (Boletín Oficial, 16/IV/1973), en su Art. 20 dispone que para registrar un cultivar, el mismo debe diferir de otros conocidos a la fecha de solicitud y debe poseer características suficientemente homogéneas y estables a través de generaciones sucesivas.

Las variedades a ser registradas comúnmente satisfacen los estándares de homogeneidad y estabilidad, sin embargo, los requerimientos de distinción son los más difíciles de establecer, en especial en materiales genéticamente emparentados. Para el registro o lanzamiento de una nueva variedad de arroz se recurre a los descriptores morfológicos (IRRI 1988) y aunque su utilización sigue siendo reglamentaria, es necesaria la búsqueda de alternativas.

Otros tipos de atributos han recibido especial atención para ser explotados como criterio de distinción de variedades. Entre ellos se encuentran los marcadores bioquímicos (proteínas, isoenzimas, metabolitos secundarios) y los basados en el análisis del ADN (ácido desoxiribonucleico).

Los análisis basados en las proteínas e isoenzimas han pasado a ser efectivamente utilizados en la caracterización

de variedades desde la década de los '70, posibilitando también la estimación de la variabilidad y las relaciones entre genotipos (Milach 1999).

El análisis del polimorfismo isoenzimático ha sido de gran valor para la caracterización, distribución y cuantificación de la diversidad genética del germoplasma de arroz en distintos países (Glaszmann 1987; Kochko 1987; Sie y Ghesquiere 1992; Cai y Morishima 1997). Blair et al. (1999), Virk et al. (2000) y Luce et al. (2001), examinando la diversidad de los recursos genéticos de arroz con distintos marcadores, han adoptado como estudio de referencia el reportado por Glaszmann (1987), quien estudió 1688 variedades de arroz asiáticas con 8 sistemas isoenzimáticos.

La identificación de variedades de arroz fue descripta empleando técnicas tales como el análisis del polimorfismo de los fragmentos de ADN amplificados al azar (RAPD, random amplified polymorphic DNA) por Ko et al. (1994), Mackill (1995) y Virk et al. (2000), el polimorfismo de la longitud de fragmentos amplificados (AFLP, amplified fragment length polymorphism) por Mackill et al. (1996) y Virk et al. (2000) y el estudio de las intersecuencias simples repetidas (ISSR, intersimple sequence repeat) por Blair et al. (1999) y Virk et al. (2000) o de secuencias simples repetidas (SSR, simple sequence repeat o microsatellites) por Garland et al. (1999) y Luce et al. (2001). Estas técnicas han mostrado una amplia variación en su capacidad de discriminación. No obstante, cada una de estas estrategias de identificación junto a los marcadores de otro tipo, aportan una invalorable información para la caracterización de variedades.

Esta creciente disponibilidad de técnicas bioquímicas y moleculares, que revelan un mayor nivel de polimorfismo y consistencia en los resultados, abre el espectro de posibilidades para que los organismos oficiales cuenten con herramientas potencialmente eficientes para la identificación, el registro y la protección de las variedades.

Si bien el uso de estos marcadores aún no está generalizado, algunos países han implementado su aplicación como complemento de las características morfológicas. Tal es el caso de Inglaterra que ha incorporado como criterio de distinción varietal para trigo (*Triticum aestivum* L.) los perfiles electroforéticos de gliadinas (Milach 1999), de Francia que para la identificación y el registro de líneas de maíz (*Zea mays* L.), trigo y cebada (*Hordeum vulgare* L.) emplean patrones isoenzimáticos (Lombard et al. 2000) y de Italia, que utilizan el análisis de hordeínas para cebada siguiendo un protocolo de la UPOV (Delogu et al. 1993).

Estos antecedentes y las características propias de las evaluaciones basadas en marcadores bioquímicos (simplicidad, alta sensibilidad, reproducibilidad, carácter no destructivo y celeridad en la obtención de resultados a un costo considerablemente menor comparado con los marcadores moleculares) los postulan como un método de elección al momento de buscar otros criterios que complementen a los existentes.

El objetivo del presente trabajo fue encontrar polimorfismos isoenzimáticos que permitan diferenciar 45 variedades de arroz empleadas en Argentina, para que en el futuro puedan ser utilizados como efectivos marcadores de distinción.

Materiales y métodos

Material vegetal

Para este estudio se utilizaron plantas de diferentes variedades de arroz (*Oryza sativa* L.), pertenecientes a todos los tipos comerciales, representando gran parte del germoplasma presente en Argentina. Dichas variedades fueron: 'Altamirano P.A.', 'Arroyo Grande P.A.', 'Bluebelle', 'Calá P.A.', 'Colonia Macías 5 C.A.', 'Chacarero P.A.', 'Chajarí P.A.', 'EMBRAPA–6–Chuí', 'CT 6919 INTA', 'Cypress', 'Don Ignacio FCAYF', 'Don Juan INTA', 'El Paso 144', 'El Paso 227', 'EPAGRI 107', 'Fortuna INTA', 'Gená P.A.', 'Guaviraví P.A.', 'Guayquiraró P.A.', 'IR 1529 INTA', 'BR/IRGA 409', 'BR/IRGA 410', 'BR/IRGA 416', 'BR/IRGA417', ' La Plata Itapé P.A.', 'La Candelaria P.A.', 'La Plata Mochi P.A.', 'Lemont', 'Mandisoví P.A.', 'Maybelle', 'Mocoi F.C.A.', 'Mocoretá P.A.', 'Montiel P.A.', 'Ñancay P.A.', 'Palmar P.A.', 'Petei F.C.A.', 'Quebracho P.A.', 'Rico', 'R.P. 2', 'EMBRAPA–7–Taim', 'Taipero P.A.', 'Tebonett', 'Villaguay P.A.', 'Yeruá P.A.' y 'Yuquerí'.

Análisis de isoenzimas

Los extractos crudos se prepararon a partir de 50 mg de hojas jóvenes en activo crecimiento de plantas obtenidas a partir de semillas germinadas en tierra estéril, en frascos de 382 cm^3 sigilados con Resinite AF–50® (Casco S.A.C., Buenos Aires) e incubados en un cuarto climatizado a 27±2°C con 14hs de fotoperíodo y con una intensidad lumínica de 116 µmol m^{-2} s^{-1}. Las muestras se homogeneizaron en un mortero con buffer de extracción: 0,1 M de Tris/HCl pH=6,8 + 2% de glicerol + 0,1% de β-mercaptoetanol + 0,01% de azul de bromofenol, en relación 0,1 g ml^{-1}. Los homogenados se centrifugaron a 14.000 rpm por 10 minutos, se fraccionaron en alícuotas y se conservaron a –70°C.

Se analizaron 5 sistemas isoenzimáticos: Fosfatasa ácida: ACP (E.C. 3.1.3.2), Diaforasa: DIA (E.C. 1.6.4.3), Esterasas: EST (E.C. 3.1.1. –), Malato deshidrogenasa: MDH (E.C. 1.1.1.37) y Siquimato deshidrogenasa: SDH (E.C. 1.1.1.25) en geles discontinuos de poliacrilamida no desnaturalizantes 3% – 7,5% (PAGE), utilizando buffer de electrodo 0,025 M de Tris/HCl + 0,192 M de glicina, pH=8,3; según Laemmli (1970).

Dependiendo del sistema isoenzimático, se sembraron entre 18 y 20 µL del homogenado por muestra. La electroforesis se llevó a cabo a 4°C y a intensidad constante de 1,2 mA/cm. El revelado se efectuó sumergiendo los geles en la mezcla de reacción específica para cada sistema enzimático: ACP, MDH y SDH según Arulsekar y Parfitt (1986); DIA según Stuber et al. (1988) y EST según Soltis et al. (1983).

Luego de la aparición de las bandas coloreadas que revelan la actividad enzimática, los geles fueron lavados varias veces con agua destilada y secados a temperatura ambiente entre hojas de papel celofán.

Se analizaron cualitativamente las bandas isoenzimáticas y se calcularon las movilidades electroforéticas relativas (R_f) para cada una de ellas.

Se representó la capacidad de diferenciación varietal de los sistemas evaluados a través del porcentaje acumulado de diferenciación varietal de cada sistema más el que surge de

la combinación aditiva de las demás isoenzimas empleadas, comenzando por los sistemas isoenzimáticos más polimórficos y siguiendo por los menos polimórficos.

Resultados y discusión

Los zimogramas obtenidos fueron claramente legibles, muy consistentes y altamente reproducibles en corridas electroforéticas independientes.

Para la identificación de 45 variedades de arroz se utilizaron los 5 sistemas isoenzimáticos más polimórficos entre los 15 analizados por Medina et al. (1998).

En los perfiles isoenzimáticos se visualizaron 57 bandas, 39 polimórficas y las restantes 18 monomórficas. En los Apéndices 1A y 1B (online) se transcriben los zimogramas indicando presencia (+) y ausencia (-) de bandas isoenzimáticas y los grupos que originaron. En la Figura 1A, 1B, 1C, 1D y 1E se esquematizan los perfiles isoenzimáticos de los 5 sistemas evaluados. En la Figura 2 se pueden observar los zimogramas de algunos de los grupos varietales formados a partir de los 5 sistemas isoenzimáticos analizados.

Del análisis de los patrones de bandas obtenidos surge que el sistema SDH ha generado la máxima cantidad de grupos (21) constituyéndose el marcador más polimórfico. Los sistemas EST y ACP han presentado una considerable variabilidad, que se tradujo en la discriminación de 15 y 14 grupos, respectivamente. Ello concuerda con Glaszmann (1987), Sie y Ghesquiere (1992), Cai y Morishima (1997) quienes también han considerado estas isoenzimas de utilidad para la diferenciación de grupos varietales. En otras especies como yam (*Dioscorea cayenensis* Lam. y *D. rotundata* Poir.), EST y SDH resultaron heteromórficos (Dansi et al. 2000), sin embargo, en kiwi (*Actinidia deliciosa* [A. Chev.] C.F. Liang et A.R. Ferguson) Messina et al. (1991) evidenciaron patrones de bandas de SDH idénticos en todos los cultivares evaluados.

MDH fue el sistema menos polimórfico permitiendo la menor agrupación. Ello coincide con lo reportado por Kochko (1987) quien analizó la variabilidad isoenzimática de variedades de arroz en África, resultando éste uno de los sistemas más monomórficos. También Tao y Sugiura (1987) observaron que dicho sistema fue el que produjo la menor cantidad de grupos en cultivares de kaki (*Diospyros kaki* Lf.).

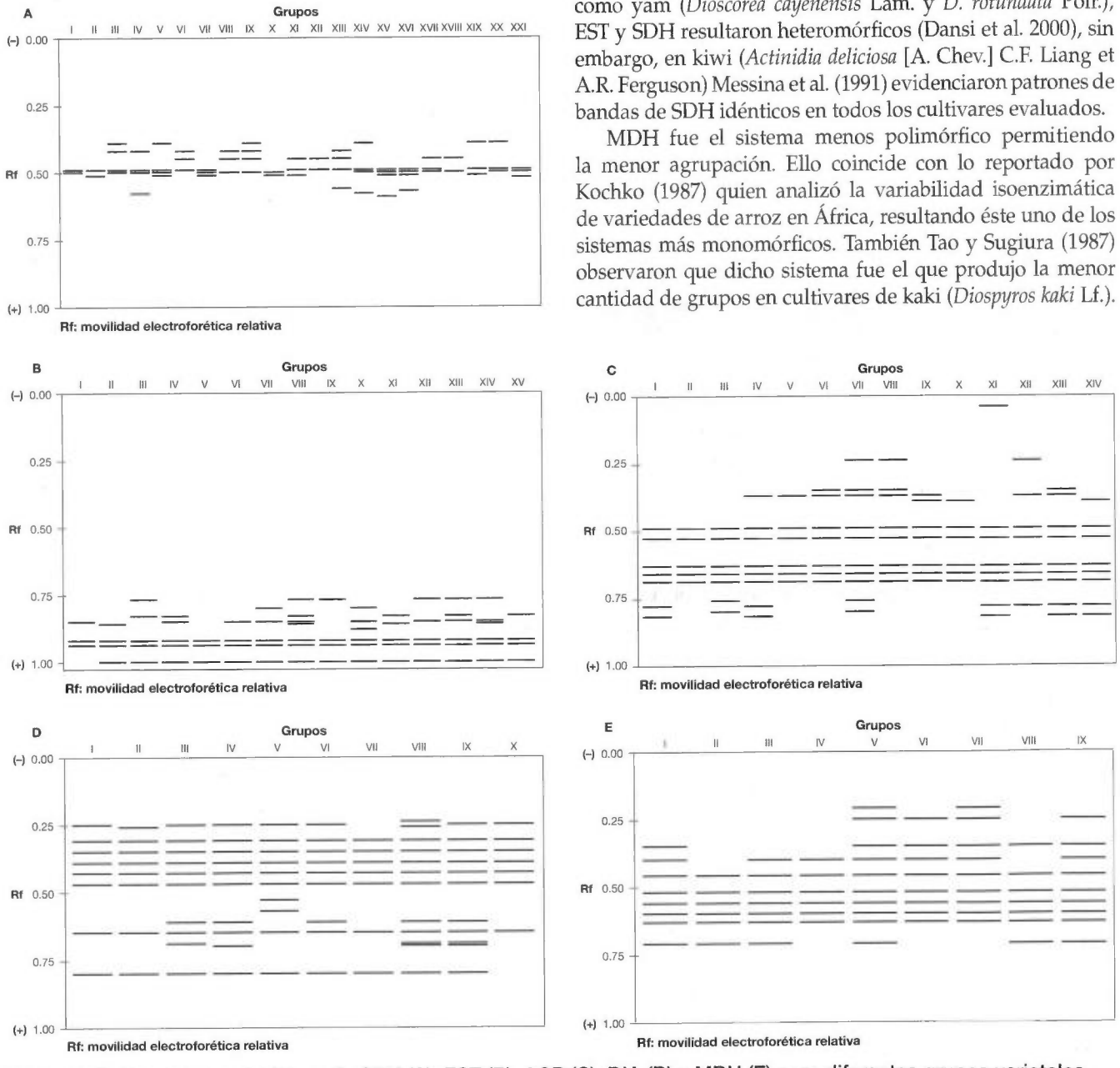


Figura 1. Patrones isoenzimáticos de SDH (A), EST (B), ACP (C), DIA (D) y MDH (E) para diferentes grupos varietales.

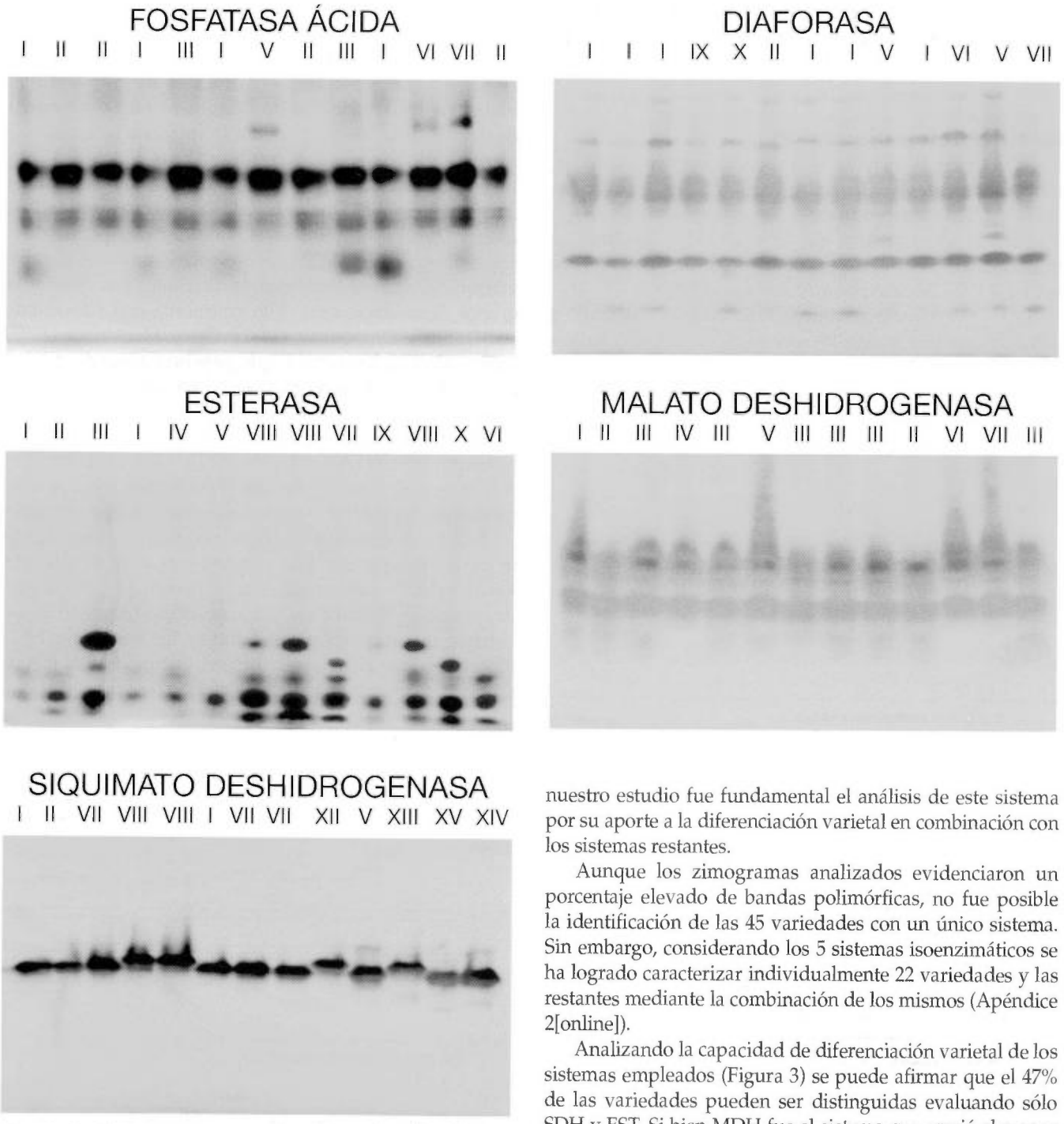


Figura 2. Zimogramas en geles de poliacrilamida no desnaturalizantes (3%–7,5%) de algunos de los grupos varietales formados a partir del análisis de 5 sistemas isoenzimáticos.

En kiwi, Messina et al. (1991) determinaron que MDH no fue útil como marcador de clasificación.

Si bien DIA no es un sistema comúnmente utilizado para detectar polimorfismos que permitan la clasificación de germoplasma de arroz (Glaszmann 1987; Kochko 1987; Sie y Ghesquiere 1992), De los Reyes et al. (1989) han reportado patrones distintos para DIA en diferentes accesiones. En nuestro estudio fue fundamental el análisis de este sistema por su aporte a la diferenciación varietal en combinación con los sistemas restantes.

Aunque los zimogramas analizados evidenciaron un porcentaje elevado de bandas polimórficas, no fue posible la identificación de las 45 variedades con un único sistema. Sin embargo, considerando los 5 sistemas isoenzimáticos se ha logrado caracterizar individualmente 22 variedades y las restantes mediante la combinación de los mismos (Apéndice 2[online]).

Analizando la capacidad de diferenciación varietal de los sistemas empleados (Figura 3) se puede afirmar que el 47% de las variedades pueden ser distinguidas evaluando sólo SDH y EST. Si bien MDH fue el sistema que arrojó el menor número de bandas polimórficas y generó la menor cantidad de grupos, demostró al mismo tiempo una gran capacidad de discriminación cuando se lo combinó con los otros sistemas ensayados.

Para la caracterización de ejemplares de distintas especies también se han empleado uno o varios sistemas isoenzimáticos. Quirós et al. (1987) analizando 6 sistemas isoenzimáticos en apio (*Apium graveolens* L.) pudieron establecer 9 grupos en 15 cultivares. Tobolski y Kemery (1992) diferenciaron 15 cultivares de *Acer rubrum* L. mediante el análisis de 7 sistemas enzimáticos que resultaron polimórficos. Maass et al. (1993) emplearon 4 sistemas para evaluar 8 accesiones de

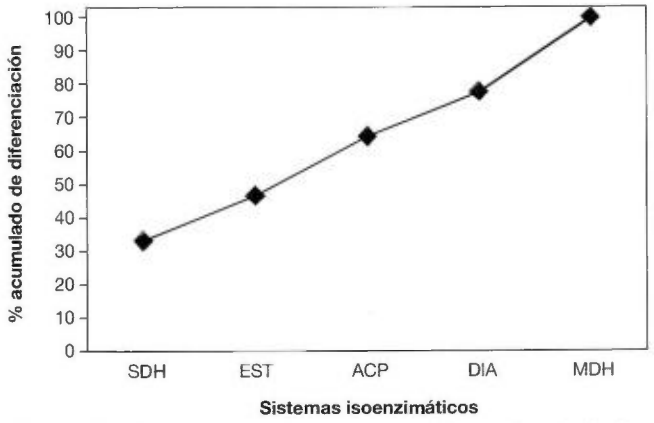


Figura 3. Capacidad de diferenciación varietal de los sistemas isoenzimáticos empleados.

maní forrajero (*Arachis Pintoi* Krap. & Greg.). Marquard y Chan (1994) con 6 sistemas isoenzimáticos distinguieron 45 cultivares de manzano silvestre (*Malus* spp.). Sin embargo, Hussain et al. (1987) caracterizaron 20 cultivares de mandioca (*Manihot esculenta* Crantz) solamente con el patrón de bandas de EST.

Para la mayoría de las especies, los exámenes de Distinción, Homogeneidad y Estabilidad (DUS) únicamente confían en la comparación de características morfológicas entre los cultivares candidatos y todos los previamente registrados que componen la colección de referencia (Lombard et al. 2000) si bien, como ha sido mencionado anteriormente, existen ejemplos del uso de marcadores bioquímicos como complementarios. Los protocolos que se basan en las características morfológicas tienen ciertas limitaciones. En primer lugar tienen un costo económicamente elevado, dado que requieren evaluaciones en distintas localidades y un cierto número de réplicas, lo que se complica aún más cuando año tras año se incrementa el tamaño de la colección. Por otra parte las características deben evaluarse a lo largo de todo el ciclo del cultivo y por más de un ciclo, dependiendo de la especie, lo que conlleva un mayor consumo de tiempo. En este contexto, los exámenes DUS podrían beneficiarse de las bondades y ventajas que les brindarían los marcadores bioquímicos y moleculares.

La identificación de variedades de arroz mediante el análisis de marcadores moleculares basados en ADN se ha llevado a cabo utilizando varias técnicas. Ko et al. (1994) utilizando 22 cebadores arbitrarios pudieron diferenciar 27 variedades de distinto origen empleando RAPD, aunque algunas de ellas presentaron altos porcentajes de similaridad (88–97%). Mackill et al. (1996) estudiaron 14 variedades con tres sistemas marcadores: AFLP, RAPD y microsatélites, siendo este último el que generó mayor polimorfismo intra e intersubespecífico. Luce et al. (2001) evaluando 16 cebadores microsatélites lograron identificar 419 variedades adaptadas a condiciones europeas, si bien 82 de ellas presentaron un alto polimorfismo intravarietal.

Aunque no sea posible la comparación de dichas técnicas sin el empleo del mismo número de marcadores, se puede afirmar que los análisis de RAPD resultan un método simple y rápido, si bien son frecuentemente criticados por el escaso polimorfismo revelado (Mackill 1995), por su falta de reproducibilidad (Karp y Edwards 1997; Lee y Henry 2001) y su poca transferibilidad de un laboratorio a otro según lo reportado por Ferreira y Grattapaglia (1998). Los marcadores microsatélites son útiles para la identificación de variedades porque brindan un alto nivel de polimorfismo (Ferreira y Grattapaglia 1998; Garland et al. 1999; Luce et al. 2001), aunque su tasa de mutabilidad es 500 veces mayor de lo normal (Jarne y Lagoda 1996), siendo ésta una de las razones por las que se explica el fenómeno del polimorfismo intravarietal, pudiendo hacer dificultoso el establecimiento de un determinado patrón identificatorio que sirva como referencia y sea estable durante un elevado número de generaciones.

Virk et al. (2000) compararon experimentalmente la capacidad de discriminación de cuatro estrategias de identificación (isoenzimas, AFLP, microsatélites e ISSR) considerando un número similar de marcadores para cada una. Ellos reportaron que a excepción de ISSR, las demás técnicas clasifican las variedades de arroz de acuerdo a Glaszmann (1987), siendo las isoenzimas y los AFLP los más eficientes. Los marcadores AFLP tienen un alto poder de discriminación debido a que generan un considerable número de bandas que pueden analizarse en un único gel, sin embargo, son técnicas que incluyen mayor número de etapas y la exigencia de un alto nivel de pureza del ADN que implica un mayor consumo de tiempo y mayores costos operativos (Ferreira y Grattapaglia 1998; Lee y Henry 2001).

Empleando 5 sistemas isoenzimáticos fue factible identificar 45 variedades de arroz cultivadas en Argentina, brindando una herramienta complementaria que podría ser considerada para el registro de cultivares. Si bien no se puede afirmar que se trata del mejor método de identificación ya que no se estudiaron otros métodos comparativamente, esta estrategia se presenta como una técnica no destructiva, de simple resolución y de alta sensibilidad que requiere muy poco material por individuo. Éste, comparado con otros sistemas de marcadores, es de bajo costo operativo y de establecimiento (Ferreira y Grattapaglia 1998). Por otra parte, la variabilidad ontogenética y aquélla debida al ambiente pueden ser eliminadas en un sistema de trabajo controlado garantizando una alta reproducibilidad del método.

Si bien las bases estatuidas para la mayoría de los exámenes DUS que se realizan para el otorgamiento de una licencia de protección y registro de una nueva variedad seguirán siendo los caracteres morfológicos y fenológicos, la UPOV, en circunstancias donde se hace difícil determinar la distinción varietal, ha aceptado el uso de marcadores bioquímicos como una alternativa (Camlin 2001) y de hecho existen algunos ejemplos de su utilización en la identificación y registro de cereales (Lombard et al. 2000).

Conclusiones

Con este trabajo se ha demostrado que es posible diferenciar 45 variedades de arroz empleadas en Argentina mediante la evaluación de cinco sistemas isoenzimáticos (ACP, DIA,

EST, MDH y SDH), siendo la electroforesis en minigeles discontinuos de poliacrilamida no desnaturalizantes un método eficiente que permite un análisis muy sensible con la obtención de resultados inmediatos. Ello, sumado a las vastas evidencias de la utilidad de los marcadores bioquímicos para la identificación varietal, inclusive en diversas especies, incrementa la probabilidad que, en un futuro cercano, sean considerados y empleados como criterio en los exámenes de distinción para el registro nacional de variedades de arroz en Argentina.

Referencias

Arulsekar S, Parfitt D. 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. HortScience 21:928–933.

Bayle DC. 1983. Isozymic variation and plant breeders rights. In: Tanskley SD, Orton TJ, editors. Isozymes in plant genetics and breeding. Part A. Elsevier Science Publishers BV, Amsterdam, Holland, pp. 129–146.

Blair MW, Panaud O, McCouch SR. 1999. Intersimple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 98:780–792.

Boletín Oficial de la República Argentina. 16/IV/1973. Ley de Semillas y Creaciones Fitogenéticas N° 20.247.

Cai HW, Morishima H. 1997. *Indica–Japonica* differentiation of Bangladesh rice cultivars detected by isozyme analysis. Rice Genetics Newsletter 14:29–30.

Camlin, MS. 2001. Possible future roles for molecular techniques in the identification and registration of new plant cultivars. In: Doré C, Dosba F, Baril C, editors. Proceeding of the International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture. Acta Horticulturae (ISHS) N° 546, Montpellier, France, pp. 289–296.

Dansi A, Mignouna HD, Zoundjihekpon J, Sangare A, Asiedu R, Ahoussou N. 2000. Using isozyme polymorphism to assess genetic variation within cultivated yams (*Dioscorea cayenensis/ Dioscorea rotundata* complex) of the Republic of Benin. Genetic Resources and Crop Evolution 47:371–383.

De los Reyes BG, Brar DS, Khush GS. 1989. Identification of four new isozyme loci in rice. Rice Genetics Newsletter 6:103–106.

Delogu G, Terzi V, Cattivelli L, Stanca AM. 1993. Le varità di orzo coltivate in Italia. Edizioni L'informatore agrario X, Verona, Italia.

Ferreira ME, Grattapaglia D. 1998. Introducción al uso de marcadores moleculares en el análisis genético. EMBRAPA–CENARGEN Documento 20. EMBRAPA–CENARGEN, Brasilia, Brasil.

Garland SH, Lewin L, Abedinia M, Henry R, Blakeney A. 1999. The use of microsatellite polymorphisms for the identification of Australian breeding lines of rice (*Oryza sativa* L.). Euphytica 108:53–63.

Glaszmann JC. 1987. Isozymes and classification of Asian rice varieties. Theoretical and Applied Genetics 74:21–30.

Hussain A, Bushuk W, Ramirez H, Roca W. 1987. Identification of cassava (*Manihot esculenta* Crantz) cultivars by electrophoretic patterns of esterase isozymes. Seed Science and Technology 15:19–22.

International Rice Research Institute. 1988. Standard evaluation system for rice (*Oryza sativa* L.). 3rd Edition. IRRI, Los Baños, Philippines, pp. 1–54.

Jadue Díaz Y. 1999. Certificación de variedades: la visión de UPOV. En: Pagliano D, editor. Calidad genética y sanitaria. Un instrumento para la competitividad de la cadena agroindustrial. IICA–PROCISUR, Montevideo, Uruguay, pp. 53–60.

Jarne P, Lagoda P. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution 11(10):424–429.

Karp A, Edwards KJ. 1997. Molecular techniques in the analysis of the extent and distribution of genetic diversity. In: Ayad WG, Hodgkin T, Jaradat A, Rao VR, editors. Molecular genetic techniques for plant genetic resources. Report of the International Plant Genetic Resources Institute (IPGRI) Workshop, 9–11 October 1995, Rome, Italy, pp. 11–22.

Ko HL, Cowan DC, Henry RJ, Graham GC, Blakeney AB, Lewin LG. 1994. Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. Euphytica 80:179–189.

Kochko A. 1987. Isozymic variability of traditional rice (*Oryza sativa* L.) in Africa. Theoretical and Applied Genetics 73:675–682.

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

Lee LS, Henry RJ. 2001. Commercial applications of plant genotyping. In: Henry RJ, editor. Plant Genotyping: the DNA fingerprinting of plants. CAB International, Wallingford, UK, pp. 265–273.

Lombard V, Baril CP, Dubreuil P, Blouet F, Zhang D. 2000. Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: consequences for varietal registration. Crop Science 40:1417–1425.

Luce C, Noyer JL, Tharreau D, Ahmadi N, Feyt H. 2001. The use of microsatellite markers to examine the diversity of the genetic resources of rice (*Oryza sativa*) adapted to European conditions. In: Doré C, Dosba F, Baril C, editors. Proceeding of The International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture. Acta Horticulturae (ISHS) N° 546, Montpellier, France, pp. 221–230.

Maass BL, Torres AM, Ocampo CH. 1993. Morphological and isozyme characterization of *Arachis Pintoi* Krap. *et* Greg. *nom. nud.* germplasm. Euphytica 70:43–52.

Mackill DJ. 1995. Classifying *japonica* rice cultivars with RAPD markers. Crop Science 35:889–894.

Mackill DJ, Zhang Z, Redoña ED, Colowit PM. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39:969–977.

Marquard R, Chan Ch R. 1994. Identification of crab apple cultivars by isozymes. In: Proceeding of the 8th Conference of the Metropolitan Tree Improvement Alliance and International Ornamental Crabapple Society. US National Arboretum, Washington DC, USA, pp. 87–96.

Medina RD, Faloci MM, Marassi MA, Mroginski LA. 1998. Caracterización isoenzimática de variedades de arroz (*Oryza sativa* L.) cultivadas en Argentina. En: Actas de la III Reunión de Comunicaciones Científicas y Tecnológicas. Vol. 3. Universidad Nacional del Nordeste. EUDENE, Corrientes, Argentina, pp. 101–104.

Messina R, Testolin R, Morgante M. 1991. Isozymes for cultivar identification in kiwifruit. HortScience 26:899–902.

Milach SCK. 1999. Disponibilidad de técnicas moleculares para la identificación varietal. En: Pagliano D, editor. Calidad genética y sanitaria. Un instrumento para la competitividad de la cadena agroindustrial. IICA–PROCISUR, Montevideo, Uruguay, pp. 30–37.

Quirós CF, Mc Grath M, Stites JL. 1987. Use of stem proteins and isozymes for the identification of celery varieties. Plant Cell Reports 6:114–117.

Sie M, Ghesquiere A. 1992. Isozyme polymorphism and classification of rice varieties from Burkina Faso in Africa. Rice Genetics Newsletter 9:75–76.

Soltis D, Haufler C, Darrow D, Gastony G. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. American Fern Journal 73:9–27.

Stuber CW, Wendel JF, Goodman MM, Smith JS. 1988. Techniques and scoring procedures for starch gel electrophoresis and enzymes from maize (*Zea mays* L.). In: Technical Bulletin 286, North Carolina, Agricultural Research Service. North Carolina State University, Raleigh, North Carolina, USA, pp. 1–87.

Tao R, Sugiura A. 1987. Cultivar identification of Japanese persimmon by leaf isozymes. HortScience 22:932–935.

Tobolski JJ, Kemery RD. 1992. Identification of red maple cultivars by isozyme analysis. HortScience 27:169–171.

Virk PS, Zhu J, Newbury HJ, Bryan GJ, Jackson MT, Ford–Lloyd BV. 2000. Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa*) germplasm. Euphytica 112:275–284.

Apéndice 1A. Patrón de bandas isoenzimáticas de 22 variedades de arroz (*Oryza sativa* L.) y grupos varietales formados (en números romanos), está disponible en http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Apéndice 1B. Patrón de bandas isoenzimáticas de 23 variedades de arroz (*Oryza sativa* L.) y grupos varietales formados (en números romanos), está disponible en http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Apéndice 2. Sistemas isoenzimáticos identificatorios para cada variedad, está disponible en http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Evaluation of variability among breeding lines and cultivars of taro (*Colocasia esculenta*) in Papua New Guinea using ISSR fingerprinting and agro-morphological characterization

T. Okpul,[1] E.S. Mace,[2*] I.D. Godwin,[2] D. Singh[1] and M.E. Wagih[3]
[1] National Agricultural Research Institute, WLLMP-Bubia, P.O. Box 1639, Lae, Papua New Guinea
[2] School of Land and Food Sciences, University of Queensland, Brisbane, QLD 4072, Australia. Fax: +61-7-3365-1188. Email: i.godwin@uq.edu.au
[3] Biotechnology Center, University of Technology, PMBS, Lae, Papua New Guinea
*Current address: Queensland Department of Primary Industries, Hermitage Research Station, 604 Yangan Road, Warwick, QLD 4370, Australia

Summary

Evaluation of variability among breeding lines and cultivars of taro (*Colocasia esculenta*) in Papua New Guinea using ISSR fingerprinting and agro-morphological characterization

Agro-morphological characterization and inter-simple sequence repeat (ISSR) DNA fingerprinting were used to evaluate variability among selected Papua New Guinean (PNG) taro (*Colocasia esculenta*) accessions. Twenty-three discriminatory descriptors selected from the International Plant Genetic Resources Institute (IPGRI, 1999) descriptor list were combined with ISSR markers to characterize 13 taro accessions. Genotyping data were obtained via polymerase chain reaction (PCR) with three ISSR primers: $(GA)_9AT$, $(GA)_9AC$ and $(ACC)_6Y$. Initial evaluation of the agro-morphological variation indicated that the total variation observed appeared to be associated with colours of corm flesh, corm cortex and petiole, stolon formation, corm shape, lamina orientation and lamina vein pattern. All genotypes were separated as distinct morphotypes, providing useful information for development of a minimum descriptor list. ISSR primers generated complex banding patterns, with primer $(GA)_9AC$ amplifying fewer but proportionately more (90%) polymorphic fragments. Fingerprinting with three ISSR primers enabled the identification of closely related genotypes and the separation of those that were distantly related. Little correlation was found in between the agro-morphological and molecular data in the clusters generated. However, the two types of data provided complementary information on the existing diversity and available genotypes that can be immediately used by the breeders.

Key words: taro, *Colocasia esculenta*, genetic resources, agro-morphology, inter-simple sequence repeats (ISSR), DNA fingerprinting

Résumé

Evaluation de la variabilité dans des lignées de sélection et des cultivars de taro (*Colocasia esculenta*) en Papouasie-Nouvelle Guinée par fingerprinting ISSR et caractérisation agro-morphologique

La caractérisation agro-morphologique et le fingerprinting des répétitions séquentielles internes simples (ISSR) de l'ADN ont été utilisés pour évaluer la variabilité parmi des accessions de taro (*Colocasia esculenta*) de Papouasie-Nouvelle Guinée. Vingt-trois descripteurs discriminants choisis dans la liste des descripteurs de l'Institut international des ressources phytogénétiques (IPGRI, 1999) ont été utilisés parallèlement à des marqueurs ISSR pour caractériser 13 accessions de taro. Les données de génotypage ont été obtenues par réaction de polymérisation en chaîne (PCR) avec trois amorces ISSR: $(GA)_9AT$, $(GA)_9AC$ et $(ACC)_6Y$. L'évaluation initiale de la variation agro-morphologique indique que la variation totale observée est associée à la coloration de la chair du bulbe, au cortex du bulbe et au pétiole, à la formation de stolons, à la forme du bulbe, à l'orientation des écailles et au profil des veines des écailles. Tous les génotypes ont été séparés en tant que morphotypes distincts, fournissant des informations utiles pour l'élaboration d'une liste minimale de descripteurs. Les amorces ISSR produisent des profils de bandes complexes, les amorces $(GA)_9AC$ amplifiant peu, mais proportionnellement plus (90 %) de fragments polymorphes. Le fingerprinting avec trois amorces ISSR permet l'identification de génotypes étroitement apparentés et la séparation de ceux qui sont plus éloignés. Peu de corrélation a été observée entre les données agro-morphologiques et les données moléculaires dans les groupes (clusters) générés. Cependant, les deux types de données fournissent des informations complémentaires sur la diversité existante et les génotypes disponibles qui peuvent être immédiatement utilisés par les sélectionneurs.

Resumen

Evaluación de la variabilidad en líneas mejoradas y cultivares de taro (*Colocasia esculenta*) en Papua Nueva Guinea, empleando huellas de ISSR y caracterización agromorfológica

Se emplearon la caracterización agromorfológica y la determinación de la huella de ADN por repetición de secuencia intersimple (ISSR) para evaluar la variabilidad entre accesiones seleccionadas de taro (*Colocasia esculenta*) en Papua Nueva Guinea (PNG). Se combinaron veintitrés descriptores discriminadores seleccionados de la lista de descriptores del Instituto Internacional de Recursos Fitogenéticos (IPGRI, 1999) en combinación con marcadores ISSR para caracterizar 13 accesiones de taro. Se obtuvieron datos de genotipos mediante la reacción en cadena de polimerasa (PCR) con tres primeres ISSR: $(GA)_9AT$, $(GA)_9AC$ y $(ACC)_6Y$. La evaluación inicial de la variación agromorfológica indicó que la variación total observada resultaba asociada con los colores de la carne del bulbo, la corteza del bulbo y el pecíolo, la formación del estolón, la forma del bulbo, la orientación de la lámina y la pauta de la vena de la lámina. Todos los genotipos se separaron como morfotipos distintos y proporcionaron información útil para el desarrollo de una lista descriptora de base. Los primeres ISSR generaron pautas complejas de bandas, y el primer $(GA)_9AC$ amplificaba menos pero proporcionalmente más fragmentos polimórficos (90%). La determinación de la huella de tres primeres ISSR permitió identificar los genotipos vinculados estrechamente entre sí y la separación de los vinculados de manera lejana. En los conglomerados generados se halló una escasa correlación entre los datos agromorfológicos y los moleculares. No obstante los dos tipos de datos proveyeron información complementaria en cuanto a la diversidad existente y los genotipos disponibles que pueden ser utilizados de inmediato por los fitomejoradores.

Introduction

Taro, *Colocasia esculenta* (L.) Schott, is one of the most important staple food crops of Pacific Island countries. It plays an important role both as a root crop and a leafy vegetable. In Papua New Guinea (PNG), taro is the third most important food crop after sweet potato (*Ipomoea batatas*) and banana (*Musa* spp.) (Sar et al. 1998). The estimated annual production of taro in PNG is approximately 440 000 tonnes (t) from an area of 77 000 hectares (ha). Taro is one of the oldest crops in this region, probably reaching the Polynesian islands 2000 years ago with human migrations and spread eastwards into the Pacific. Two principal botanical varieties of taro are recognized, namely *C. esculenta* var. *esculenta*, commonly known as dasheen, and *C. esculenta* var. *antiquorum*, commonly known as eddoe. The dasheen variety is distinguishable by its large central corm, with suckers and stolons, whereas the eddoe variety has a small central corm and a large number of smaller cormels (Purseglove 1972).

Taro cultivars in the Pacific exhibit remarkable morphological variation. This variation may be associated with cross-pollination and sexual recombination, and perhaps mutation, followed by intensive selection by isolated human communities in diverse environments (Rao et al. 1998). It is also likely that continuous vegetative propagation and selection has contributed to the phenotypic diversity observed on farms and in the wild in PNG (Tanimoto and Matsumoto 1986; Ivancic et al. 1995; Okpul et al. 2003). The origins of existing landraces, or farmer varieties, can only be elucidated by assessing genetic diversity to establish varietal identity and the extent and distribution of genetic variation.

The study of agro-morphological variability is the traditional method of assessing genetic diversity. Several descriptors have been developed for characterizing taro (IBPGR 1980; TANSAO 1998; IPGRI 1999). Agro-morphological characterization, however, is often hindered by the limited number of polymorphic features and the need for multiple test environments (Kresovich et al. 1997). Additionally, characters useful for genetic studies often have gross effects on phenotype and can be recessive and rare in nature (Marshall 1997). Such limitations have resulted in the increased use of molecular approaches in assessing genetic diversity (Karp et al. 1997), and in particular the emergence of polymerase chain reaction (PCR)-based markers. DNA markers with high levels of polymorphism permit fine-scale genetic characterization of germplasm if distributed throughout the genome. In addition they avoid the complications of environmental effects.

Cytogenetic investigations (Yen and Wheeler 1968; Kuruvilla and Singh 1981; Coates et al. 1988) have established taro to be a cytologically polymorphic species with $2n = 2x = 28$ (diploid) and $2n = 3x = 42$ (triploid) karyotypes. Biochemical studies using isoenzymes (Lebot and Aradhya 1991) detected a narrow genetic base in Pacific Island taros, exposing their vulnerability to new biotic and abiotic stresses.

A range of techniques have been used more recently to study DNA variation to characterize taro germplasm. Restriction fragment length polymorphism (RFLP) analysis of ribosomal and mitochondrial DNA has been performed on taros from Australia, PNG, Asia and the Pacific Islands (Matthews 1990; Matthews et al. 1992). Irwin et al. (1998) investigated taro genetic diversity using RAPD markers and could distinguish between Hawaiian morphotypes, diploids and triploids that exhibited monomorphic zymotypes in the study of Lebot and Aradhya (1991). Similarly, a high level of amplified fragment length polymorphism (AFLP) variation was observed between wild and traditional cultivars, and between *Colocasia* and *Xanthosoma* (van Eck et al. 1998; TANSAO 2000; Kreike et al. 2003).

More recently, characterization using simple sequence repeats (SSR) and inter-simple sequence repeat (ISSR) markers was undertaken for both *C. esculenta* and *Xanthosoma* (Godwin et al. 2001; Mace and Godwin 2002). In other crop species, SSR and ISSR markers have been widely exploited for varietal identification (Nagaoh and Ogihara 1997; Bredemeijer et al. 1998; Gilbert et al. 1999; Maunder et al. 1999; Prevost and Wilkinson 1999; Virk et al. 1999; Huang and Sun 2000; Métais et al. 2000; Prasad et al. 2000). PCR-based SSR markers are very powerful as they are co-dominant and multi-allelic as well as highly polymorphic. However, the major drawback of SSRs is the cost and time necessary for their development. In contrast, the ISSR approach requires no prior sequence knowledge and can be immediately applied to any plant species. Although there is no single dominant marker system that meets all the needs, ISSR markers are considered to be rapid, robust and provide more informative data sets with less effort and cost than other dominant molecular marker techniques (Salimath et al. 1995; Yang et al. 1996; Godwin et al. 1997).

This study was aimed at characterizing several important cultivars and promising hybrids of taro in PNG by combining agro-morphological characterization with ISSR fingerprinting. Some notes on developing a minimum descriptor list for Pacific Island taros are also presented.

Materials and methods

Plant material

Thirteen taro accessions (Table 1) from the taro working collection maintained *ex situ* at Bubia (146°4′E, 6°41′S), Wet Lowland–Mainland Program of the PNG National Agricultural Research Institute, were assessed. These accessions were all of the dasheen type, with a large central corm. Bubia is 20 m asl and receives a mean annual rainfall of 2870 mm. Individual accessions were planted in single rows of eight plants spaced at 0.5 m between plants and 1.0 m between rows. An application of 50 kg N/ha in the form of urea was applied at three months to boost growth. Weeds were controlled manually as required.

Agro-morphological characterization

Accessions were characterized using 22 discriminatory descriptors of the International Plant Genetic Resources Institute taro descriptor list (IPGRI 1999). This is a comprehensive set

Table 1. Local names and origin of 13 taro accessions selected for agro-morphological and molecular characterization

Accession No.	Local name	Place of origin
Bangkok	Unknown	Thailand
BC-669	Pim	Western Highland, PNG
BC-741	Kinikini	Milne Bay, PNG
BC-773	Momora	Morobe, PNG
BC-790	Momowa	Morobe, PNG
BC-793	Yagi	Morobe, PNG
BC-826	Numkowec-koko	Morobe, PNG
BC-827	Hoyawe	Morobe, PNG
BC-843	Numkowec-sisip	Morobe, PNG
C2-E7	Breeding line	NARI, PNG
C2-E8	Breeding line	NARI, PNG
Ph-17	Unknown	PNG
Ph-21	Unknown	PNG

NARI = National Agricultural Research Institute.

of descriptors useful for general characterization of varieties. Only those that discriminated among our samples were used for the present analysis (Table 2). Above-ground traits were scored at five months after planting, while underground traits were evaluated at six months during harvest. Each trait was scored for qualitative (interval) data.

Cluster analysis was performed on standardized data based on the Euclidean distance coefficient and unweighted pair-group method (Sokal and Michener 1958; UPGMA). The dendrogram was generated using the TREE sub-program of the software package NTSYS-pc (Numerical Taxonomic System, Exeter Software), version 2.202i (Rohlf 1998). Principal component analysis (PCA) was also performed, to confirm the result and identify those traits that were influential in explaining the observed variation, using software S-Plus 2000 (MathSoft Inc.). Those principal components (PCs) with eigen values greater than one were selected as outlined by Jeffers (1967). Correlation values between the traits and their respective PCs were also obtained according to the method of Matus et al. (1996), where those characters with correlation values ≥0.6 were considered as relevant for that PC. Cluster analysis was performed on the PC scores where the resultant dissimilarity matrix was later compared with the ISSR data by calculating the product-moment correlation (r) between matrices and the Mantel's (Mantel 1967, cited in Rohlf 1998) test statistics (Z) using the MXCOMP sub-program of NTSYS-pc. A test for association between traits was performed for the more elaborate IPGRI descriptor list.

ISSR fingerprinting

Young unrolled leaves were harvested for DNA extraction. Total genomic DNA was extracted using the Nucleon PhytoPure system (Amersham plc) with minor modification. The DNA was re-suspended into a final volume of 60 µl TE buffer and quantified using a fluorimeter (TKO 100, Hoefer Scientific Instruments). Approximately 4 µl of an estimated concentration of 2.5 ng/ µl of genomic DNA was added to PCR master mix of reagents comprised of 2.5 mM $MgCl_2$, 2 µl of 10x buffer (Promega), 0.6 µM of each dNTPs, 0.5 µM primer and 1 unit of *Taq* polymerase (Promega). ISSR primers $(GA)_9AT$, $(GA)_9AC$ and $(ACC)_6Y$, initially screened by Nurzuhairawaty (2000), were used. PCR amplifications were performed in a PTC-200 Peltier Thermocycler (MJ Research) with a hot start for 2 minutes at 94°C; followed by 35 cycles of denaturing at 94°C for 15 seconds; annealing at 37°C for 15 seconds; and product extension for 5 minutes at 72°C. The ISSR amplification products were resolved using 10% polyacrylamide gel electrophoresis using a Mini PROTEAN 3 system (Bio Rad Inc.) and visualized by silver staining. Sizes of the amplified fragments were determined in comparison to a 100 bp ladder (New England Biolab).

A binary data matrix was generated for all accessions based on banding patterns observed at a particular locus, i.e. the presence (1) or absence (0) of each specific ISSR marker. Two replicate DNA extractions were used to test for consistency of band profiles generated by the primers. Scoring was done by eye on three independent occasions and compiled into a data matrix. Genetic similarity between accessions was estimated using Jaccard's coefficient (Jaccard 1908). The resultant similarity matrices were then clustered based on UPGMA. PCA was also performed on the ISSR data matrix using software S-Plus 2000. Cluster analysis was then performed on the PC scores where the resultant dissimilarity matrix was compared with those derived from the agro-morphological data based on Mantel's matrix correlation. Additionally, a multi-dimensional scaling analysis (MDS) was performed on the ISSR data set to confirm the presence of clusters within the data set, as identified through the UPGMA analysis.

Table 2. Morphological characters selected from IPGRI and TANSAO descriptor lists

IPGRI No.	Trait	Trait code	Principal Components Explained[#]
1	Position of leaf	LP	
2	Lamina color	LC	
3	Lamina variegation	LV	
4	Type of variegation	TV	
5	Color of variegation	CV	
6	Petiole junction pattern	PJP	
7	Petiole junction color	PJC	
8	Leaf main vein color	LVC	
9	Leaf vein pattern	LVP	3,4
10	Petiole color-top	PCT	3
11	Petiole color-bottom	PCB	3
12	Petiole stripe	PS	
13	Petiole stripe color	PSC	1,2
14	Basal ring color	BRC	
15	Flowering	F	
16	Corm branching	CB	
17	Corm shape	CS	3,5
18	Corm cortex color	CCC	1
19	Corm flesh color	CFlC	1
20	Corm fiber color	CFbC	
21	Plant height	PH	
22	Number stolon	ST	
23	Number sucker	SU	

[#] See Table 3 for more detail.

Results and discussion

Agro-morphological diversity

Cluster analysis separated the 13 accessions as different genotypes with Euclidean distance coefficients ranging from 3.69 (between C2-E8 and BC-826) to 8.58 (Ph-17 grouping with remaining 12 genotypes) (Figure 1).

Ordination among the different accessions showed that the first six PCs had eigen values greater than 1.00 and cumulatively accounted for 93% of the total variation (Table 3). The first component explained 31% of the variation and was associated with petiole stripe colour (PSC), corm flesh colour (CFlC) and corm cortex colour (CCC). The second PC explained 24% of the variation and was associated with PSC. The third component explained 20% of the variation and was mainly associated with leaf lamina vein patterns (LVP), colours of the bottom (PCB) and top (PCT) portions of the petiole and corm shape (CS). The fourth component was mainly associated with LVP and explained only 8.5% of the variation, with subsequent PCs explaining 5% or less of the variation.

Significant correlations were observed for CV and PCT, PCT and PJP, PCB and PCT, side shoot numbers (ST, SU) and CS, and corm colours (CCC, CFlC, CFbC and BRC) respectively (Appendix 1 [online]).

ISSR fingerprint diversity

The 3'-anchored tri- and di-nucleotide ISSR primers $(ACC)_6Y$, $(GA)_9AT$ and $(GA)_9AC$ generated complex banding profiles, with 39 scorable bands in all (Table 4). No variation in the presence or absence of bands was found between the replicated runs, although there were slight changes observed in band intensity. The ISSR fragment sizes detectable ranged from 200 to 1300 bp. The number of bands and polymorphisms detected differed with each primer (Table 4). Primer $(GA)_9AC$ amplified fewer bands (10) but more polymorphism (90%) compared to 69% for both $(GA)_9AT$ and $(ACC)_6Y$.

Cluster analysis based on Jacard's coefficient and UPGMA produced a cophenetic correlation value of r=0.87, indicating a good fit of the resulting cluster analysis with the original data set. The resultant dendrogram (Figure 2) revealed levels of genetic similarity ranging from 64% to 95% with two clusters formed at 80%. A 95% level of genetic similarity was found between C2-E8 (improved line) and BC-843 (cultivar). Ph-17 and Ph-21 exhibited about 80% similarity. As expected, the Thai wild type, 'Bangkok', was genotypically distinct (64% level of similarity) from all PNG accessions tested. The two clusters identified were also confirmed by the MDS analysis (Figure 3).

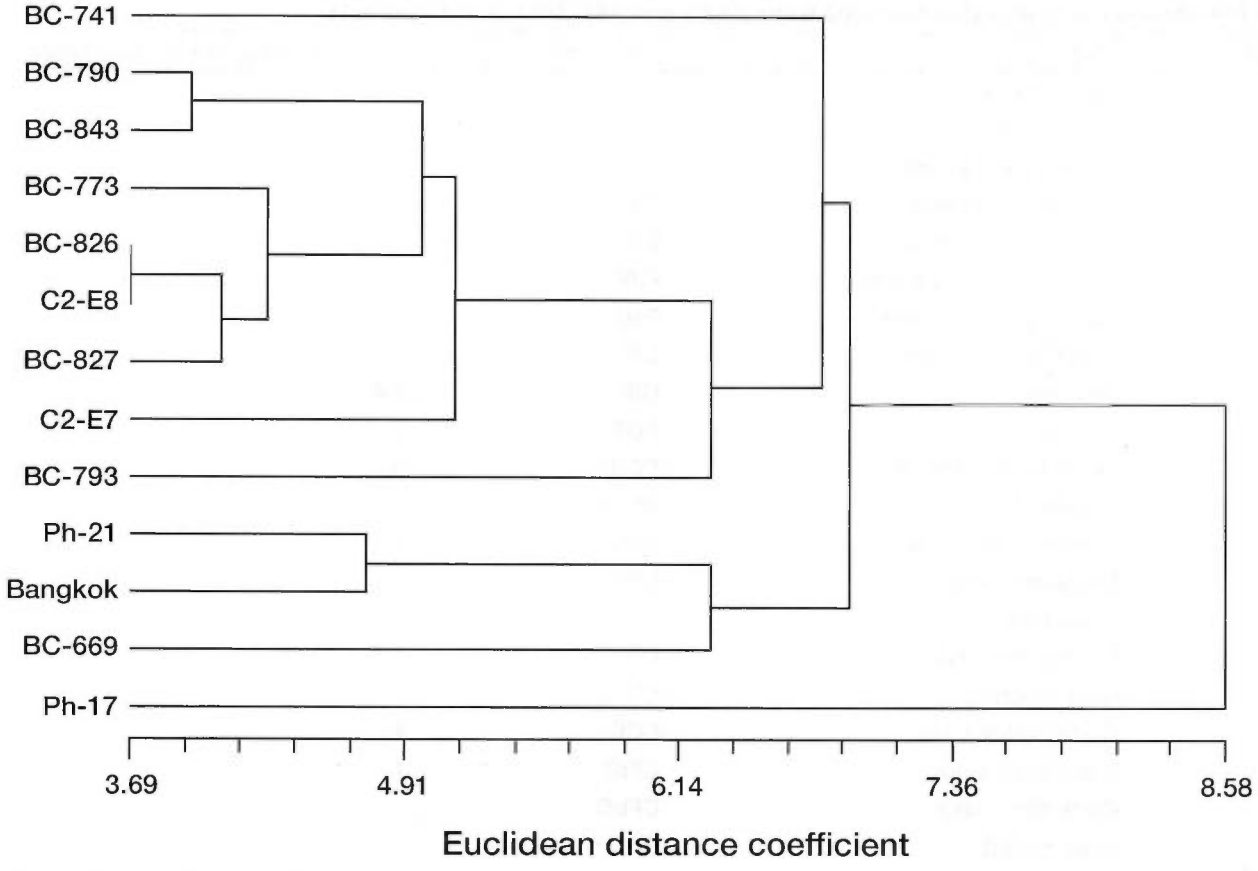


Figure 1. Dendrogram illustrating dissimilarities based on Euclidean distance coefficient and UPGMA clustering methodology for the agro-morphological data provided by 13 accessions and 23 IPGRI descriptors.

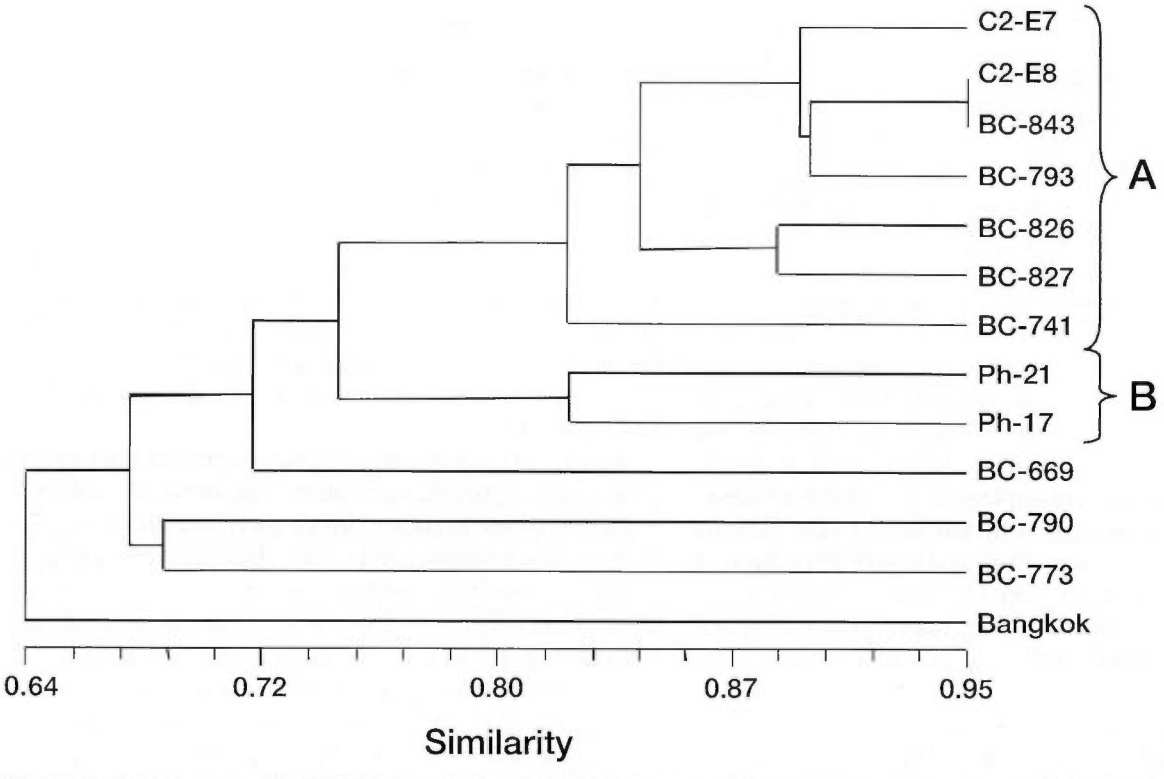


Figure 2. Dendrogram illustrating genotypic similarities among 13 accessions using Jaccard's similarity coefficient and UPGMA clustering methodology based on 39 ISSR markers.

Table 3. Eigen value, percentage of variability explained by each principal component (PC) and accumulated variability derived from agro-morphological characterization

	Principal component					
	1	2	3	4	5	6
Eigen value	1.83	1.72	1.65	1.33	1.21	1.13
Variability (%)	30.55	23.62	20.35	8.45	5.9	4.38
Accumulated variability (%)	30.55	54.17	74.52	82.97	88.9	93.3
LP	-0.03	0.45	-0.21	0.38	0.66†	0.44
LC	-0.03	-0.18	-0.04	-0.11	trace	-0.10
LV	0.09	-0.03	-0.13	-0.07	-0.04	trace
TV	0.18	-0.05	-0.25	-0.13	-0.08	trace
CV	0.13	-0.05	-0.24	-0.02	-0.07	0.05
PJP	0.03	-0.17	-0.32	0.06	-0.04	0.15
PJC	0.04	-0.37	-0.31	-0.10	0.50	0.36
LVP	0.15	-0.21	-0.66†	-0.94†	-0.16	0.33
PCT	-0.25	-0.26	-0.76†	0.24	0.27	-0.36
PCB	0.03	-0.12	-0.77†	-0.04	0.27	-0.56
PS	0.17	0.16	-0.11	-0.05	-0.02	-0.01
PSC	-0.88†	-1.28†	0.15	0.25	-0.09	0.13
BRC	0.37	-0.28	0.11	0.10	-0.03	-0.18
F	-0.2	-0.07	-0.13	0.18	-0.10	-0.05
CB	-0.05	0.01	0.02	trace‡	0.03	0.10
CS	0.20	-0.42	0.62†	-0.38	0.65†	0.03
CCC	0.95†	-0.46	-0.07	0.22	-0.02	0.08
CFlC	0.91†	-0.22	-0.18	0.50	-0.07	0.12
CFbC	0.58	-0.49	0.15	-0.03	-0.20	0.04
PH	0.04	0.02	-0.07	-0.04	trace	-0.13
ST	-0.18	0.07	-0.38	0.16	-0.22	0.37
SU	0.30	-0.10	0.28	-0.25	0.24	-0.36

†Relevant when explaining the component (after Matus et al. 1996)
‡Trace values <0.01.

Table 4. Number of bands scored and polymorphism detected using three ISSR primers in 13 taro accessions

Primer	$(GA)_9AT$	$(GA)_9AC$	$(ACC)_6Y$	Total
Number of bands detected	13	10	16	39
No. of polymorphic bands	9	9	13	29
% of polymorphic bands	69.2	90	68.7	74.4

Correlation between morphological and ISSR fingerprint data

The Mantel test for association among the dissimilarity matrices derived from ISSR and agro-morphological data indicated a poor matrix correlation of r=-0.09 ($P > 0.05$), showing that these differing methods discriminated very differently among these genotypes.

Use of discriminatory and stable agro-morphological traits can provide basic information to complement molecular data. However, construction of a comprehensive descriptor list is time-consuming and prone to error; many characters are sensitive to environmental fluctuations and other traits are divided so finely that it is sometimes difficult to make the distinction between alternative states within a descriptor. Identification of discriminatory and stable traits, especially those that are highly discriminating, is necessary to improve efficiency in characterization and management of larger germplasm collections. This is particularly the case for vegetatively propagated crops like taro, where there is little published data on the mode of inheritance of morphological traits.

Morphological characterization, based on 23 agro-morphological descriptors, enabled the separation of 13 accessions as different morphotypes. Corm, petiole and leaf colours, CS and LVP were important traits in elucidating the observed variation and identity of the accessions. Significant correlations were observed between several traits. Similar observations were also reported by Manzano et al. (2001)

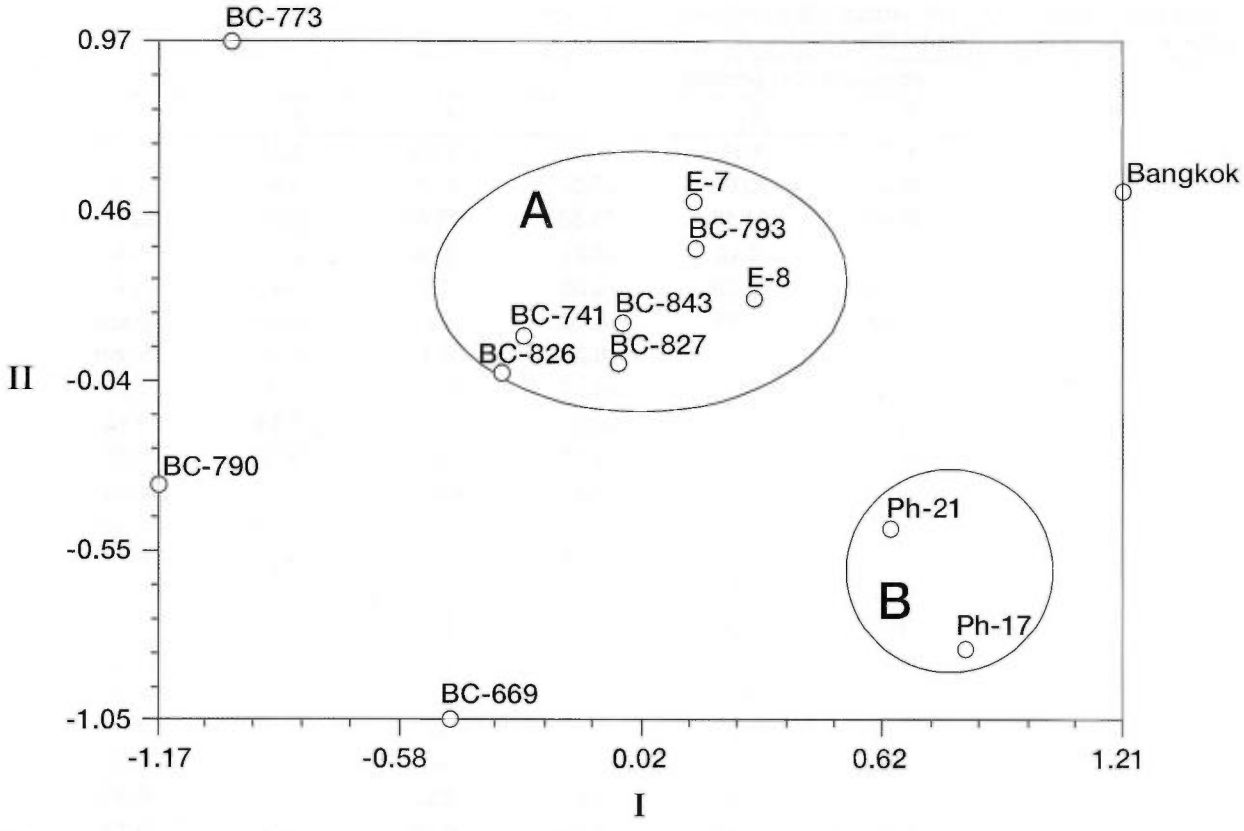


Figure 3. Groups formed from the first two multi-dimensional scale axes from the ISSR data set based on 39 markers.

in a study of Cuban taro germplasm. Our results should be interpreted with caution because of the small sample size. However, they reveal considerable redundancy in descriptor items relating to lamina variegation (LC, TV, CV), petiole colour variations (PS, PSC) and type of side shoot (ST, SU). The correlated traits should each be considered as a single descriptor item to avoid diminishing marginal returns to the use of increasing numbers of descriptor items.

The tested morphotypes were also confirmed to be unique genotypes by ISSR fingerprinting, where a high level of diversity was observed in the PNG accessions, up to approximately 30% dissimilarity. Ph-17 and Ph-21 exhibited about 80% similarity. Irwin et al. (1998) using RAPD markers also detected close genetic similarity (90%) between Ph-17 and Ph-21. The close genetic similarity of Ph-17 and Ph-21 as revealed by ISSR markers contrasted with the results of the agro-morphological analyses, where Ph-17 was found to be morphologically distinct. However, in other situations the results from the genotyping and agro-morphological characterizations were more closely aligned; farmers have classified BC-843 ('Numokwec-sisip') and BC-826 ('Numkowec-koko') as morphological variants based on their white and red basal ring colours (Okpul et al. 2003). ISSR fingerprinting has shown that they are also genotypically different, clustering together only at a level of approximately 85% similarity. Breeding line C2-E8 showed 95% homology with BC-843 ('Numkowec-sisip'). The latter was used as a parent in the breeding work of Singh and Okpul (2000), since it is highly preferred by farmers. The observed genetic relationship indicates that the genetic constitution of C2-E8 is mainly derived from cv. Numkowec-sisip, with additionally acquired traits of high yield and resistance to leaf blight caused by *Phytophthora colocasiae* (Racib.). Interestingly, C2-E8 and BC-843 are morphologically very distinct.

The 'Bangkok' accession grouped with the 12 genotypes from PNG only at a level of 65% similarity. This accession is essentially a wild-type derived from a Himalayan ecotype, and is an exotic introduction to PNG rather than a conserved selection. With the limited sample size, it is impossible to compare levels of diversity existing among Southeast Asian and PNG taros.

Godwin et al. (2001) previously noted primer $(GA)_9AC$ amplified proportionately more polymorphism than other primers in taro, and we found a similar result. With just three ISSR primers, closely related genotypes can be distinguished (C2-E8 and BC 843) as well as distantly related genotypes (e.g. the 'Bangkok' and PNG accessions). For larger sample sets, it may be advantageous to increase the number of primers.

Conclusion

ISSR markers have been shown to be useful in fingerprinting and analysing diversity in taro germplasm collections and breeding programmes. In our analysis, phenotypic variation and genotypic variation showed little correlation. A similar

disparity has been reported in many crops, including rye-grass (Roldán-Ruiz et al. 2001). Nevertheless, both approaches provided useful complementary information on the existing diversity. Both forms of information can be of immediate use for breeders.

Acknowledgement

Support from the Taro Virus Indexing and DNA Fingerprinting Project (funded by the Australian Centre for International Agricultural Research), the Taro Genetic Resources: Conservation and Utilization project (TaroGen, funded by AusAID) and the TANSAO project (funded by the European Commission) is gratefully acknowledged. The authors also thank Mr J. Guaf for technical assistance.

References

Bredemeijer GMM, Arens P, Wouters D, Visser D, Vosman B. 1998. The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. Theorical andApplied Genetics 97:584–590.

Coates DJ, Yen DE, Gaffey PM. 1988. Chromosome variation in taro, *Colocasia esculenta*: implications for origin in the Pacific. Cytologia 53:551–560.

Gilbert JE, Lewis RV, Windson MJ, Caligari PDS. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theorical and Applied Genetics 98:1125–1131.

Godwin ID, Aitken EAB, Smith LW. 1997. Application of inter-simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18:1524–1528.

Godwin ID, Mace ES, Nurzuhairawaity. 2001. Genotyping Pacific Island taro (*Colocasia esculenta* (L.) Schott) germplasm. In: Plant Genotyping—the DNA fingerprinting of plants (Henry, R. ed.). pp. 109–128. CAB International, UK.

Huang JC, Sun M. 2000. Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by Inter-Simple Sequence Repeat (ISSR) and restriction analysis of chloroplast DNA. Theorical and Applied Genetics 100:1050–1060.

IBPGR. 1980. Descriptor list for *Colocasia*. International Board for Plant Genetic Resources, Rome, Italy.

IPGRI. 1999. Descriptor list for *Colocasia*. International Plant Genetic Resources Institute, Rome, Italy.

Irwin SV, Kaufusi P, Banks K, de la Pena R, Cho J. 1998. Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. Euphytica 99:183–189.

Ivancic A, Simin A, Ososo E, Okpul T. 1995. Wild taro (*Colocasia esculenta* (L.) Schott) populations in Papua New Guinea. Papua New Guinea Journal of Agriculture, Forestry and Fisheries 38(1):31–45.

Jaccard P. 1908. Nouvelles recherches sue la distribution florale. Bulletin de la Société Vaundoise des Sciences Naturelles 44:223–270.

Jeffers JNR. 1967. Two case studies in the application of principal component analysis. Applied Statistics 16:225–236.

Karp A, Kresovich S, Bhat KV, Ayad WG, Hodgkin T. 1997. Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome, Italy.

Kreike CM, Van Eck HJ, LebotV. 2004. Genetic Diversity of taro (*Colocasia esculenta* (L.) Schott) in South-East Asia and the Pacific. Theorical and Applied Genetics (in press).

Kresovich S, McFerson JR, Westman AL. 1997. Using molecular markers in genebanks: identity, duplication, contamination and regeneration. In: Molecular genetic techniques for plant genetic resources. Report of an IPGRI workshop, 9–11 October 1995 (Ayad WG, Hodgkin T, Jaradat A, Rao VR (eds) International Plant Genetic Resources Institute, Rome, Italy. pp. 23–38.

Kuruvilla KM, Singh A. 1981. Karyotypic and electrophoretic studies on taro and its origin. Euphytica 30:405–413.

Lebot V, Aradhya KM. 1991. Isozyme variation in taro (*Colocasia esculenta* (L.) Schott) from Asia and Oceania. Euphytica 56:55–66.

Mace ES, Godwin ID. 2000. Using SSRs to determine genetic diversity in taro. The Satellite 3(1):4–6.

Mace ES, Godwin ID. 2002. Development and characterization of polymorphic microsatellite markers in taro (*Colocasia esculenta*). Genome 45 (5):823–832.

Mantel NA. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Manzano AR, Nodals AAR, Gutierrez MIR, Mayor ZF, Alfonso LC. 2001. Morphological and isozyme variability of taro (*Colocasia esculenta*) germplasm in Cuba. Plant Genetic Resources Newsletter 126:31–40.

Marshall DF. 1997. Meeting training needs in developing countries. In: Molecular genetic techniques for plant genetic resources: Report of an IPGRI Workshop (9–11 October 1995). Ayad WG, Hodgkin T, Jaradat A, Rao VN. (eds). International Plant Genetic Resource Institute, Rome, Italy. pp. 128–132.

Matthews PJ. 1990. The origin, dispersal and domestication of taro. PhD Thesis, Australian National University, Canberra, Australia.

Matthews PJ, Matsushita Y, Sato T, Hirai M. 1992. Ribosomal and mitochondrial DNA variation in Japanese taro (*Colocasia esculenta* L. Schott.). Japanese Journal of Breeding 42:825–833.

Matus I, Gonzales IM, del Poso A. 1996. Evaluation of phenotypic variation in a Chillean collection of garlic (*Allium sativum* L.) clones using multivariate analysis. Plant Genetic Resources Newsletter 117:31–36.

Maunder M, Culham A, Bordeu A, Allainguillaumes J, Wilkinson M. 1999. Genetic diversity and pedigree for *Sophora toromiro* (Leguminsae): A tree extinct in the wild. Molecular Ecology 8:725–738.

Métais I, Aubry C, Hamon B, Jalouzot R, Peltier D. 2000. Description and analysis of genetic diversity between commercial bean lines (*Phaseolous vulgaris* L.). Theorical and Applied Genetics 101:1207–1214.

Nagaoh T, Ogihara Y. 1997. Application of Inter-Simple Sequence Repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theorical and Applied Genetics 94:597–602.

Nurzuhairawaty. 2000. The use of ISSR markers to study diversity in Pacific Island taro germplasm. Unpublished MAgrSt thesis. The University of Queensland, Brisbane, Australia.

Okpul T, Gunua T, Wagih ME. 2004. The selection of a core sample of Papua New Guinean taro (*Colocasia esculenta* (L.) Schott) using agro-morphological characteristics. Genetic Resources and Crop Evolution 51:671–678

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK. 2000. The use of microsatellies for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theorical and Applied Genetics 100:584–592.

Prevost A, Wilkinson MJ. 1999. A new system of comparing PCR primers applied to SSR fingerprinting of potato cultivars. Theorical and Applied Genetics 98:107–112.

Purseglove JW. 1972. Tropical Crops. *Monocotyledons I*. Longman, London, UK. 334 pp.

Rohlf FJ. 1998. NTSYS-pc: Numerical Taxonomy and Multivariate System. Version 2.02i Exeter Software, New York, USA.

Roldán-Ruiz I, van Eeuwijk FA, Gilliland TJ. 2001. A comparative study of molecular and morphological methods of describing the relationships between perennial ryegrass (*Lolium perenne* L.) varieties. Theorical and Applied Genetics 103:1138–1150.

Salimath SS, Oliviera AC, Godwin ID, Bennetzen JL. 1995. Assessment of genome origin and genetic diversity in the genus *Eleusine* with DNA markers. Genome 38:757–763.

Sar SA, Wayi BM, Ghodake RD. 1998. Review of research in Papua New Guinea for sustainable production of taro (*Colocasia esculenta*). Tropical Agriculture (Trinidad) 75:134–138.

Singh D, Okpul T. 2000. Evaluation of 12 taro (*Colocasia esculenta* (L.) Schott) leaf blight resistant clones for yield and eating quality in Papua New Guinea. *SABRAO* Journal of Plant Breeding and Genetics 32:39–45.

Sokal RR, Michener CP. 1958. A statistical method for evaluating systematic relationships. University of Kansas Science Bulletin 38:1409–1438.

Tanimoto T, Matsumoto T. 1986. Variation of morphological characters and isozyme patterns in Japanese cultivars of *Colocasia esculenta* Schott and *Colocasia gigantea* Hook. Japanese Journal of Plant Breeding 36:100–111.

TANSAO. 1998. Taro Network for Southeast Asia and Oceania: 1998 Annual Report. CIRAD, Port Vila, Vanuatu.

van Eck HJ, Han T, Jacobsen E. 1998. Evaluation of the genetic diversity in taro (*Colocasia esculenta*) using AFLP fingerprinting. In: TANSAO 1998 Annual Report. CIRAD, Port Vila, Vanuatu. pp. 78–82.

Virk PS, Pooni HS, Syed NH, Kearsey MJ. 1999. Fast and reliable genotype validation using microsatellite markers in *Arabidopsis thaliana*. Theorical and Applied Genetics 98:462–464.

Yang W, de Oliviera AC, Godwin ID, Schertz K, Bennetzen JL. 1996. Comparison of DNA marker technology in characterizing plant genome diversity: Variability in Chinese sorghums. Crop Science 36:1669–1667.

Yen DE, Wheeler JM. 1968. Introduction of taro into the Pacific: the indication of chromosome numbers. Ethnobotany 7:259–267.

Appendix 1. Correlation among the variable descriptors using the IPGRI descriptor list is available online from http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

The effect of seed oil content on viability assessment using tetrazolium: a case study using 171 species

C.B. Wood,[1,2] S. Miles,[1] C. Rix,[1] J. Terry[1] and M.I. Daws[1]
[1]Seed Conservation Department, Royal Botanic Gardens Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK. Email: m.daws@rbgkew.org.uk
[2]Current address: The Graduate School, Mary Newman Building, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, UK.

Summary

The effect of seed oil content on viability assessment using tetrazolium: a case study using 171 species

In this paper we compare seed viability estimates using the topographical tetrazolium (TZ) stain with germination data for 171 species from 27 families, with a particular emphasis on 6 families. TZ consistently underestimated viability compared with germination testing for species in the Asteraceae (19 species), Brassicaceae (17 species) and Solonaceae (16 species), possibly as a result of stain permeation problems into oily seeds (typically ca. 20–30% in species of these families). TZ also underestimated viability in Aizoaceae (19 species), but oil contents are unknown for this group of species. Viability and germination data were more comparable in Fabaceae (15 species) and Poaceae (59 species), which typically have low oil contents (ca. 5–8%) seeds. These findings suggest the need for TZ protocol improvements, especially for high oil content seeds. In contrast, TZ gave higher values than the germination test for some species, indicating the need for germination protocol improvements of biodiverse species. Overall, the results emphasize the need to be cautious when interpreting TZ tests, particularly for species where there are no published international guidelines.

Key words: germination, oil, protocol, tetrazolium, viability

Résumé

Effet de la teneur en huile des graines sur l'évaluation de leur viabilité en utilisant le tétrazolium : étude de cas sur 171 espèces

Dans cet article, nous comparons des estimations de viabilité de graines en utilisant une coloration topographique au tétrazolium (TZ) et des données de germination relatives à 171 espèces de 27 familles, avec une attention particulière pour 6 familles. TZ sous-estime systématiquement la viabilité en comparaison des tests de germination chez des Asteraceae (19 espèces), Brassicaceae (17 espèces) et Solanaceae (16 espèces), peut-être en raison de problèmes de pénétration du colorant dans l'huile des graines (typiquement dans env. 20 à 30 % des espèces de ces familles). TZ sous-estime également la viabilité chez les Aizoaceae (19 espèces), mais les teneurs en huile sont inconnues dans ce groupe d'espèces. Les données de viabilité et de germination sont comparables chez les Fabaceae (15 espèces) et les Poaceae (59 espèces), dont les graines ont typiquement de faibles teneurs en huile (env. 5 à 8 %). Ces résultats montrent qu'il est nécessaire d'améliorer le protocole de coloration au TZ, en particulier dans le cas de graines à teneur élevée en huile. En revanche, TZ donne des valeurs plus élevées que le test de germination pour certaines espèces, indiquant la nécessité d'améliorer les protocoles de germination. Dans leur ensemble, les résultats incitent à la prudence dans l'interprétation des tests TZ, notamment, dans le cas d'espèces pour lesquelles aucune recommandation internationale n'a été publiée.

Resumen

Efectos del contenido de aceite de las semillas al emplear tetrazolio para evaluar su viabilidad: estudio de caso sobre 171 especies

En este documento comparamos la evaluación de la viabilidad de semillas empleando tintura topográfica de tetrazolio (TZ) con los datos de germinación de 121 especies de 27 familias, destacando en particular 6 familias. El TZ subestimaba de manera constante la viabilidad comparado con las pruebas de germinación de las especies Asteraceae (19 especies), Brassicaceae (17 especies) y Solonaceae (16 especies), probablemente debido a problemas de permeación de la tintura en las semillas aceitosas (típicamente ca. 20–30% en especies de esas familias). El TZ también subestimaba la viabilidad de Aizoaceae (19 especies), aunque se desconoce el contenido de aceite de ese grupo de especies. Los datos de viabilidad y germinación fueron más comparables para Fabaceae (15 especies) y Poaceae (59 especies), que por lo general tienen semillas de bajo contenido de aceite (ca. 5–8 %). Estos resultados sugieren la necesidad de mejorar el protocolo de TZ, especialmente en lo relativo a semillas de alto contenido de aceite. Para algunas especies, por el contrario, el TZ dio valores más altos que la prueba de germinación, lo que indicaba la necesidad de mejorar el protocolo de germinación de especies biodiversas. En general los resultados destacan la necesidad de obrar con cautela al interpretar las pruebas de TZ, en particular para especies de las que no se han publicado directrices internacionales.

Introduction

Tetrazolium (TZ) salts have been used for over 60 years to give a rapid assessment of seed viability (Kuhn and Jerchel 1941). A topographical method of assessment was developed for cereals (Lakon 1942) and later for coniferous species (Lakon 1949). Since then, the test has been widely adopted, e.g. by the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA). International rules for seed testing using TZ have been produced (AOSA 2000; ISTA 1999), including recommendations for the determination of seed viability in 89 taxa (ISTA 1999). In the TZ test, a colourless solution of tetrazolium chloride is reduced using dehydrogenase enzymes within viable tissues to form a stable, non-diffusable and distinctive red dye (2,3,5-triphenyl formazan) (Seidler 1991). The distribution and extent of red stained areas in treated seeds are examined when seed viability is assessed; in the absence of active enzymes, i.e. in dead tissues, there is no stain development.

The TZ test has been used to diagnose seed quality problems in a range of species, including numerous crops (Moore 1962), grasses and legumes (Porter et al. 1947), cotton (Lambou 1953), conifers (Parker, 1953) and orchids (Van Waes and Deberg 1986). It is used primarily as a rapid estimate of seed viability and is an important means of distinguishing between dead and dormant seeds (Gosling 2003). The test can also be used to examine the effects

of environmental conditions on seed viability, such as responses to desiccation and storage (Dickie et al. 1991; Kovach and Bradford 1992).

Although quite widely applied, the TZ test has limitations because of the idiosyncrasies raised by the particular species under test (Justice 1972; Bhodthipuks et al. 1996; Gosling 2003). For example, the visual interpretation of staining can be subjective (Howarth and Stanwood 1993) and requires experience, especially for small or darkly coloured seeds (Harty et al. 1972; Copeland and McDonald 1985; Gosling 2003). Thus, the wider application of the TZ test in seed biology is a considerable challenge.

In this study we focus mainly on six families: Aizoaceae, Asteraceae, Brassicaceae, Fabaceae, Poaceae and Solonaceae. The Aizoaceae is a comparatively small, pan-tropical family (128 genera, 1850 species) with most species concentrated in South Africa (Mabberly 1997). They are predominantly used as greenhouse ornamentals or landscape plants in warmer regions. The Asteraceae is an extremely large (1528 genera, 22 750 species) cosmopolitan family (Mabberly 1997). Many of the species are of medicinal, food (e.g. sunflower) or ornamental value. The Brassicaceae is a smaller family (365 genera and 3250 species) (Mabberly 1997) consisting of shrubs and herbs, many found in temperate arid areas of the world. Some species produce mustard-oil glucosides and capers (*Capparis spinosa*) that are of economic value. The Fabaceae is a large family (642 genera, 18 000 species) with a global distribution (Mabberly 1997). Many species are important agriculturally as food for humans and livestock, and as garden ornamentals. The Poaceae (grass family) is a large (668 genera, 9500 species), widespread family, found in all climates and regions and is the source of the most important crops (Mabberly 1997). The Solonaceae is a cosmopolitan family (comprising 94 genera and 2950 species), which is found mainly in Australia, Central and South America (Mabberly 1997). Their economic uses include food, ornamentals, as a source of alkaloids used in medicine and tobacco.

This work is part of a programme aimed at investigating the effectiveness of vital staining for seed viability testing (Wood et al. 2003; Wood and Pritchard 2004) and here we compare results of TZ analyses and germination tests on 171 species from 27 families.

Materials and methods

Seed material

The results of germination studies and TZ analysis for 145 wild plant species in the 6 main families investigated (see Appendix 1 [online]), conducted over the course of ca. 20 years, are presented based on data collated within the Seed Bank Database (Royal Botanic Gardens, Kew). To provide additional information, 26 species in 21 families (see Appendix 1[online]) are also included. All seed lots came from the Royal Botanic Garden, Kew's Millennium Seed Bank collections. Upon receipt at the seed bank, seeds were dried at 15°C and 15% relative humidity and subsequently removed from attached fruit tissues. Following cleaning, seeds were hermetically sealed in containers (various) and stored at –20°C until used (weeks to years, depending on species).

Seed germination

Initially, germination tests were conducted at a range of constant temperatures with the choice of temperatures dependent on whether a particular species was of temperate or tropical origin. Tropical species were germinated at some or all of the following temperatures; 20, 25 and 30°C (±1°C), while temperate species were germinated at 10 and 15°C (±1°C). For the vast majority of species, 1×50 seeds per test were sown on the surface of 1% agar in water in either 55 or 90 mm diameter Petri dishes, depending on seed size. In some cases the seed numbers varied from that specified above and these changes are detailed in Appendix 1(online). Seeds received either an 8 or 12 h period of irradiance (see Appendix 1[online]), the levels of which will have varied over the course of 20 years, but are likely to have been ca. 10 μmol m^{-2} s^{-1}. For species where germination at constant temperatures exceeded 70%, the seed lot was assumed to be non-dormant and no further germination tests were conducted.

Seed lots that failed to reach 70% germination were subjected to a range of alternative germination test conditions based on a knowledge of seed germination requirements for particular families as reported in the scientific literature (see Baskin and Baskin 1998). Treatments included alternating temperatures [25/10, 35/10, 33/19 or 23/9°C with the elevated temperature applied for either 8 (25/10 or 35/10°C) or 12 h (33/19 or 23/9°C)], mechanical scarification, removal of the palea and lemma (for species in the Poaceae) and the addition of GA_3 (0.722 mM) or KNO_3 (1 mM) to the agar substrate. For scarification, the seed coat was chipped using a scalpel blade. Appendix 1 (online) details the germination conditions under which we observed the highest level of germination for each taxon. While these conditions did not necessarily result in 100% seed germination, they represent our best estimate of the total seed lot germinability. For seed lots that did not germinate rapidly, the test lasted for at least 4 weeks.

TZ test

After establishing the 'optimal' germination conditions, a TZ test was performed. A 1% aqueous solution of 2,3,5-triphenyl tetrazolium chloride buffered to pH 7 using potassium dihydrogen orthophosphate (KH_2PO_4) and disodium hydrogen orthophosphate dehydrate ($Na_2HPO_4 \cdot H_2O$) was prepared according to international guidelines (ISTA 1999). Prior to testing, seeds were rehydrated for 24 h over water at 20°C followed by further imbibition on 1% agar in water at 20°C for 48 h. Species with an impermeable seed coat (mainly in the Fabaceae) were mechanically scarified prior to rehydration.

Following imbibition, seeds were prepared according to the general ISTA recommendations on seed dissection

for the specified families (ISTA 1985). Thus for species in the Aizoaceae, the testa was punctured near the centre; for Asteraceae, seeds were cut longitudinally completely through the mid-section of the distal half; for the Brassicaceae, the seed coat was cut through the mid-section near the distal half; for the Fabaceae, a cut was made through the coat over the entire cotyledon length and near the mid-section length; for the Solanaceae, a deep incision was made from the mid-section to the radicle (and cotyledon) tip; for the Poaceae, seeds were cut longitudinally through the embryo and three-quarters of the endosperm (ISTA 1999). Prepared seeds were submersed in the test solution in Petri dishes. The dishes were then wrapped in aluminium foil and incubated at 25°C for 24 h. Seeds were fully dissected to reveal the staining patterns for evaluation. Seeds were assumed to be viable when all seed tissues, e.g. axis and cotyledons, were uniformly stained red as opposed to pink. Patchily stained seeds were assumed to be viable when the axis and part (>50%) of the cotyledons were stained red and inviable when the axis was unstained or only partially stained. Typically, n=1×50 seeds were used per seed lot, although this did differ (minimum 1×20) depending on species/seed lot (see Appendix 1[online]).

Statistical analysis

To determine whether, for individual species, there was a significant difference between the level of germination and the estimate of viability (from TZ) a two-tailed proportions test using the normal approximation to the binomial distribution and a Pearson's Chi-squared statistic was implemented in S-Plus 2000 (Mathsoft Inc., Massachusetts, USA).

For the six selected families, a sign test was used to assess whether the TZ test provided an accurate assessment of viability. The test was applied by calculating the number of species for which viability, as assessed by TZ, was (1) greater, (2) lower or (3) the same as the observed level of germination. Subsequently, the significance of the number of species for which the estimate of viability differed from the estimate of germinability was tested using the binomial distribution (Sokal and Rohlf 1995).

Results and discussion

Germination testing

For 89 of the 145 species tested here, no information regarding germination is presented in the general literature (Baskin and Baskin 1998).

Alternating temperatures stimulated germination in the majority of the 19 species in the Aizoaceae and 9 more showed improved germination following chipping of the seed coat (Appendix 1 [online]). According to Baskin and Baskin (1998), physiological dormancy is the only type to occur in this family, and our results with alternating temperatures support this suggestion. However, the beneficial effects of chipping suggest that physical dormancy may also be present in some species. Whether the effect of chipping was mediated by an increase in water or gas uptake or by a reduced physical restraint to embryo expansion is not yet clear.

Our results with the Asteraceae also suggest that physiological dormancy is present in many of these species; alternating temperatures were superior to constant temperatures for 7 of the 19 species studied, which concurs with Baskin and Baskin (1998), who report that physiological dormancy is typical for this family. Testa removal facilitated germination in some species, probably by enabling embryo growth (Teketay 1993).

A majority of species in the Brassicaceae responded to alternating temperatures. In addition, germination benefited when GA_3 and/or mechanical scarification were applied. Many of the species tested belong to the genus *Cleome*. Thus, it appears that physiological dormancy is widespread in this group of species and may, potentially, be combined with physical dormancy. There is little information on seed germination for this genus and related species previously classified within the Capparaceae (Baskin and Baskin 1998). Clearly, further work is needed on this group of species.

Germination of the Poaceae frequently required alternating temperatures and the addition of KNO_3 to the germination media. This suggests the presence of physiological dormancy, which, according to Baskin and Baskin (1998), is the only type of dormancy observed in this family. In addition, removal of covering structures (palea and lemma) facilitated germination in many species. The effect of palea and lemma removal has previously been reported for grasses and interpreted as the embryo having insufficient growth potential to overcome the mechanical restraint imposed by these structures (Baskin and Baskin 1998).

The 16 species in the Solonaceae all responded to either GA_3 or alternating temperatures, indicating the presence of physiological dormancy, as suggested by Baskin and Baskin (1998). All the species examined in the Fabaceae responded favourably to mechanical scarification. This is not surprising as species in this family typically have seeds that develop water-impermeable seed coats when dehydrated to low moisture contents (Baskin and Baskin 1998).

TZ analysis

In the first analysis, looking at the effect of viability assessment using TZ compared with germination in 26 species in 21 families revealed three distinct responses (Figure 1A and B). For 13 species (*Aeonium percarneum, Amaranthus dubius, Asphodeline lutea, Berchemia zeyheri, Brachyotum microdon, Clethra delavayi, Erythronium montanum, Moehringia trinervia, Nigella damascena, Opuntia echinocarpa, Veronica officinalis, V. triphyllos*, and *Xiphidium caeruleum* var *caeruleum*), TZ and germination values were not significantly different (p>0.05). However, this was not the case for the other species investigated. For these, two other distinct responses were observed. For five species, results for TZ analysis were significantly higher (p<0.05) than those of a germination test (Figure 1A and B). This is likely to reflect the presence of physiological dormancy in seeds of *Vaccaria pyrimidata, Juncus ambiguus* (Baskin and Baskin 1998)

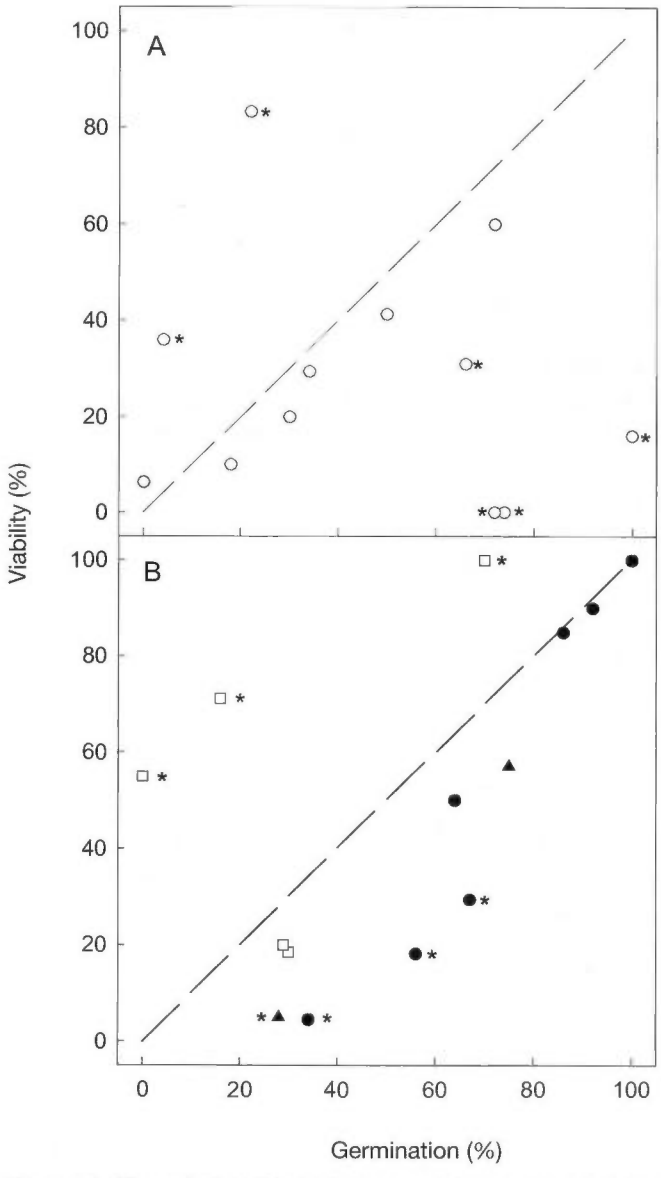


Figure 1. The relationship between maximum germination and viability assessment via TZ staining for (A) 12 species in 11 families for which oil contents were not available and (B) 14 species in 13 families for which oil contents were available (□=0–10%; ▲=10–20%; ●=>20% oil content), either for the particular species in question or species in the same genus. Asterisks adjacent to symbols indicate a significant difference ($p<0.05$) between viability estimates and germination percentage.

and *Carex pendula* (Schültz 2000). The results indicate a similar phenomenon in *Cercidiphyllum japonicum* and *Argemone cf. platyceras* for which nothing was previously known about their germination requirements. The usefulness of the TZ test for highlighting seed dormancy problems has also been recently shown by Newton et al. (2002) for a range of species in the Restionaceae.

In eight species, the viability assessment with TZ was significantly lower than that observed for germination ($p<0.05$; Figure 1A and B). A review of the data sheets revealed the frequent occurrence of pink rather than red stained embryos. Pink staining of embryos has been noted previously in Brazil nut (*Bertholletia excelsa*) and has been demonstrated to indicate seed viability in this species (Camargo et al. 1997). Brazil nut is a highly oily species (61.5%; SID5, Tweddle et al. 2003) and one possible cause of the pink staining could be poor permeation of the TZ stain into the embryo. To assess whether the presence of oil in seeds may have contributed to the observed under-estimation of viability, oil contents either for the species or genus level (from SID5, Tweddle et al. 2003) were reviewed. Data were available for 14 taxa (see Figure 1B). This assessment suggested that there could be an association between seed oil content and the accuracy of the TZ viability assessment. Three of the species with an oil content >20% and one species with an oil content between 10 and 20% had a significantly higher level of germination than viability (assessed by TZ; $p<0.05$). In addition, for five species with an oil content <10% germination was either significantly lower or not significantly different from the estimate of viability (Figure 1B).

To test this possibility further, a wider analysis was performed on the six largest families investigated. For two of the families (Fabaceae and Poaceae), TZ gave a relatively accurate assessment of viability, i.e. much of the data were scattered around the null line (Figure 2). In these families, there was no significant bias for TZ to either overestimate or under-estimate viability (Sign Test, $p>0.05$). For both legumes and grasses, considerable effort has been expended to determine TZ test protocols. For example, in the ISTA Rules (ISTA 1999) guidelines for the use of TZ are presented for 8 and 29 taxa in the Fabaceae and Poaceae, respectively. However, for the four additional families (Aizoaceae, Asteraceae, Brassicaceae and Solonaceae; Figure 2) TZ significantly (Sign Test, $p\leq0.02$) under-estimated viability. These families are either poorly represented or absent from the ISTA Guidelines (ISTA 1999).

A review of the literature revealed that three of the four problem families typically have seeds with comparatively high oil content and mean values across the families ranging from 22 to 29% (Table 1; Tweddle et al. 2003); no data were available for the Aizoaceae. In contrast, legume and grass seeds are generally lower in oil, with means across the families of <10% (Table 1). These data support the notion that high lipid content of seeds reduces their permeability to the stain and thus hinders its development.

It is interesting to note that among agricultural and horticultural species, lettuce (Asteraceae; 35.4% oil content; Eckey 1954) and oil seed rape (Brassicaceae; 22–49% oil content; Eckey 1954) are absent from the ISTA guidelines (ISTA 1999). Although soybean is an oil crop, it has a comparatively low oil content (ca. 19.6%; Eckey 1954). Interestingly, there are a number of published reports on the use of TZ for this species (Da Costa and Marcos-Filho 1994; Prijic et al. 1998). Accepting that oil content may affect the ease of use of the TZ test, it is important to consider what type of method changes might improve test accuracy. Longer treatment times or warmer temperatures seem worthy of consideration. Of particular interest is the use of vacuum

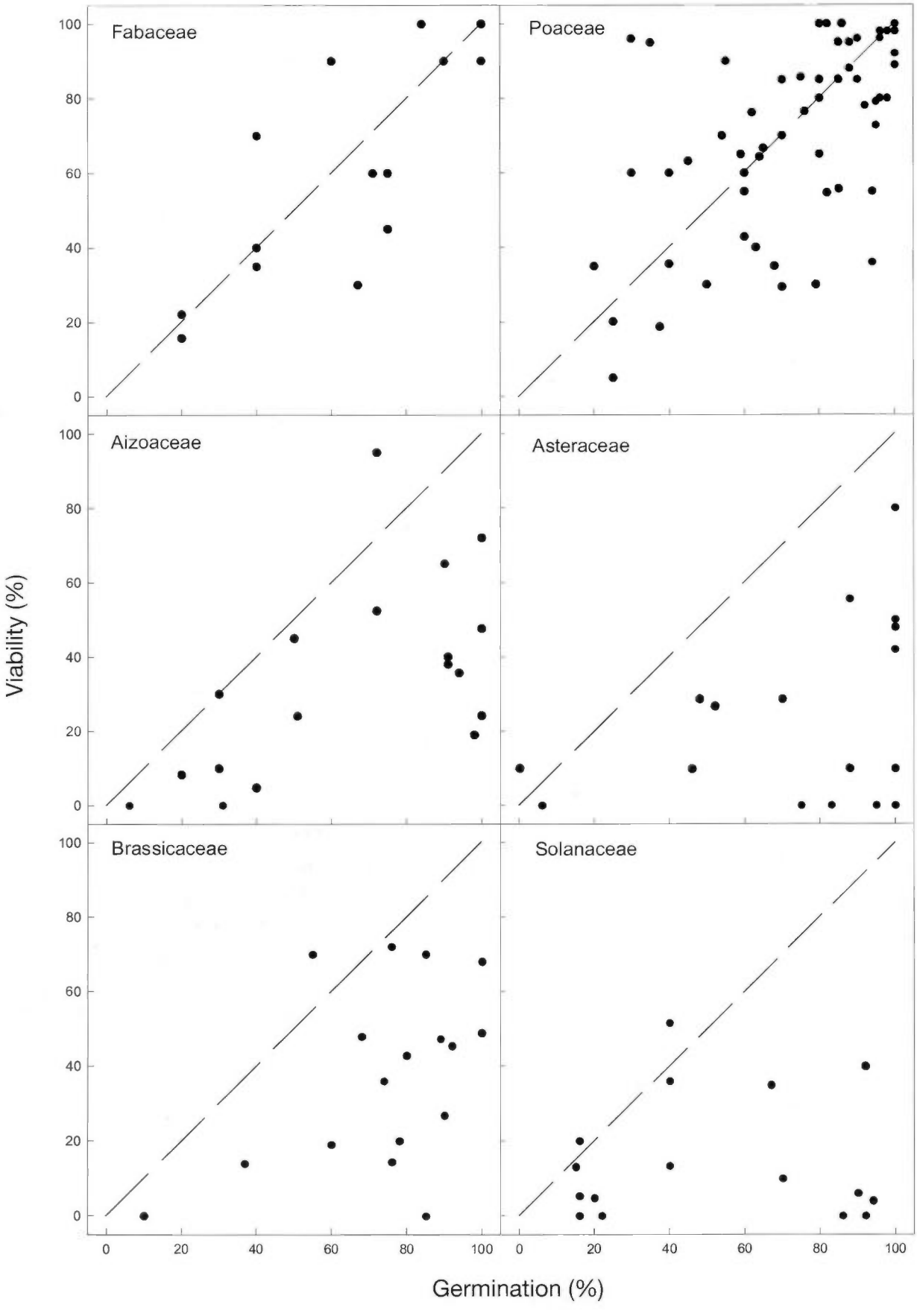


Figure 2. The relationship between maximum germination and viability assessment via TZ staining for 145 species drawn from Fabaceae, Poaceae, Aizoaceae, Asteraceae, Brassicaceae and Solonaceae.

Table 1. Oil contents for a representative range of species for five of the study families from SID5 (Tweddle et al. 2003)

Family	Number of species for which oil content is available†	Mean and median oil content from SID5 (%) [range]
Asteraceae	340	23.3, 22.6 [1.0–61.2]
Brassicaceae	137	29.4, 30.4 [0.9–47.4]
Fabaceae	705	8.3, 6.6 [0.4–48.9]
Poaceae	131	5.3, 4.0 [0.8–19.6]
Solanaceae	33	22.4, 21.3 [6.3–41.3]

†For some species there is more than one record.

infiltration, as *Pinus sylvestris* (mean oil content for the genus *Pinus* from Tweddle et al. [2003] is 39%) seed staining can be improved using this method to force TZ stain into the seed (Savonen 1999).

Conclusion

In conclusion, our analyses, based on a wide range of species, indicate that the TZ test gives a reasonably accurate determination of seed viability in species of some families; for some species though, protocol improvement for both the germination and TZ test conditions are needed to ensure a 1:1 relationship between these measures of seed 'quality'.

Acknowledgements

We thank Dr H.W. Pritchard for advice and comments on the manuscript and Frances Briscoe and Hazel Cleland for support. Financial support for this work from the Millennium Commission, the Wellcome Trust and Orange plc is gratefully acknowledged. The Royal Botanic Gardens, Kew receives grant-aided support from Defra, UK.

References

AOSA. 2000. Tetrazolium testing handbook, no. 29, 1999. Association of Official Seed Analysts, Las Cruces, New Mexico, USA.

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

Bhodthipuks J, Pukittayacamee P, Saelim S, Wang BSP, Yu SL. 1996. Viability testing of tropical tree seed as a tool for the conservation of endangered Western Terrestrial orchids. Australian Journal of Botany 49:619–628.

Camargo IP, de Carvalho MLM, Vieira M das GG. 1997. Evaluation of the deterioration in Brazil nut seeds by the tetrazolium test. Pesquisa Agropecuaria Brasileira 32:835–839.

Copeland LO, McDonald MB. 1985. Principles of seed science and technology (3rd ed.). Springer, Berlin, Germany.

Da Costa NP, Marcos-Filho J. 1994. Alternative methodology for the tetrazolium test for soybean seeds. Seed Science and Technology 22:9–17.

Dickie JB, May K, Morris SVA, Titley SE. 1991. The effects of desiccation on seed survival in *Acer platanoides* L. and *Acer pseudoplatanus* L. Seed Science Research 1:149–162.

Eckey EW. 1954. Vegetable fats and oils. Reinhold Publishing Corporation, New York, USA.

Gosling PG. 2003. Viability testing. In: Smith RD Dickie JB, Linington SH, Pritchard HW, Probert RJ, editors. Seed Conservation: Turning Science into Practice. Royal Botanic Gardens, Kew, UK. pp. 445–481.

Harty RL, Paleg LG, Aspinall D. 1972. Quantitative reduction of triphenyl tetrazolium chloride as a measure of seed viability in cereal seeds. Australian Journal of Experimental Agriculture and Animal Husbandry 12:517–522.

Howarth MS, Stanwood PC. 1993. Tetrazolium staining viability test using color image processing. Transactions of the American Society of Agricultural Engineers 36:1937–1940.

ISTA. 1985. Handbook on tetrazolium testing (2nd ed.). International Seed Testing Association, Zurich, Switzerland.

ISTA. 1999. International rules for seed testing: rules 1999. Seed Science and Technology 27, supplement, pp. 201–244.

Justice OL. 1972. Essentials of seed testing: rules 1999. Annexes supplement to: Seed Science and Technology 45:115–120.

Kovach DA, Bradford KJ. 1992. Imbibitional damage and desiccation tolerance of wild-rice (*Zizania palustris*) seeds. Journal of Experimental Botany 43:747–757.

Kuhn R, Jerchel D. 1941. Reduktion von Tetrazoliumsalzen durch Bakterien, garende Hefe und keimende Samen. Berichte der Deutshen Botanischen Gesellschaft 60:299–305.

Lakon G. 1942. Topographischer Nachweis der Keimfähigkeit der Getreidefrüchte durch Tetrazolium-Verfahren [The 'vigour' of seeds and its determination by the topographical terazolium method]. Saatgutwirtschaft 2:37–41.

Lakon G. 1949. The topographical tetrazolium method for determining the germination capacity of seeds. Plant Physiology 24:389–394.

Lambou MG. 1953. 2,3,5-triphenyl tetrazolium chloride as a rapid indicator of viability in cotton seeds. Science 117:690–693.

Mabberly DJ. 1997. The plant-book: a portable dictionary of the vascular plants (2nd ed.). Cambridge University Press, Cambridge, UK.

Moore P. 1962. TZ checks your seed quality. Crops and Soils 15:10–12.

Newton RJ, Bond WJ, Farrant JM. 2002. Seed development, morphology and quality testing in selected species of the nut fruited Restionaceae. South African Journal of Botany 68:226–230.

Parker J. 1953. Some applications and limitations of tetrazolium chloride. Science 118:77–79.

Porter RH, Durrell M, Romm HJ. 1947. The use of 2,3,5-triphenyl tetrazolium chloride as a measure of seed germinability. Plant Physiology 22:149–159.

Prijic L, Jovanovic M, Glamoclija D. 1998. Germination and vigour of wrinkled and greenish soybean seed. Seed Science and Technology 26:377–383.

Savonen EM. 1999. An improvement to the topographic testing of Scots pine (*Pinus sylvestris* L.) seeds. Seed Science and Technology 27:49–57.

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

Seidler E. 1991. The tetrazolium-formazan system: design and histochemistry. Progress in Histo-chemistry and Cytochemistry 24:1–86.

Sokal RR, Rohlf FJ. 1995. Biometry. W.H. Freeman and Company, New York, USA.

Teketay D. 1993. Germination ecology of *Vernonia galamensis* (Cass.) Less. var. *ethiopica* M.G. Gilbert a new industrial oil seed crop. Tropical Ecology 34:67–74.

Tweddle JC, Turner RM, Dickie JB. 2003. Seed Information Database (release 5.0, July 2003), http://www.rbgkew.org.uk/data/sid.

Van Waes JM, Deberg PC. 1986. Adaptation of the tetrazolium method for testing the seed viability and scanning electron microscopy of some of the Western European orchids. Physiologia Plantarum 66:435–442.

Wood CB, Pritchard HW. 2004. Determination of intra-specific variation in orchid seed viability using fluorescein diacetate. Seed Science and Technology 32:629–635.

Wood CB, Pritchard HW, Mugambi JK. 2003. Fluorescein diacetate and orchid seed viability in UK and Kenyan species. In: Smith RD, Dickie JB, Linington SH, Pritchard HW, Probert RJ, editors. Seed Conservation: Turning Science into Practice. Royal Botanic Gardens, Kew UK. pp. 493-506.

Appendix 1. Species for which percentage germination and viability estimates (from TZ) were available, and the conditions under which maximum germination was observed is available online from http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Exploration and collecting expedition on the Cheradi islands (southern Italy), 2004

G. Laghetti,[1]✉ G. Sonnante,[1] S. Cifarelli[1] and K. Hammer[2]
[1]Institute of Plant Genetics (IGV), C.N.R., I-70126 Bari, Italy
[2]Institute of Crop Science, University of Kassel, D-37213 Witzenhausen, Germany

Summary
Exploration and collecting expedition on the Cheradi islands (southern Italy), 2004
In the spring of 2004, a collecting mission to the Cheradi islands (southern Italy) was carried out within an exploration cycle aimed at safeguarding autochthonous crop genetic resources still present in Italian minor islands. Nowadays, autochthonous vegetation of Cheradi is scarce because the large cultivated fields of the recent past have been abandoned. In this first and preliminary expedition, 34 taxa were found and classified belonging to 16 botanical families (e.g. *Vicia ervilia, Beta vulgaris* subsp. *maritima*, wild *Daucus carota*). Five populations of *Cynara cardunculus* var. *sylvestris* were also sampled and collected. Some small areas on the Cheradi islands still currently represent, botanically, very interesting places, well protected from human intervention that would merit more in-depth study.

Key words: agricultural biodiversity, Cheradi islands, collecting, crop genetic erosion, exploration

Résumé
Mission d'exploration et de collecte dans les îles Cheradi (Sud de l'Italie), 2004
Au printemps 2004, une mission de collecte dans les îles Cheradi (Sud de l'Italie) a été effectuée dans le cadre d'un cycle d'exploration afin de sauvegarder les ressources génétiques des plantes cultivées autochtones encore présentes dans les petites îles italiennes. Actuellement, la végétation autochtone de Cheradi a fortement régressé en raison de la taille excessive des parcelles cultivées dans un passé récent et, à présent, abandonnées. Lors de cette première expédition préliminaire, 34 taxons appartenant à 16 familles différentes ont été observés (par exemple, *Vicia ervilia, Beta vulgaris* subsp. *maritima, Daucus carota* sauvage). Des échantillons de cinq populations de *Cynara cardunculus* var. *sylvestris* ont également été collectés. Certaines petites zones des îles Cheradi constituent, aujourd'hui encore, des sites très intéressants du point de vue botanique, bien protégées de l'anthropisation, et elles mériteraient une étude plus approfondie.

Resumen
Expedición de exploración y recolección en las islas Cheradi (Italia meridional) en 2004
En la primavera de 2004 se llevó a cabo una misión de recolección en las islas Cheradi (Italia meridional) en el marco de un ciclo de exploraciones tendientes a salvaguardar los recursos genéticos de cultivos autóctonos que aún se encuentran en las islas menores de Italia. La vegetación autóctona de Cheradi está hoy fuertemente reducida a causa de los extensos campos que fueron cultivados en el pasado reciente y están abandonados en la actualidad. En esta primer expedición preliminar se encontraron 34 taxa que se clasificaron como pertenecientes a 16 familias botánicas (por ejemplo *Vicia ervilia, Beta vulgaris* subsp. *maritima, Daucus carota* silvestre). También se muestrearon y recogieron cinco poblaciones de *Cynara cardunculus* var. *Sylvestris*. En la actualidad algunas zonas pequeñas de las islas Cheradi son aún lugares muy interesantes del punto de vista botánico, bien protegidos de la antropización y que merecerían un estudio más profundo.

Introduction
At the end of spring 2004, a collecting team from the Institute of Plant Genetics (IGV), Bari (Italy) carried out a preliminary collecting expedition to the Cheradi islands (Italy). Apart from some botanical explorations for wild flora (Manni 1793; Novembre 1965–67; Medagli 1999), no expedition to the Cheradi islands for collecting crop genetic resources had been reported before. The research was planned within the special programme, "Safeguarding crop genetic resources of Italian small islands" begun in 1994 by IGV and the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) of Gatersleben (Germany) and then continued by Kassel University, Germany (Laghetti 1997).

The Cheradi islands are located in the inner part of the Gulf of Taranto ringing round the 'Mar Grande' (Figure 1). This archipelago of southern Italy has two main islands—San Pietro, the largest island, is 117 ha and San Paolo is 5 ha—and originated through several phases starting from the Pleistocene (Mastronuzzi et al. 1999). The climate is very mild with an annual average temperature of about 17°C. The Cheradi islands fall within the Eumediterranean rainfall typology.

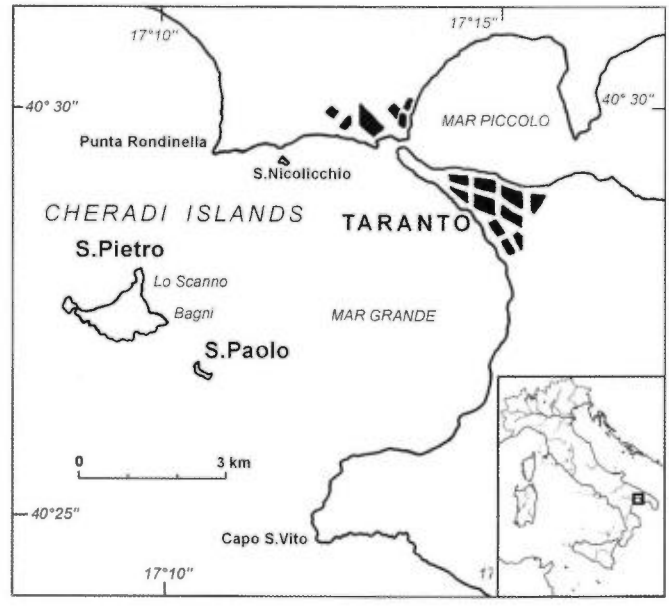


Figure 1. Geographical position of the Cheradi islands, Italy (drawing by Mr S. Cifarelli).

At present the Cheradi islands are under the control of the Ministry of the Navy and landing is allowed only to Navy personnel. San Paolo is uninhabited (Figure 2) while San Pietro is used only by the military for service reasons.

The main aim of the first exploration was to identify local crop genetic resources, to be collected afterwards, and to gather information on their degree of genetic erosion and ethnobotanical data.

Background

The evolutionary progress of wild flora on the Cheradi islands, together with its latest census, have been well described by Medagli (1999). Today, autochthonous vegetation of the Cheradi islands (i.e. maquis, garigue and spontaneous wood) is strongly reduced because of the large cultivated fields of the recent past which, at present, are completely abandoned.

Novembre (1965–67) reports that there were already some cultivated areas during the Roman age but only with colonization by Basiliani monks was the land extensively tilled and the natural wood was removed for cultivating wheat and olive groves. After these people went away, the Cheradi islands remained uninhabited and natural vegetation grew again. At the beginning of the 18th century, the 'Masseria del Capitolo' (the Capitolo's farm) was built and large areas were tilled for sowing, but at the end of that century, the islands were again unpopulated and rich in woods. In the 19th century, a quarter of San Pietro's surface was covered by wood but, thanks to two new farms, agriculture was again practised. In 1842, the 'Masseria del Capitolo' was rented to a farmer who cultivated crops such as oat, durum wheat, bread wheat ('maiorica' variety), 'grano francese' ('French wheat') (*Triticum* sp.), barley, cotton (called 'bambaggia'), broad beans, hay of 'dolica' (*Lathyrus clymenum*), fig trees and Indian figs (Mastronuzzi and Marzo 1999). In the 20th century, for military purposes, crop growing was drastically reduced and a pine forest was planted to hide the local military works (Figure 3). In 1960, there was an attempt to cultivate tobacco but it was a failure because of the bad quality of products (Medagli 1999). It is also well known that on the Cheradi islands, vegetable crops do not grow well because of water shortage and hardness. Additional notes about agricultural activities on the Cheradi islands in the past have been reported by Carducci (1771), Tanzi (1902) and Blandamura (1925).

Results

During the mission, a census of crop genetic resources growing on the islands of San Paolo and San Pietro was carried out (Appendix 1 [online]) with the intention of collecting them later, during the right maturity time. In addition to the 34 taxa observed, some other plants were present on San Pietro island according to Manni (1793), but were not found either by Medagli (1999) or by us, such as *Rumex acutus* (used to treat scabies), *Rubia tinctorium* (used as vegetable dye) and *Hyacinthus comosus* (bulbs greatly appreciated gastronomically). However, five populations of *Cynara cardunculus* var. *sylvestris* were sampled and collected (Figure 4). This specific collecting activity on wild artichoke is included in a research project by IGV considering several Mediterranean areas (Pignone and Sonnante 2004).

Figure 2. Wild vegetation on the uninhabited San Paolo island.

Figure 3. The introduced pine forest (*Pinus halepensis*) on San Pietro island.

Figure 4. Population of *Cynara cardunculus* var. *sylvestris*, the probable wild progenitor of the cultivated artichoke (San Pietro island).

Conclusion

In conclusion, some small areas on the Cheradi islands still currently represent, botanically, very interesting places, well protected from human intervention (apart from the military presence during the last century) that would merit more in-depth study.

Acknowledgements

Our warmest thanks to Capitano di Corvetta Adriano Strazzeri (Commandant of the Cheradi islands) and to 1° Maresciallo LT Antonio Miale of Marina Militare in Taranto for courtesy, assistance and support offered during our exploration on the Cheradi islands. Particular thanks go to the Fondazione Marittima Ammiraglio Michelagnoli in Taranto that kindly provided free books and valuable information during the collecting expedition. Special acknowledgements also go to our colleague Dr Antonino De Lisi (IGV) for his worthy help during the planning of the mission.

References

Blandamura G. 1925. Choerades Insulae. Le Cheradi dello Jonio. Tip. Arcivescovile, Taranto, Italy.

Carducci GA. 1771. Della Delicie Trentine di Tommaso Nicolò d'Aquino. Stamperia Raimondana, Naples, Italy.

Hammer K, Cifarelli S, Perrino P, Laghetti G. 2004. Dynamics of *Rubus ulmifolius* Schott var. *anaplothyrsus* Sudre and other cultivated blackberries in Italy. Genetic Resources and Crop Evolution 51:237–239.

Laghetti G. 1997. Recent meetings on biodiversity in Italy. (Country news: Italy). In: Heywood VH, editor. Medusa Newsletter, No. 1. Reading, UK, p. 15.

Manni P.A. 1793. Ricerche botaniche nell'isola di Santa Pelagia, Taranto. In: von Salis-Marschlins CU, editor. Reisen in verschieden Provinzen des Konigreichs Neapel. Zurich, 1793 (Republished In: Nel Regno di Napoli, viaggi attraverso varie provincie nel 1789, 94–95, Vecchi V. (ed.), Trani (BA), Italy).

Mastronuzzi G, Marzo P, curators. 1999. Le isole Chéradi fra natura leggenda e storia. Fondazione Marittima Ammiraglio Michelagnoli, Taranto, Italy, pp. 125–128.

Mastronuzzi G, Palmentola G, Sansò P. 1999. La storia geologica. In: Mastronuzzi G, Marzo P, curators. Le isole Chéradi fra natura leggenda e storia. Fondazione Marittima Ammiraglio Michelagnoli, Taranto, Italy, pp. 31–41.

Medagli P. 1999. Aspetti della flora e della vegetazione. In: Mastronuzzi G, Marzo P, curators. Le isole Chéradi fra natura leggenda e storia. Fondazione Marittima Ammiraglio Michelagnoli, Taranto, Italy, pp. 181–189.

Novembre D. 1965–67. Le isole Cheradi. Annals of Florence University, Facoltà di Lettere Filosofia e Magistero 3:423–448.

Pignone D, Sonnante G. 2004. Wild artichokes of South Italy: did the story begin here? Genetic Resources and Crop Evolution 51:577–580.

Tanzi GF. 1902. L'Archivio di Stato di Lecce. Note e documenti. Stabilimento Tipografico, Giurdignano (Le), Italy.

Appendix 1. Plant genetic resources growing on the Cheradi islands is available online from http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Collecting crop genetic resources in the Mediterranean agricultural islands: Corsica (Part I—northern Corsica)

S. Bullitta,[1]✉ S. Cifarelli,[2] Th. Gladis,[3] K. Hammer[3] and G. Laghetti[2]
[1]CNR-ISPAAM Sez. Sassari, Via Enrico de Nicola, I-07100 Sassari, Italy. Email: bullitta@cspm.ss.cnr.it
[2]Istituto di Genetica Vegetale-CNR, I-70126 Bari, Italy
[3]Kassel University, Dept. Agrobiodiversity, Steinstr. 19, D-37213 Witzenhausen, Germany

Summary

Collecting crop genetic resources in the Mediterranean agricultural islands: Corsica (Part I—northern Corsica)

The collecting mission on Corsica is part of a new exploration cycle opened in 2002 with a mission to the Maltese archipelago. The main aim is the safeguard of autochthonous crop genetic resources still present on Mediterranean islands. This new exploratory cycle follows the eight-year research accomplished for the collection of crop genetic resources from the small Italian islands. Corsica, with a surface area of 8722 km^2, is the fourth largest Mediterranean island. Because of its prevalent mountain areas with a high number of small villages not readily accessible, a first exploratory mission was started in the northern part of the island in September 2003, with the southern part being visited in 2004. In our first expedition, 112 valuable landraces were collected, which belong to several species of pulses, vegetables, cereals, forages, spices and condiments. The progressive genetic erosion of crop genetic resources was clearly evident from the abandoned orchards found around all the villages that were visited. Because of the genetic erosion risks, the building of a local genebank might help to keep alive the local agricultural traditions and resources.

Key words: agricultural biodiversity, collection, crop genetic erosion, Mediterranean islands, northern Corsica

Résumé

Collecte de ressources génétiques de plantes cultivées dans les îles méditerranéennes abritant une activité agricole : Corse (Partie I—Corse du Nord)

La mission de collecte en Corse s'inscrit dans un nouveau cycle d'exploration initié en 2002 par une mission dans l'archipel de Malte. L'objectif principal est de sauvegarder les ressources génétiques de plantes cultivées autochtones encore existantes dans les îles méditerranéennes. Ce nouveau cycle d'exploration fait suite à un programme de recherche de huit ans qui avait pour but de collecter les ressources génétiques de plantes cultivées dans les petites îles italiennes. La Corse, avec une superficie de 8 722 km^2, est la quatrième île méditerranéenne par sa taille. Les zones montagneuses étant prédominantes, avec un grand nombre de petits villages difficilement accessibles, une première mission exploratoire a débuté dans le Nord de l'île en septembre 2003 tandis que le Sud a été exploré en 2004. Lors de notre première expédition, 112 variétés régionales ont été collectées, appartenant à plusieurs espèces de légumineuses, légumes, céréales, espèces fourragères, épices et condiments. L'érosion génétique progressive des ressources génétiques de plantes cultivées est clairement attestée par les vergers abandonnés autour de tous les villages que nous avons visités. En raison des risques d'érosion génétique, la constitution d'une banque de gènes locale pourrait certainement contribuer à perpétuer les traditions agricoles et les ressources locales.

Resumen

Recolección de recursos genéticos de cultivos en las islas agrícolas del Mediterráneo: Córcega (Parte I - Córcega septentrional)

La misión de recolección en Córcega forma parte de un nuevo ciclo de exploración abierto en 2002 con una misión al archipiélago maltés. El propósito principal es salvaguardar los recursos genéticos de cultivos autóctonos aún presentes en las islas del Mediterráneo. Este nuevo ciclo exporatorio sigue a los ocho años de investigación llevados a cabo para recoger recursos genéticos de cultivos en las pequeñas islas de Italia. Córcega, con una superficie de 8.722 km^2 es la cuarta isla en tamaño del Mediterráneo. Debido a sus zonas en prevalencia montañosas con gran número de pequeños pueblos de no fácil acceso, en septiembre de 2003 se inició una primera misión exploratoria en la zona septentrional de la isla, mientras que la zona sur se visitó en 2004. En nuestra primera expedición se recogieron 112 valiosas razas locales pertenecientes a numerosas especies de legumbres y hortalizas, cereales, forrajes, especias y condimentos. La progresiva erosión genética de los recursos genéticos de los cultivos quedaba perfectamente evidenciada en las huertas abandonadas en torno a todas las aldeas visitadas. Debido a los riesgos de erosión genética, el establecimiento de un banco local de genes podría ciertamente ayudar a mantener vivos los recursos genéticos y las tradiciones agrícolas locales.

Introduction

A collecting mission was carried out in northern Corsica in September 2003. This mission is the second of a new exploratory cycle started in 2002 in the Maltese archipelago (Laghetti et al. 2004), after the accomplishment of a previous exploration in the small Italian islands, which started in 1994 and ended in 2002 (Laghetti et al. 1996, 1998, 1999a,b, 2001, 2002, 2003a,b). The team came from CNR-IGV Istituto di Genetica Vegetale (former Germplasm Institute of Bari, Italy), University of Kassel (Germany) and CNR-ISPAAM Section of Sassari (former Centro Pascoli Mediterranei, Sardinia, Italy).

The results of all the previous collecting missions confirm the authors' idea that small islands represent the last refuge for landraces and are also richer in crop genetic resources than other mainland areas (Hammer et al. 2001; Laghetti et al. 2003a). So, as in the previous missions, our aim was to safeguard the autochthonous crop genetic resources still present and to compare the results with those from the large neighbouring island of Sardinia (Gladis et al. 1994; Pignone et al. 1997; Laghetti et al. 1999b) or other Mediterranean islands using checklists of cultivated plants (e.g. Della 1999).

There are nearly 10 000 islands in the Mediterranean basin, of all sizes and origins, a result of a varied paleogeography; their wide range of sizes, altitudes, substrates and morphologies, as well as human activities, have led to the evolution of a highly diversified flora (Delanoe and de Montmollin 1996). Moreover, many of the cultivated crops originated in the Mediterranean area, which was rich in ancient local varieties, now mostly disappeared because of the progressive genetic erosion caused by the spread of cultivated crop species and varieties, increase of abandoned agricultural areas and strong urbanization.

Corsica island covers a surface area of 8722 km^2 and has a population of 255 000 inhabitants. It is located 14 km from the north Sardinian coast, 160 km from the French coast (cap Martin) and 82 km from the Italian coast (Tuscany) (Figure 1). The highest elevation is Monte Cinto (2710 m asl).

The climate is typically Mediterranean with hot summers (23°C minimum and 36°C maximum temperatures along the coast and from 8 to 26°C on the mountains) and cold winters (0°C minimum and 18°C maximum temperatures). The average annual rainfall is ca. 900 mm, which is unevenly distributed, mainly during the winter months.

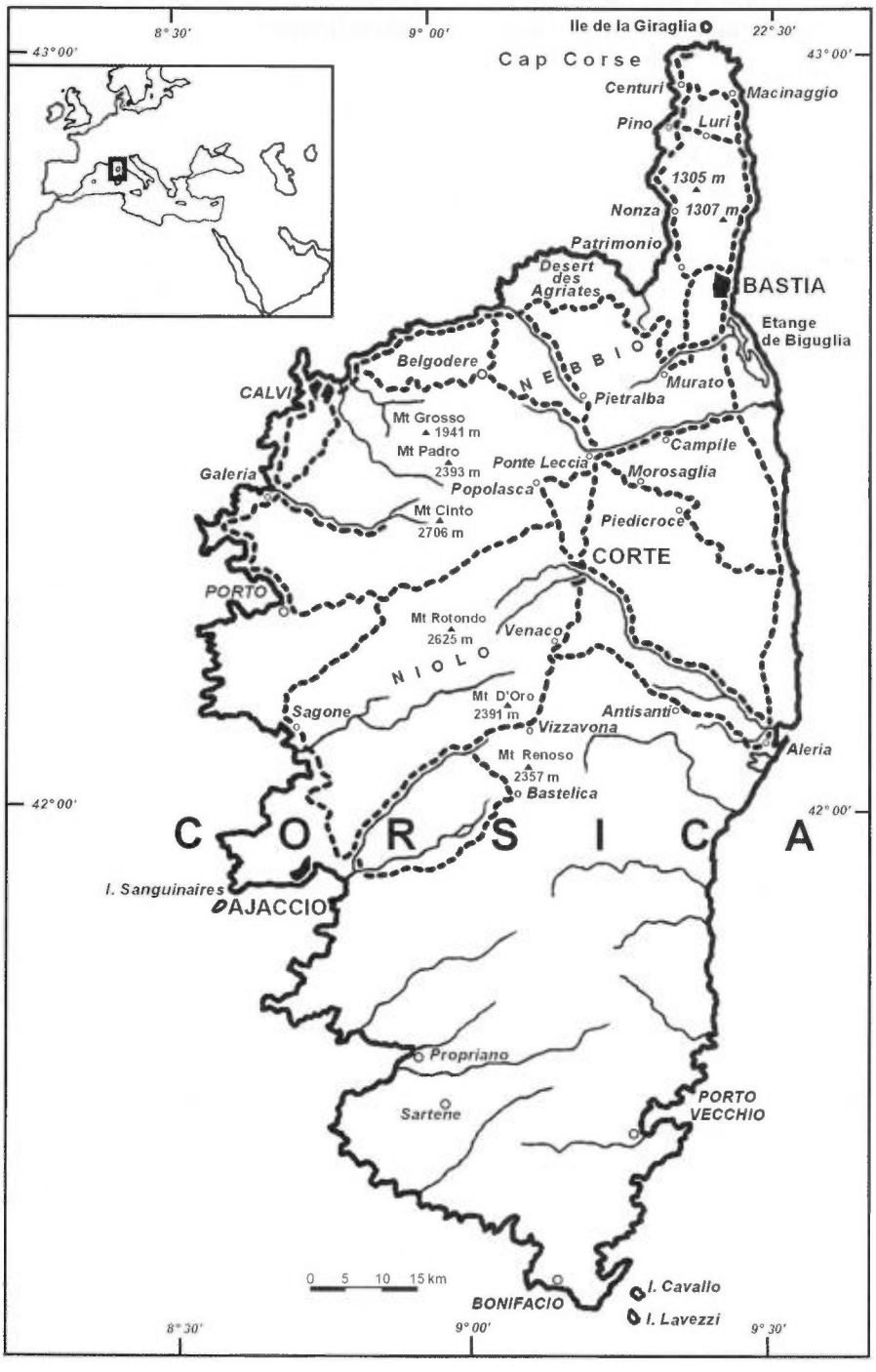


Figure 1. Geographical position of Corsica (France) and collection route (broken line) (drawing by S. Cifarelli).

The local vegetation can be divided into three groups, forest, scrub (mainly *Cistus* spp. formations and *Genista* at higher altitudes) and herbaceous communities; the local flora includes ca. 2380 indigenous plants species and sub-species and 489 xenophytes, of which 174 have naturalized, 190 are sub-spontaneous and 125 are considered to be adventives (EPIDEMIE research project; see Jeanmonod 1987). About 75% of the island is covered by forests or maquis; only 15% is cultivated land. The evergreen and impenetrable undergrowth of maquis characterizes the landscape, covering more than half of the island's surface. The aromatic scent of its flowers and herbs is well known (http://www.terracorsa.info/flora.html). Corsica has 131 endemic plant species and a further 75 corso-sardinian species. This is considered to be a high proportion in relation to the relatively small surface of the island. In the alpine zones, nearly half the species are endemic (http://www.corsica-isula.com/mountains.htm#%20%20Flora%20&%20Fauna [Appendix 1{online}]). The 'Projet Flore de Corse' coordinated by the Botanical Garden of Genève studies the wild flora and vegetation in detail (http://www.cjb.unige.ch/fdc/fdc1.html). About 351 000 ha of land are part of the Regional Park, created in 1971 and including 138 municipal communities (Parc Naturel Régional de la Corse, 2003; http://www.parc-naturel-corse.com). There are also six natural reserves and protected littoral areas. Coasts cover 1047 km with about 300 km of beaches.

Tourism is the most important of the economic activities, with almost 2 million tourists visiting the island each year (Ravis-Giorgione 1991).

Figure 2. Interviewing a Corsican farmer working in his traditional kitchen garden in the countryside of Campu. These kitchen gardens are rich in landraces.

Figure 3. Mrs. Sammarcelli showing the typical variety of *Lagenaria siceraria* traditionally used as a container for drinks or, when cut in half, as a dish.

Agricultural activities are based on extensive animal breeding, characterized by moving to summer pastures in the central areas, olive and chestnut trees and, especially in the eastern plain, vegetables, fruit tree plantations and vineyards for wine making.

The wild and endemic plants of the Regional Park of Corsica have been well studied (Gamisans and Marzocchi 1996), but there have been few attempts to study and find out vernacular names of utilized plants (except e.g. Pellet and Pellet 2002) or to check the regional flora of cultivated plants.

Collecting approach

The main sources of the collected materials were farmers or home gardens kept by elderly people (Figure 2). Some samples were collected from abandoned orchards. Passport data were recorded at each collection site, using data from a hand-held GPS system. Additional information on Corsican agriculture was obtained by interviewing local specialists from the ICALPE in Corte, the Regional Park of Corsica in Ajaccio, the Association Jardins de Cap Corse in Santa Severa-Luri and local growers. Further details on the sampling methods and exploration strategies are reported by Hammer et al. (1997, 1999) and Laghetti et al. (1996).

Results and discussion

Today's cultivated crops on the island are olives, grapes, citrus and wheat. Timber and cork production are also important. Chestnut, fig and other fruit production are of local importance. Animal husbandry (mainly sheep rearing), the utilization of the regional, semi-domesticated pig landrace and production of local cheese are other significant agricultural specialities of this island.

Almost all collection sites were located in small orchards close to the tiny villages peculiar to the distribution of Corsica's inhabitants. Some orchards were abandoned, some others were run by elderly people (Figure 3). From the terraced lands and the remains of cultivated fruit crops invaded by weeds, the decrease occurring in the agricultural activities during the last decades was clearly evident. This assumption was confirmed by all of the villagers interviewed. A remarkable initiative regarding Cap Corse traditional crops conservation was that of Madame Seguy running 'Les Jardins Traditionnels du Cap Corse' in Santa Severa-Luri where she multiplied local vegetable landraces (onions, beans, peas, broad beans, eggplants, peppers) and old fruit varieties (mainly pears, apples, figs, peaches, cherries and plums).

In total, 112 accessions were collected, belonging to 42 genera. The Leguminosae family was the most represented with seven different genera and seven species plus another five species to be determined; it was also the richest with respect to the 22 accessions collected. Regarding the uses of the accessions, most of them were vegetables, followed by pulses, cereals, spices and condiments, forages and ornamentals. A detailed list of the collected material is reported in Appendix 2 (online).

Cereals

It was very difficult to find cereal landraces, particularly wheat. In a broad valley close to Belgodere it was possible to find a sample of *Triticum aestivum* L. em. Fiori et Paol. mixed with a few seeds of *Hordeum* sp. and *Avena* sp. (the latter had seeds varying in colour). Another accession of *T. aestivum* (local name 'blè barbue') was provided by Mrs Seguy from 'Les Jardins Traditionnels de Cap Corse' in Santa Severa-Luri. In addition, one accession of *T. aestivum* was provided by a local teacher of Pietralba, who multiplied and stored the landrace for over 30 years. Local wheat landraces were cultivated in terraces until the Second World War, but then they disappeared (Figure 4). Until the 1970s, larger areas of cultivated old landraces of wheat could be found in the plains (pers. com. Ms M. Sammarcelli).

One accession of *A. sativa* L. was found in Lu Pinu; another six *Avena* spp., still to be classified, were collected. Some of them belong to wild species.

Two maize landraces (*Zea mays* L.) were found; both present a high variability, with irregular shapes and various colours of grain even within the same cob.

Pulses

Eleven accessions of common beans (*Phaseolus vulgaris* L.) were collected. Among the two faba bean (*Vicia faba* L.) accessions, one was a mixture of *V. faba* var. *equina* Pers. and var. *faba*. One accession of *Pisum sativum* L. s.l. was acquired by a farmer; because of its variability in seed size, colour and shape, it is probably a mixture of three different types. A black seeded accession of *Vigna unguiculata* (L.) Walp. was also collected.

Around the ruins of the Seneca tower close to Luri, at an elevation of 600 m asl, a small sample of *Pisum elatius* Steven ex M. Bieb. was found. One interesting accession of *Lupinus albus* L. was found growing both as a cultivated plant and as a weed in the surroundings of Calvi.

Vegetables

Four accessions of *Cynara scolymus* L. were collected, while only one accession of the perennial wild artichoke (*C. cardunculus* L. var. *sylvestris* Lam.) was collected (Figure 5). Specific collecting activities on wild artichoke are included in a research project by IGV considering several Italian (e.g. 'Monti della Tolfa' in Latium, Apulia, Calabria, Basilicata regions) and Mediterranean areas (Pignone and Sonnante 2004).

Three samples of citron melon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai var. *citroides* (Bailey) Mansf.) (named by locals as 'Pateca' or 'Pateca a confitura') were collected. The fruit of this variety is not eaten by local people, but only used for making jams (Figure 6). The flesh and rind are used for making preserves and pickles. The citron melon is often referred to as stock melon or preserving melon; its raw fruits are inedible. They resemble icebox watermelons because they are round to oval, up to 15cm long, white or light green with darker green stripes and have a smooth surface. The flesh is white and so tough that the fruits can be bounced on the floor with only a small chance of bursting. The smell is comparable to that of *C. lanatus* var. *caffer* (Schrad.) Mansf., but the seeds are not as often marbled as those of the common watermelon. The low, vining plants have both male and female flowers and require bees for pollination. Because of the close relationship to watermelon, cross-pollination between the two may occur if grown side by side. Where desired, they should be grown isolated from each other and in a manner similar to watermelons and cantaloupes. The citron melon will be the object of a separate paper with further information.

Only one accession of *Lagenaria siceraria* (Molina) Standl. was acquired. In former times, the fruits of this species were used as containers for drinks or, cut into half, they were used as dishes (Figure 3). The 'Corsian plate' is a very old and famous traditional European variety of this species. Sweet varieties, used as a vegetable, are reported from Asia only

Figure 4. A true wheat landrace relic from the Pietralba countryside (*Triticum aestivum* L. convar. *semirigidum* A. Filat. et Dorof. subconvar. *semirigidum* A. Filat. et Dorof. var. *variabile* [Kudr.] A. Filat. and the closely related var. *graecum* [Koern.] Mansf.), with bread made from the flour of this landrace. This landrace was widely cultivated on the terraced farms of Corsica prior to the Second World War; today it is almost extinct.

Figure 5. The head of a wild artichoke (*Cynara cardunculus* var. *sylvestris*), from a rare population growing near to Aleria.

(http://www.genres.de/infos/kalebasse). Further collected material from the Cucurbitaceae family are one accession of *Cucurbita pepo* L. (small sized and arc shaped), one of *C. maxima* Duch., two of *C. moschata* Duch. and one of *Cucumis sativus* L. (small sized and irregularly shaped).

Six samples of tomato (*Lycopersicon esculentum* Mill.) were found: two had very large fruit, where a single fruit could reach 1 kg in weight, with the shape irregular and ribbed; fruit of the other four were round and small. Two local landraces of egg plant (*Solanum melongena* L.) were acquired, both of small size. One was dark violet and had the peculiarity of being yellow coloured only in the upper part, whilst the other one was completely white.

The two accessions of wild lettuce (*Lactuca* sp.) found are probably introgressions from cultivated plants.

Five accessions of *Allium cepa* L. were found, all different in size, from small to big, and in colour, from white to violet and brown; one was an old landrace called 'Cipolla Corsa' and was very large sized (Figure 7). Two samples each of wild garlic (*A. ampeloprasum* L. s.l.) and leek (*A. porrum* L.) were collected. Two other *Allium* spp. have yet to be identified. One was characterized by aerial bulbils instead of flowers and seeds (Figure 8). Wild garlic is often locally used for cooking soups and other typical dishes. This species tends to become a weed but is tolerated as a useful wild plant in gardens and fields.

Two accessions of *Beta vulgaris* L. were collected, both belong to semicultivated types.

Seeds of two accessions of *Brassica oleracea* L. were found, one of which was an old, tall-growing landrace called 'Cavolo di Portogallo', used as a leaf vegetable.

Spices, condiments and officinals

In the area of Cap Corse, information was acquired about the use of officinal plants, spices and condiments. *Borago officinalis* L. (local names: 'Frisgia', 'Frisgiula', 'Burrascia', 'Burrarca', 'Burrace' and 'Burracine') is an important spice, used for tisanes.

Seeds of *Foeniculum vulgare* Mill. were acquired. This plant is used by locals not only as a condiment in dish preparations but especially for preparing the liqueur 'Pastis'. Seeds of

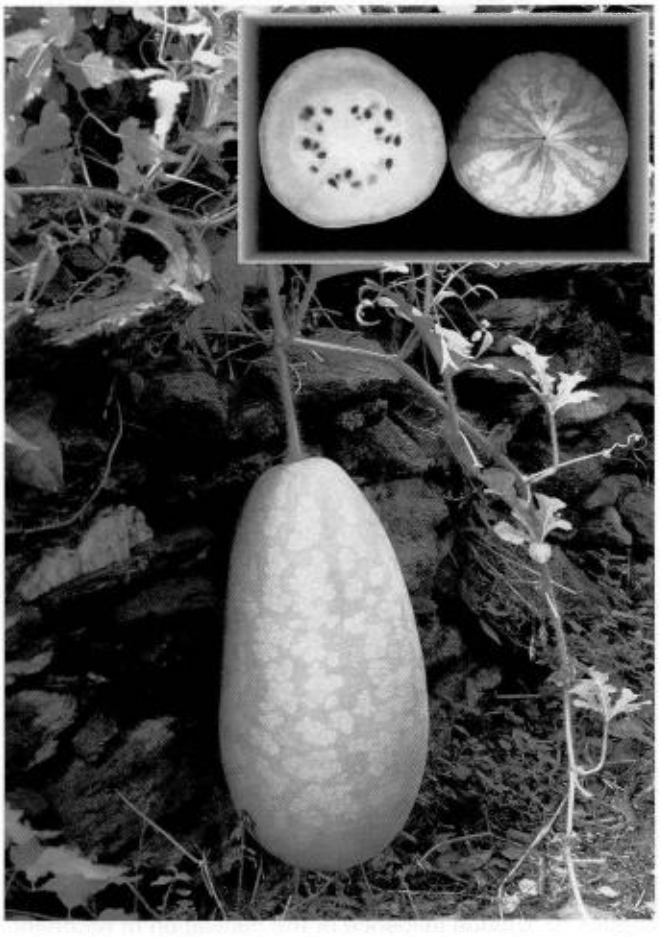


Figure 6. The fruit of the typical Corsican citron melon locally known as, 'Pateca a confitura'.

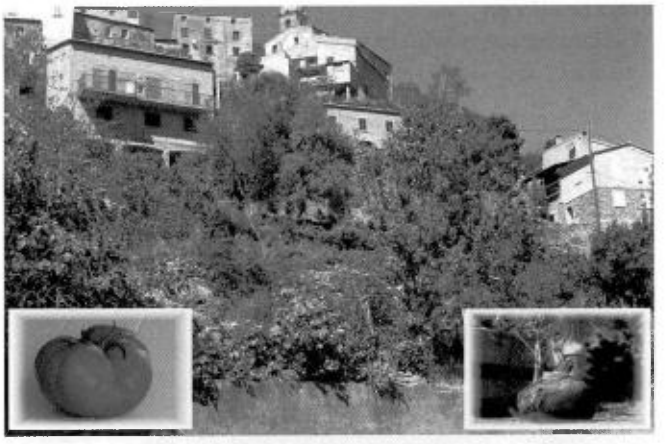


Figure 7. A small garden where traditional Corsican crops are still painstakingly cultivated. The inserts show the typical onion variety 'Cipolla Corsa' and a local variety of tomato from Pratu di Ghjuvellina.

Figure 8. Close-up of a wild garlic characterized by aerial bulbils.

Petroselinum crispum (Mill.) Nym. ex A.W. Hill, used as a condiment, were gathered. An accession of *Coriandrum sativum* L. was found, probably recently introduced by immigrants (Figure 9).

Calamintha nepeta (L.) Savi (local name: 'Nepita') is used as a flavour for tomato sauce and as a condiment for cooking fish. Two accessions of *Ocimum basilicum* L. were collected, with both having deep green and large leaves and a very strong smell. One accession of *Origanum vulgare* L. (local names: 'Carnepulia', 'Carepuglia', 'Cunella', 'Persa', 'Pimpinella' and 'Piverella') was found in the area of Campitello. This population had reddish leaves. One accession of *Salvia officinalis* L. was found. It was an old type with small leaves.

One accession of *Chrysanthemum parthenium* (L.) Bernh. was collected in a small orchard. It was probably used as an officinal plant in the past but now is only used as an ornamental.

Wild relatives

Vegetative parts of wild or weedy growing *Apium* sp., *Mentha aquatica* L. and *Melissa officinalis* L. were sampled to be grown and better classified in the laboratory. Additionally, three accessions of wild *Cichorium intybus* L. were collected, one of which grew very tall.

Other crops

Although no samples were taken, avocado trees (*Persea americana* Mill.) and kakee plum (*Diospyros kaki* L.f.) were found, not in big stands but just as small groups or isolated plants. What is remarkable is the cultivation of fruit-bearing date palms (*Phoenix dactylifera* L.), but these are merely used as ornamentals (see Ravis-Giorgione 1991, p. 246).

A sample of *Gomphocarpus fruticosus* (L.) Ait. f. was collected in a field near Belgodere. In the past, it was cultivated as a surrogate for cotton and now it has spread into the uncultivated fields of the area and has become an invasive species occupying devastated areas and parched river beds.

Seed samples were taken from *Canna edulis* Ker-Gawler at Pruneto, close to the coast. It was only used as an ornamental, but in the area of origin the roots of the plant are eaten. The giant tree dahlia (*D. imperialis* Roezl ex Ortgie), originating from Central America, was another interesting find. One old

Figure 9. Dried *Coriandrum sativum* specimens, probably introduced by north African immigrants.

Figure 10. Unique evidence of the cultivation of *Nicotiana rustica* L. found in a home garden in Muracu during the mission.

plant was observed surviving in the Mediterranean climate for several years.

Other remarkable samples were *Isatis tinctoria* (at Murati), *Lavatera arborea* L., *Opuntia ficus-indica* (L.) Mill. (with completely circular and extremely spiny platyclades and red fleshed fruit) and *Nicotiana rustica* L. The Indian tobacco was cultivated in a home garden in remembrance of the smoking father of the owner (Figure 10). Further species collected during this mission will be reported after determination in a later report.

From well-known examples such as *Agave americana* L., *Opuntia* spp. etc., a new cultivated plant began to invade ruderal as well as native areas: *Anredera cordifolia* (Ten.) Steenis originates from southern America and is cultivated for its edible bulbs and foliage. Sometimes used as a clamberer and screen from view, the dioecious plant grows and expands very fast and can overgrow all other plants. At the moment, only one sex seems to be present in the region. A breeding population of this competitive species would be a disaster (see Gladis et al. 2001).

A botanical peculiarity are fruit, occurring e.g. in Cylindropuntias (*Opuntia* chain *imbricata*/Haworth/DC.). This species is a common garden plant and even tends to become invasive.

Conclusion

While threats facing the flora of the Mediterranean islands are undoubtedly known by the scientific community, less attention is paid to the specific activities related to the preservation of crop genetic resources. The Mediterranean Island Plant Specialists Group (MIPSG), officially formed at the beginning of 1995, is a group of specialists concentrated on the conservation of Mediterranean island flora. No other network exists specifically devoted to safeguard the crop genetic resources of Mediterranean islands. Efforts to assure conservation of crop genetic resources are surely justified, not only for cultural and scientific purposes but also because of the increasing interest in typical local products, considered to be a prospective economic activity related to the development of tourism in the islands. For this reason, the collecting missions we started in the Italian islands and the subsequent explorations of the other Mediterranean islands are a unique opportunity for monitoring what is still present and make the plant material available for future uses by means of storing the seeds in genebanks. Without such activities, genetic erosion, diminished cultivation of local varieties and increasing influence of invasive neophytic or hemerophytic species will mean that the precious patrimony of ancient varieties will soon be irremediably lost. The southern part of Corsica will be, hopefully, visited in 2004.

Acknowledgments

Monsieur Michel Dubost from ICALPE in Corte, Madame Nivaggioli from Parc Naturel Régional de la Corse in Ajaccio, Madame Seguy from 'Les Jardins Traditionnels de Cap Corse' in Luri, Madame Michèlle Sammarcelli in Pietralba and Monsieur and Madame Cesari from Antisanti are gratefully acknowledged for their valuable information about Corsican agriculture. Mr Mario Capula from Castelsardo is gratefully acknowledged as an interpreter for the Corsican language.

A special thanks to Mrs Lucia Stimolo, working in the Conservation Service of IGV, for her unremitting zeal in seed processing after the collecting phase. In addition, a special thanks to Prof A. Filatenko for his accurate classification of the wheat landrace from Pietralba.

References

Delanoe O, de Montmollin B. 1996. Conservation of Mediterranean island plants. 1. Strategy for Action. IUCN, Gland, Switzerland.

Della A. 1999. The Cyprus Flora in checklist format. Native or naturalized–cultivated–endemics–rarities–additions. IPGRI/Agricultural Research Institute, Ministry of Agriculture, Natural Resources and Environment Nicosia, Cyprus. J.G. Cassoulides & Son Ltd, Nicosia, 299pp.

Gamisans J, Marzocchi J-F. 1996. La Flore endemique de la Corse. EDISUD, Aix en Provence, France, 208pp.

Gladis Th, Hammer K, Laghetti G, Delogu GF, Perrino P. 1994. Report of a collecting mission in Sardinia, autumn 1993. Plant Genetic Resources Newsletter 89:18–20.

Gladis Th, Arrowsmith N, Hammer K. 2001. Hemerophyta—a special case of invasive organisms. Schriften zu Genetischen Ressourcen 16:23–29.

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, Poland, pp. 22–32.

Jeanmonod D, editor. 1987. Compléments au prodome de la flore corse. Éd. des Conservatoire et Jardin Botaniques de la ville de Genève (Projet Flore Corse).

Laghetti G, Hammer K, Perrino P. 1996. Plant genetic resources in Pantelleria and Pelagie archipelago, Italy: collecting and conservation of local crop germplasm. 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.

Laghetti G, Perrino P, Cifarelli S, Hammer K. 2003a. Collecting landraces and wild relatives in Tremiti islands, Italy. Plant Genetic Resources Newsletter 133:1–7.

Laghetti G, Perrino P, Cifarelli S, Bullitta S, Hammer K. 2003b. Collecting of plant genetic resources in Italy, 2001. Plant Genetic Resources Newsletter 136:1–6.

Laghetti G, Scicluna-Spiteri A, Attard E, Perrino P, Cifarelli S, Hammer K. 2004. Collecting crop genetic resources in the Mediterranean agricultural islands: Maltese archipelago. Plant Genetic Resources Newsletter 139:11–16

Parc Naturel Régional de la Corse, editor. 2003. Arburi, Arbe, Arbigliule—Savoirs populaires sur les plantes de Corse.

Pellet H, Pellet S. 2002. Arbe, Fiori, Funghi—Plantes Savoureuses de Corse, Albiana.

Pignone D, Sonnante G. 2004. Wild artichokes of South Italy: did the story begin here? Genetic Resources and Crop Evolution 51:577–580.

Pignone D, Hammer K, Gladis Th, Perrino P. 1997. Collecting in southern Sardinia (Italy), 1995. FAO/IPGRI PGR Newsletter 109:7–10.

Ravis-Giorgione G. 1991. Le guide de la Corse: Ajaccio, Bastia, Calvi, Bonifacio, Corte, l'Ile-Rousse, Sartène, Porto-Vecchio, le parc naturel régional. La Manufacture, Besançon, France, 384 pp.

Internet site references

Terra Corsa, I Muvrini and Corsica, Corsican Flora. 2005 October 30 last update. Available from: http://www.terracorsa.info/index.html/. Date accessed: 2 November 2005.

Corsica-discover the Mediterranean island from the inside! 2005 October 3 last update. Available from: http://www.corsica-isula.com . Date accessed: 2 November 2005.

Conservatoire et Jardin botaniques del la Ville de Genève. Le project Flore de Corse. 2004 July 21 last update. Available from: www.ville-ge.ch/cjb/. Date accessed: 2 November 2005.

Parc naturel régional de Corse. 2004 September 14 last updated. Available from: http://www.parc-naturel-corse.com/presentation/. Date accessed: 2 November 2005.

Gemüse des Jahres 2002: Der Flaschenkürbis, homepage des VEN, Verein zur Erhaltung der Nutzpflanzenvielfalt e. V. 2002 May 14 last update. Available from: http://www.nutzpflanzenvielfalt.de/. Date accessed: 2 November 2005.

Appendix 1. Excerpts from http://www.corsica-isula.com/mountains.htm#%20%20Flora%20&%20Fauna is available online from http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Appendix 2. Number of accessions collected in northern Corsica is available online from http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Genetic resource survey of mandarin orange (*Citrus reticulata* Blanco) in the northeastern Himalayan region of India

A. Das,[1]✉ B. Mondal,[1] J. Sarkar[1] and S. Chaudhuri[2]

[1] Department of Plant Physiology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal – 741252, India. Email: anianiruddha@vsnl.net

[2] Department of Plant Pathology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal – 741252, India

Summary

Genetic resource survey of mandarin orange (*Citrus reticulata* Blanco) in the northeastern Himalayan region of India

A genetic resource survey for the mandarin orange plant (*Citrus reticulata* Blanco) was conducted in 18 locations covering 5 states of the northeastern Himalayan region of India. Fifty-four potential elite plants were selected for different characters such as plant type, fruit quality, yield and disease and pest tolerance from these locations. Significant variation in these characters was noticed not only among plants from different locations but also among plants from the same location. Random amplified polymorphic DNA (RAPD) profiling of the selected plants also showed this variation to the same extent and established that the variation has a strong genetic basis. Nucellar and zygotic progenies of the selected plant types have been developed through embryo culture and are being maintained in the nursery. This study concludes that it is necessary to explore more thoroughly the wide genetic diversity of the mandarin orange in the northeastern Himalayan region for the sake of plant improvement as well as conservation of plant genetic resources.

Key words: mandarin orange, diversity, resource survey

Résumé

Etude des ressources génétiques de la mandarine (*Citrus reticulata* Blanco) dans la région himalayenne du Nord-Est de l'Inde

Une étude des ressources génétiques de la mandarine (*Citrus reticulata* Blanco) a été réalisée en 18 localités dans 5 États de la région himalayenne du Nord-Est de l'Inde. Cinquante-quatre lignées élites potentielles pour différents caractères tels que le type de plante, la qualité du fruit, le rendement, et la résistance aux maladies et aux ravageurs ont été sélectionnées dans ces localités. Des variations significatives de ces caractères ont été observées non seulement entre plantes de différentes localités mais également entre plantes d'une même localité. Le profil RAPD (Random amplified polymorphic DNA ou amplification aléatoire de l'ADN polymorphe) des plantes sélectionnées présente également des variations du même degré et indique que la variation est essentiellement de nature génétique. Les descendances nucellaire et zygotique des types de plante sélectionnés ont été multipliées par culture d'embryons et conservées en pépinière. Cette étude démontre la nécessité d'étudier plus intensément l'importante diversité génétique de la mandarine dans la région himalayenne du Nord-Est en vue de l'amélioration végétale ainsi que de la conservation des ressources phytogénétiques.

Resumen

Examen del recurso genético de la naranja mandarina (*Citrus reticulata* Blanco) en la región del Himalaya nordoriental en la India

En 18 ubicaciones que cubren 5 estados de la región nordoriental del Himalaya en la India se llevó a cabo un examen del recurso genético de la planta de naranja mandarina (*Citrus reticulata* Blanco). En esas ubicaciones se seleccionaron cincuenta y cuatro plantas que forman una elite potencial por diferentes caracteres como tipo de planta, calidad del fruto, rendimiento y tolerancia a las plagas y enfermedades. Se notaron significativas variaciones en estos caracteres no solo entre las plantas de diferentes ubicaciones sino también entre las de una misma ubicación. El perfil del polimorfismo del ADN amplificado al azar (RAPD) de las plantas seleccionadas también mostraba esta variación en el mismo grado, dejando en claro que la misma es de base fuertemente genética. Mediante el cultivo de embriones se desarrollaron progenies nucelares y cigóticas de plantas de los tipos seleccionados, que se conservan en vivero. Este estudio determinó que es necesario explorar con mayor amplitud la gran diversidad genética de la naranja mandarina en la región nordoriental del Himalaya para lograr el fitomejoramiento y la conservación de los recursos fitogenéticos.

Introduction

The loose-skinned mandarin orange (*Citrus reticulata* Blanco) is one of the most economically important and popular fruits in the world, constituting about 41% of the total citrus fruits produced in India. The northeastern Himalayan region is one of the three major centres of mandarin orange cultivation in India. About 1600 ha, distributed over nine states—Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Tripura, Sikkim and the Darjeeling district of West Bengal (see Figure 1)—is under mandarin orange cultivation, where this high-value crop contributes substantially to the region's small farm economy (Ghosh and Singh 1993). The eastern Himalaya region, in general, is considered to be one of the primary gene centres of citrus worldwide, and large variability in the germplasm has been noted in the region. The northeastern Himalayan region is distinguished by its rich source of diversity of mandarin orange and may also be treated as a centre of origin for this crop (Ray and Deka 2000). A few ecotypes, including 'Darjeeling' mandarin and 'Khasi' mandarin, are excellent in quality and have good export potential.

In hilly terrain, the crop is cultivated on small farm terraces where the application of production technology is minimal. The crop suffers from low productivity, with widespread decline of the existing orchards mainly due to poor genetic quality, heterogeneity of planting material and poor management practices traditionally used in this

Figure 1. The northeastern Himalayan region of India. Mandarin orange growing area marked by thick dark border.

region (Ghosh and Singh 1993). The crop is generally cross-pollinated in nature; consequently wide variations in the plant types within and among different agro-ecological locations have become apparent over the centuries. Proper identification of cultivars or biotypes, and their maintenance as a genetic resource for future utilization, is a primary task for the development of mandarin orange cultivation in this region (Ray and Deka 2000). With this in mind, a peer and participatory survey in different locations within this region was performed in the search for phenotypically elite clones of mandarin orange, and the results are presented in this paper. Molecular identification of the plant types and the development of *in vitro* true-to-type progeny and their maintenance as a plant genetic resource followed the survey work.

Materials and methods

The survey was carried out during the fruit ripening and harvesting season of mandarin orange from September to December of the years 2001 and 2002. The survey area covered mandarin orange growing areas of five northeastern hilly states of India, namely West Bengal, Sikkim, Assam, Meghalaya and Arunachal Pradesh. Based on local information, 18 traditional locations for concentrated orange cultivation were selected and visited. After detailed discussions with the farmers, nurserymen and growers of the traditional orchards at each location, and based on their experience, choice and observation, elite plants in respect of different characters were selected. Field observations on the selected plants were recorded and a fruit sample from each plant was collected. Different physical and chemical parameters of fruit quality were measured in the laboratory. The parameters for study of plant type and fruit quality were chosen following the International Board for Plant Genetic Resources (IBPGR 1988) guidelines.

A set of nucellar and zygotic progeny plants of all the selected plants was developed and these progeny plants are being maintained in the nursery using a simple *in vitro* embryo culture technique for citrus, standardized in this laboratory (Das et al. 2000). DNA-fingerprinting of the selected elite plants of different locations using RAPD markers (universal operon primers) was performed following Coletta Filho et al. (1998) in order to estimate the actual genetic variation existing among the selected elite phenotypes of mandarin orange.

Results and discussion

Fifty-four presumptive elite plants of mandarin orange from 18 locations covering 5 states of the northeastern Himalayan region of India were selected, as summarized in Table 1. Phenotypically elite plants of mandarin orange were selected on the basis of different characters such as yield, plant type (see Figure 2), field resistance and pest and disease resistance, as shown in Table 2. Results of the survey indicated that there is much variation for these characters within and between the

Table 1. Area surveyed and plant diversity of mandarin orange plants (*Citrus reticulata* Blanco) collected

Area surveyed	Number of locations	Number of accessions
West Bengal, Darjeeling Hills (Kalimpong and Darjeeling Subdivision)	5	21
Sikkim, South Sikkim	2	2
Arunachal Pradesh, Logum Jini District	2	10
Assam, Upper and Lower Assam (Kamrup and Tinsukia District)	7	18
Meghalaya (West Khasi Hills)	2	3
Total	18	54

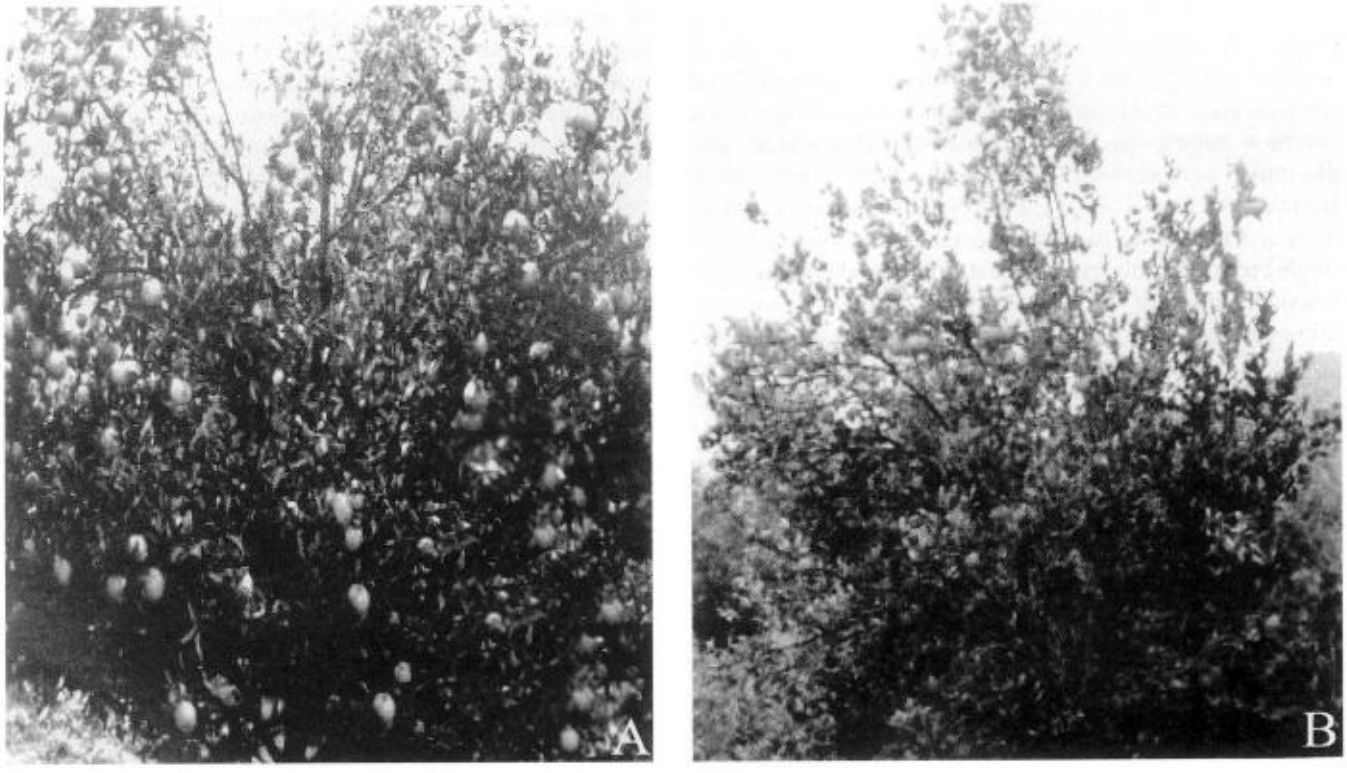


Figure 2. Open dome and erect closed type mandarin orange plants.

Table 2. Variation of characters observed within the selected mandarin orange plants

Character	Range of variation
Field characters	
Canopy type	Erect closed to open dome
Age of plant	12–75 years
Source	Mainly seed, very few are air-layered
Bearing habit	Regular, intermediate, alternate
Average number of fruits/plant	150–5000
Harvesting time	Early October–late December
Disease/pest tolerance and susceptibility	*Phytophthora* foot rot, *Xanthomonus*, *Tristeza*
Physical characters of fruits	
Weight with peel (g)	65–210
Size (horizontal circumference in cm)	15–24
Peel thickness (mm)	1.5–2.7
Number of locules/fruit	8–11
Number of seeds/fruit	3–23
Number of nucellar embryos/seed	1–5
Pulp weight (g)	9–27
Juice content (ml/fruit)	22–90
Chemical parameters of fruit	
TSS (Brix value)	9.03–11.53
Total sugar content (%)	4.46–11.01
Acidity (%)	0.3–0.9
Ascorbic acid content (mg/100 ml)	8.10–63.46

locations, both for seed and air-layered plants of mandarin orange. The observed variability was not exclusively related to soil or management factors alone. Results of physical and chemical analysis of the fruit samples also indicated significant variation among the plants cultivated within similar and dissimilar agro-ecological situations of the northeastern Himalayan region. There was evidence of significant variation in these fruit quality parameters even among the plants of a single orchard arising from a similar source and under similar management. Fruit characteristics such as low seed number, thin peel, high juice percentage and high sugar and ascorbic acid (vitamin C) content are important and may be utilized in order to satisfy the demand for quality fruit of mandarin orange in the international market (Ghosh and Singh 1993).

The number of nucellar embryos per seed developing as individual plants was also variable among the plant selections. Nucellar polyembryony of *Citrus* spp. is counted as an advantage in the propagation of citrus, as plantlets originating from the mother tissue are considered to be true to the parental type. Nucellar tissues from an immature ovule artificially cultured *in vitro* may yield several embryos and true-to-parental-type plantlets as compared with the usual process of seed germination in the soil (Button and Kochba 1993). Using the *in vitro* embryo culture technique developed in this laboratory (Das et al. 2000), at the early stage of seed germination nucellar and zygotic embryos are excised and cultured to generate multiple plantlets (Figure 3). A set of nucellar progeny of 54 selected plant types was developed and maintained in the nursery. Additional plantlets, generated in the same way from zygotic embryos, are also being maintained as they may provide the stock of a genetically diverse population of these selected elite types (Figure 4).

Random amplified polymorphic DNA (RAPD) analysis of the selected elite plants from different locations using

Figure 3. Development of multiple shoots from a single nucellar embryo of mandarin orange in standardized culture medium at 60 days after inoculation.

Figure 4. Development of nucellar and zygotic seedlings from the selected elite plants of mandarin orange.

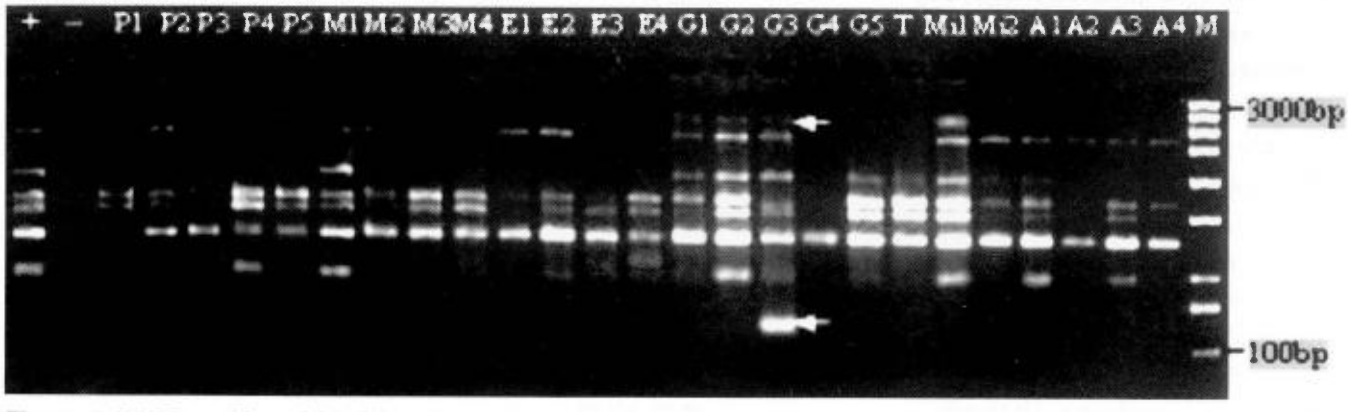


Figure 5. RAPD profiling of 25 different mandarin orange plants.

universal markers has shown wide genetic diversity among the plants, not only those from different locations but also among the plants from a single location (Das et al. 2004). A typical result of RAPD analysis using the universal primer OPAA02 of a subset of 25 elite plants from different locations is shown in Figure 5. The overall results of this analysis have not only established genetic variability among plants of a single orchard but also have shown that plants taken from agro-climatic situations as diverse and distinct as Arunachal Pradesh and the Darjeeling district of West Bengal can share genetic similarities.

Conclusion

The northeastern Himalayan region of India is a rich source of genetic diversity of the high-value fruit crop, mandarin orange (*Citrus reticulata* Blanco). The extent of variation in the plant types, fruit quality parameters and other characters is such that it not only exists among the plants from different locations but also among the plants from a single location. This observed variability is not exclusively due to environmental factors, but rather has a genetic basis that is clearly reflected in the results of the RAPD profiling of the selected plants from different locations. Considering the overall importance of this fruit crop and the urgent necessity for a plant improvement programme, elite stocks in respect of different characters need to be identified and properly maintained. A sample of 54 elite plant types has been selected, analyzed, genetically marked and their *in vitro*-developed progeny are being maintained. Further initiatives to explore the wide diversity of mandarin orange in the northeastern Himalayan region are desperately needed and should be supported in the interests of plant improvement as well as conservation of plant genetic resources.

References

Button J, Kochba J. 1993.Tissue culture in citrus industry. In: Reinert J, Bajaj YPS, editors. Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Springer, Berlin, Germany. pp. 71–91.

Coletta Filho HD, Machado MA, Targon MLPN, Moreira MCPQDG, Pompeu J Jr. 1998. Analysis of the genetic diversity among mandarins (*Citrus* spp.) using RAPD markers. Euphytica 102:133–139.

Das A, Mondal B, Sarkar J, Sarkar S. 2004. RAPD profiling of some elite clones of mandarin orange (*Citrus reticulata* Blanco) in the North Eastern Himalayan Region of India. Journal of Horticultural Science and Biotechnology 79:850-854.

Das A, Paul AK, Chaudhuri S. 2000. Micropropagation of sweet orange, *Citrus sinensis* Osbeck, for development of nucellar seedlings. Indian Journal of Experimental Biology 38:269–272.

Ghosh SP, Singh RB. 1993. Citrus in South Asia. FAO/RAPA Publication No. 1993/24. Bangkok, Thailand. pp. 70

IBPGR. 1988. Descriptors for Citrus. International Board for Plant Genetic Resources, Rome, Italy. 27 p.

Ray BK, Deka PC. 2000. Numerical taxonomic study of different mandarin oranges using morphological characters. Indian Journal of Genetics 60:227–232.

Qualitative and physiological properties of 'Pompia', a citron-like fruit

S. D'Aquino,[1]✉ F. Fronteddu,[2] M. Usai[3] and A. Palma[1]

[1] Istituto di scienze delle produzioni alimentari (ISPA)- CNR, Via dei Mille, 48 - 7100 Sassari (Sardegna), Italy. Tel.:+39 079-233466. Fax: 079-232047. Email: salvatore.daquino@ispa.cnr.it

[2] Ente Regionale di Sviluppo e Assistenza Tecnica in Agricolture (ERSAT) – Servizio Territoriale del Nuorese, Centro Zonale di Siniscola. via Caprera n.8, 09123 - Cagliari (Sardegna), Italy

[3] Dipartimento di Scienze del Farmaco–Università degli Studi di Sassari. Dipartimento di Scienze del Farmaco-Università degli Studi di Sassari - Via Muroni 23A, 07100 Sassari (Sardegna), Italy

Summary

Qualitative and physiological properties of 'Pompia', a citron-like fruit

Pompia is a citron-like tree of unknown origin traditionally cultivated in Sardinia, Italy. The tree is similar to both the lemon and citron with very large oblate, thick-peeled fruit that turns yellow when fully mature. This fruit is used in traditional Sardinian sweets. The juice is very similar to lemon juice and can be used to dress salads. The objective of the trial was to follow physiological, morphological and internal qualitative changes that occur from the end of October to the end of March, the period during which the fruits are harvested. During the five month observation period, little increase in weight occurred, while the colour of the peel turned from deep green to yellow. Respiration activity decreased progressively, while a slight increasing trend was observed in internal CO_2 concentration. Ethylene was produced in very low amounts. From October to March a marked reduction of juice vitamin C content occurred, whereas titratable acidity and total soluble solids decreased moderately.

Key words: Citrus, Pompia, chemical characteristics, respiration, ethylene

Résumé

Propriétés qualitatives et physiologiques du « pompia », fruit de type cédrat

Le pompia est un arbre de type cédratier, d'origine inconnue, traditionnellement cultivé en Sardaigne, Italie. L'arbre ressemble à la fois au citronnier et au cédratier, avec un fruit très gros, oblong, à écorce épaisse qui devient jaune à maturité complète. Ce fruit est utilisé dans la confiserie traditionnelle en Sardaigne. Le jus ressemble à celui du citron et peut être utilisé pour assaisonner les salades. L'essai avait pour but de suivre les changements physiologiques, morphologiques et qualitatifs internes qui se produisent de la fin octobre à la fin mars, période durant laquelle les fruits sont récoltés. Durant la période d'observation de cinq mois, une légère augmentation du poids est observée, tandis que la couleur de l'écorce du fruit passe du vert sombre au jaune. La respiration diminue progressivement, tandis que la concentration interne en CO_2 augmente légèrement. L'éthylène est produit en très grande quantité. Entre octobre et mars se produit une nette réduction de la teneur en vitamine C dans le jus, tandis que l'acidité titrable et les solides solubles totaux diminuent légèrement.

Resumen

Propiedades fisiológicas y cualitativas de 'Pompia', un fruto parecido a la cidra

La pompia es un árbol de origen desconocido parecido a la cidra y cultivado en Cerdeña, Italia. El árbol es similar tanto al limonero como a la cidra y tiene un fruto muy grande achatado en los polos y de piel gruesa que se vuelve amarilla cuando madura completamente. Este fruto se utiliza para la confección de dulces sardos tradicionales. El jugo es muy similar al de limón y puede utilizárselo para aliñar ensaladas. El objeto del ensayo fue seguir los cambios fisiológicos, morfológicos y cualitativos internos que tienen lugar desde fines de octubre hasta fines de marzo, período en el que se cosecha el fruto. Durante el período de observación de cinco meses se registró un pequeño aumento del peso, mientras el color de la cáscara pasaba del verde oscuro al amarillo. La actividad de respiración disminuyó paulatinamente mientras se observaba una ligera tendencia en aumento en la concentración interna de CO_2. Se produjeron cantidades muy pequeñas de etileno. Entre octubre y marzo tuvo lugar una marcada reducción del contenido de vitamina C en el jugo, mientras que la acidez titulable y los sólidos solubles totales disminuyeron moderadamente.

Introduction

Citrus is one of the richest botanical groups of plants with thousands of known cultivars worldwide. The botanical classification of *Citrus* is very controversial; in fact, the number of species is not well defined and differs according to various botanists. Tanaka (1961) states that the genus *Citrus* includes 157 species whilst Swingle and Reece (1967) indicate only 16 species exist. More than in other botanical groups, citrus individuals of different species can cross-breed with high frequency, resulting in fertile hybrids which, in turn, create new individuals for cross-pollination. For example, Barret and Rodhes (1976) suggested that lemon is probably a trihybrid between *C. medica*, *C. grandis* and *Microcitrus*.

This justifies the suspicion that traditional botanical classification considers a group of individuals as species that are, in reality, not a species but a botanical variety. In fact, in line with recent studies, the number of true species in the *Citrus* genus is only 3–4 and all the traditional species should be considered as botanical groups or hybrids derived from these 3–4 species.

'Pompia' is a type of *Citrus* of unknown origin that has been cultivated in Sardinia for centuries. Manca dell'Arca cited it at the end of the 18th century (1780); historical documents dating back to 1760 (Cherchi Paba, 1974–1977) state that it was well known and cultivated in Milis (Oristano, central-west Sardinia). Nowadays Pompia is present all over the island, but is primarily cultivated in central-east Sardinia where there are also some specialized orchards.

Pompia trees bear large, oblate fruit that can weigh up to 500–600 g (Figure 1). The rind colour changes gradually from a deep green to yellow at full maturity (Figure 2). The albedo

Figure 1. Pompia trees with semi-mature fruit.

Figure 2. Pompia fruit rind colour changes gradually from a deep green to yellow at full maturity.

is very thick. The juice is similar to lemon juice; because the essential oils are different from those of any other citrus fruit, the juice has a distinctive flavour. The juice is used in place of lemon juice to dress special dishes and salads. However, peeled and sliced fruit are especially prized by Sardinians as the primary ingredient in a special Sardinian dessert cake. Recently, this speciality has gained popularity with tourists and is now offered as a typical local product. The peel is also candied and used as an ingredient in fruit-cake and candy. In addition, the rind can be immersed in ethanol thus extracting the essential oils which are then used to produce a typical liquor.

The growing popularityof Pompia has led to this study of the main physiological, biometric and chemical characteristics of Pompia fruit over the five-month ripening period, from the end of October, when fruit are still completely green, to March–April when the peel turns to a deep yellow colour.

Materials and methods

The experiment was carried out in a commercial orchard located in Siniscola (eastern Sardinia). Shoots were grafted onto sour orange trees and planted at 6m x 5m in 1990. During 2000–2001, the most important highly heritable characters, namely, the shape and habit of the tree, the colour of shoot tips, characteristics of the leaves, flowers and fruits and seeds in addition to the phenological phase were recorded according to the Ministero dell'Agricoltura e delle Foreste and the Società Orticola Italiana (1985) descriptors list for lemon.

Six trees located in different parts of the orchard were chosen for periodic harvesting of fruit over the growing period for chemical, morphological and physiological assessments. At each picking date, a sample of 48 fruits of medium size and free of visible defects were collected. In particular, from each tree two fruits from every cardinal point at 1–1.5 m from the ground were chosen. Pomological assessments included: weight, volume, longitudinal diameter (LG), maximum transversal diameter (HTD) and minimum transversal diameter (LTD). Colour of the peel was determined either subjectively—by estimating the percentage of yellow-coloured surface—or objectively by taking measurements on a total of 48 fruits on three equatorial points with a Minolta CR-300 chromameter (Minolta, Osaka, Japan) according to the Commission Internationale de l'Eclarage (CIE) (L*, a*, b*) colour scale. By the means of a 2-mm diameter needle applied to an Effegì penetrometer, the resistance of the peel to puncture was determined, while deformation, as mm reduction of the transversal diameter of the fruit, was measured by applying a 3-kg weight on the transversal diameter for 10 s. Peel thickness (mm) was measured at four sites 90 degrees distant one to another on the equatorial section. The colour of the flesh was assessed in two points of the equatorial section using the same chromatometer and scale as for peel. For each fruit the number of segments and seeds were also counted. The fruits were juiced and the percentage of juice was calculated. Chemical analysis of the juice included: pH, tritatable acidity (TA), total soluble solids (TSS) and vitamin C. TA was determined by titrating 10 mL of juice against 0.1 N NaOH to end point (8.2) and expressed as % of citric acid; TSS, as °Brix, were determined by the means of a digital refractometer (Atago, Tokyo, Japan), whilst vitamin C was evaluated by the indophenol titration method, as described by Ting and Rouseff (1986).

Respiration activity, ethylene production rate and endogenous gas composition were assessed on a total of 12 fruits by choosing at random 3 fruits from each cardinal point out of the 12 collected. Specifically, individual fruits were closed in 3-litre jars for 3 hours. The lid of each jar was fitted with two rubber septa. Through the two septa, two needles connected with a combined CO_2/O_2 analyzer (Combi Check 9800-1, PBI-Dansensor A/S, Denmarck) were inserted. A pump was used to continually circulate air through the first needle from the headspace. The air, after being analyzed, was re-injected through the second needle into the jar. The analysis was continued until the values of CO_2 and O_2 were constant; this took about 2 min. One hour after sealing a 2-mL sample

from each jar was withdrawn with a syringe for ethylene measurement that was determined by gas chromatography (D'Aquino et al. 1996). The fruit was then removed from the jars and left for six hours at 20°C; after that the fruits were used to determine endogenous atmosphere. Specifically, a 4-mL sample of air was withdrawn from each fruit by inserting a syringe needle into the stem scar through the calyx cavity, with the fruit submerged in water. From this sample 1 mL gas was used for ethylene determination and another 1-mL sample for CO_2 and O_2 assessment. Ethylene, CO_2 and O_2 were determined by gas chromatography using two different Varian 3300 gas chromatographs fitted with a FID and a TCD, respectively. Operating conditions and columns were the same as reported by D'Aquino et al. (1996).

Data were subjected to analysis of variance and the LSD was used for means separation using the Statgraphics software (Manugistics, version 5 professional, 2000).

Results

Table 1 shows the principal characteristics of Pompia trees. The Pompia tree is vigorous, has an upright habit that becomes large if not pruned, with sparse branches. The leaves are large, simple, ovate, with entire margin, while petiole wings are absent. The colour of the tips of the shoots is purple. The flowers are large, mainly grouped in inflorescences, less frequently isolated and the colour of the petal is purple-white. In Table 2 the main features of fruit are reported. The fruit shape is oblate, with a smooth/rough/bumpy surface, depending on the period of flowering and the position of the fruit; generally fruit borne inside the canopy are smooth while those more exposed to the light are rough or bumpy. The peel is very rich in essential oils. The essential oils resemble those of lemon but with a distinctive delicate and fragrant aroma. The peel is moderately hard and difficult to peel when fruit are green, but can be removed fairly easily when fruit are ripe. The membranes of the segments are quite delicate and the adherence of the segments to each other is moderate. Fruit axis is hollow and the cross section of the fruit is irregular. The segments present large vesicles, which are quite juicy. Fruits are strongly attached to the tree.

Data reported in Table 3 related to the main biometric parameters are the average of the records collected from October to March, since slight changes occurred during the sampling period. The fruit is very large with the average weight being 320 g; however, there is a wide variability in fruit weight with some fruits weighing more than 500 g and others less than 200 g. Fruit size depends heavily on yield; when trees have a low yield the fruit is larger, sometimes reaching 700 g. The rind is very thick with the average value being 1.5 cm; values lower than 1 cm were never registered. The number of segments is variable and ranges from 11 to 13. The average number of seeds was 10, but a great variability was observed with some fruit having 4–5 seeds and others having more than 15. The seeds are polyembryonic (3–4 embryos per seed); this supports the hypothesis that Pompia is a hybrid since true citron varieties are monoembryonic.

Table 1. Pompia tree characteristics

Principal tree characteristics	Description
Vigour of tree	Medium-high
Shape of tree	Ellipsoid-oblate
Habit	Upright
Density of branches	Sparse
Scion trunk surface	Smooth
Colour of shoot tip	Purple
Surface of shoot tip	Glabrous
Vegetative life cycle	Evergreen
Type of leaf	Simple
Colour of leaf	Green
Length of petiole relative to leaf lamina	Brevipetiolate
Petiole wings	Absent
Leaf shape	Ovate
Leaf margin	Entire
Arrangement of flowers	Mainly in inflorescence, less solitary
Size of flower	Large
Colour of flower	Petals white above, purplish below

Table 2. Qualitative characteristics of Pompia fruit

Qualitative characteristics	Description
Fruit shape	Oblate
Shape of base of fruit	Truncate with 6–10 deep furrows
Surface of epicarp	Rough (bumpy) or smooth
Adherence of epicarp to mesocarp	Moderate
Nature of oil glands	Conspicuous
Amount of oil	Elevated
Adherence of segments to each other	Moderate
Toughness of skin around segments	Delicate
Fruit axis	Hollow
Cross-section of axis	Irregular
Texture of pulp	Firm
Size of vesicles	Large
Shape of vesicles	Thick
Juice in endocarp	Medium
Colour of juice	Pale yellow
Aroma of juice	Strong
Attachment of fruit to tree	Strong
Shape of seeds	Ovoid

Table 3. Morphological and biometric parameters of Pompia fruit

Parameters	Average*	Standard error (n = 252)
Fruit weight (g)	320	12.74
Longitudinal diameter (H) (mm)	74	1.166
Max width (L) (mm)	102	1.217
Min width (mm)	95	1.43
Shape index (H/L)	0.725	
Peel thickness (mm)	15	0.057
Number of seeds per fruit	10	0.90
Number of segments per fruit	12	0.284
Number of embryos per seed	3–4	-

* The value of each parameter is the average of seven samplings (36 fruit per sample) carried out from October to March.

Table 4. Evolution of rind colour, specific weight, juice percentage, deformation and resistance to puncture of the rind of Pompia fruit from October to March

Sampling period	Yellow coloured rind (%)	Specific weight	Juice (%)	Deformation (mm)	Resistance to puncture (g)
31 October 2000	17.58 a	0.71 d	20.8 d	2.24 a	1374 d
16 November 2000	37.75 b	0.67 c	19.4 cd	2.50 a	1373 d
1 December 2000	91.96 c	0.66 bc	18.9 bcd	2.43 a	1145 c
22 December 2000	100.00 d	0.65 b	18.3 bc	2.33 a	1059 b
19 January 2001	100.00 d	0.64 b	16.8 ab	3.01 b	821 a
15 February 2001	100.00 d	0.62 a	15.7 a	3.35 c	853 a
12 March 2001	100.00 d	0.61 a	15.5 a	4.07 d	808 a

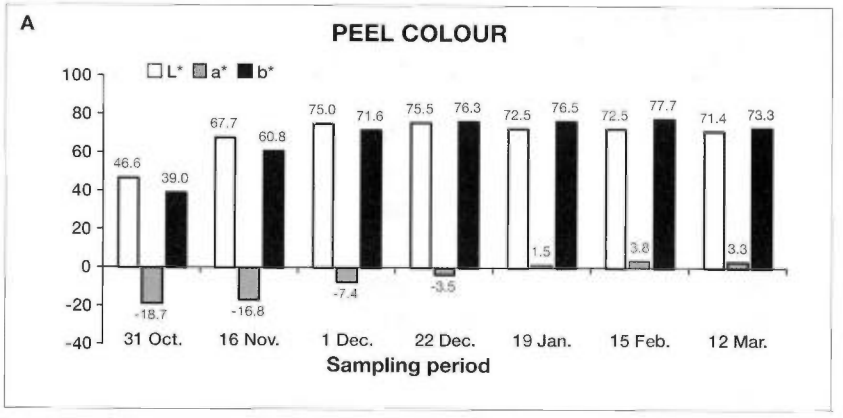
At the beginning of October fruit were still completely green; at the first inspection 17% of the peel was yellow whereas at the 4th inspection the peel was completely yellow (Table 4). From 31 October to 15 November objective analysis of the peel indicated a sharp increase in the L* value; after 15 November only little variations occurred. However, a* values increased progressively over the sampling period with the final value being slightly higher than 0. The b* component was always positive; it increased sharply during the first three inspections and was fairly constant at the subsequent sampling periods (Figure 3A). Objective measurements show that the evolution of the colour of the peel was completed by December with the final values of b* and a* indicating a strong yellow component with a very weak red constituent. However, the flesh underwent little change with the a* component remaining negative and the b* component remaining positive, but considerably less than the b* values of the peel (Figure 3B).

As maturation progressed specific weight decreased progressively (Table 4). A significant decrease was also detected for juice percentage which diminished from 20.8% on 30 October to 15.5% on 12 March (Table 4). Also puncture resistance decreased significantly during the sampling period, while deformation increased significantly only from January on, indicating no important relationship with resistance to puncture (Table 4).

Titratable acidity, pH, total soluble solids and vitamin C, which are reported in Table 5, were typical of lemons. Over the sampling period, pH increased progressively and ranged between 2.35 and 2.69. Titratable acidity accounted for about 90% of the total soluble solids. Both parameters underwent a slight reduction over the sampling period. However, vitamin C content showed the most dramatic change during ripening with a reduction of 33.6% from the first sampling (33.97 mg/100 mL of juice) to the last one. Chemical parameters are very similar to those of lemons grown in similar environmental conditions (D'Aquino et al. 1998; D'Aquino et al. 2002).

Endogenous CO_2 increased progressively over the ripening period; at the first sampling CO_2 was 1.62 kPa, while at the last sampling it was 2.83 kPa (Figure 4A). Conversely endogenous O_2 decreased from 18.9 to 17.4 kPa (Figure 2A). Although a large variability among the single fruits was observed at each inspection, endogenous C_2H_4 showed an increasing trend but with a final value of 0.13 $\mu L.L^{-1}$ (Figure 2A). Respiration activity showed a decreasing trend with a reduction of about 35% at the last assessment with respect to the first one (Figure 4B).

As maturation progressed, C_2H_4 production increased slightly; however, as for endogenous C_2H_4, a very large variability was observed amongst the single fruits and differences, from the statistical point of view, were inconsistent (Figure 2A). The overall physiological behaviour of Pompia fruit resembles that of lemon. In different experiments with



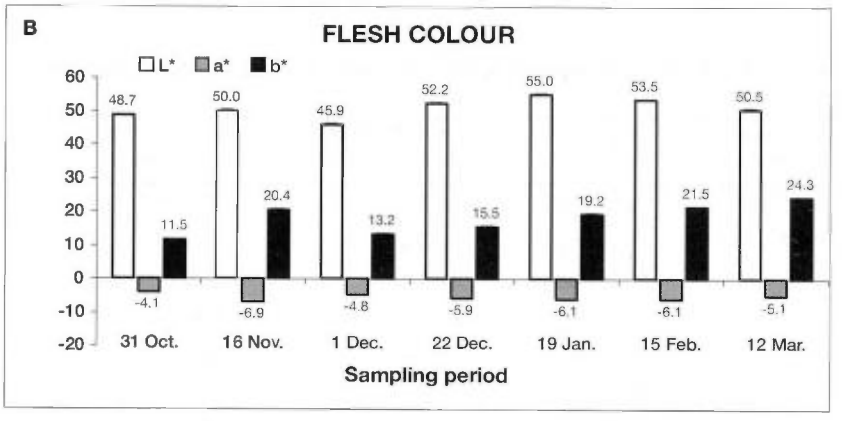
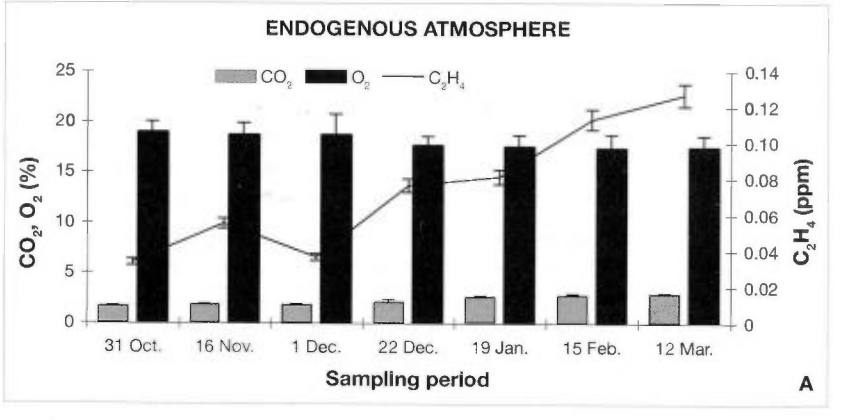


Figure 3. Changes in peel colour (A) and flesh colour (B) over the sampling period. For each sampling period 48 fruits were used.



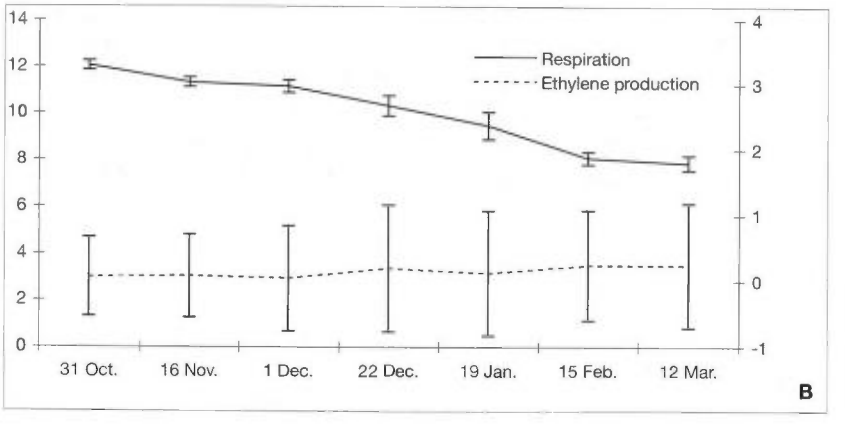


Figure 4. Evolution of endogenous carbon dioxide, oxygen and ethylene (A), respiration activity and ethylene production rate (B) over the sampling period. Vertical bars indicate the standard error (n = 12).

Table 5. Evolution of pH, titratable acidity, total soluble solids and vitamin C of Pompia juice from October to March

Sampling period	pH	Tritatable acidity (g citric acid/100 mL)	Total soluble solids (°Brix)	Vitamin C (mg ascorbic acid/100 mL)
31 October 2000	2.35 a	6.74 d	7.51 d	33.97
16 November 2000	2.43 b	6.66 cd	7.29 c	35.39
1 December 2000	2.48 bc	6.62 cd	7.02 b	29.54
22 December 2000	2.52 c	6.49 c	6.90 b	28.22
19 January 2001	2.53 c	6.45 bc	6.98 b	27.31
15 February 2001	2.61 d	6.24 b	6.55 a	26.78
12 March 2001	2.69 e	5.99 a	6.48 a	22.54

lemon cultivars, we have noted similar trends and intensities of respiratory activity (data not shown). The slight but gradual reduction in respiration activity intensity observed, which was counteracted by a concomitant raise of endogenous CO_2 and decrease of endogenous O_2, is typical of citrus fruit (Agabbio et al. 2000; Eaks 1970). In addition to the physiological reduction of metabolic activity due to ageing, this can also be explained by a concomitant reduction of permeability to rind gases probably from the continuous accumulation of wax on the rind that occurs with fruit maturation (El-Otmani and Coggins 1985), which leads to a rise of endogenous CO_2 and a consequent reduction of respiration activity.

Conclusion

Although it is not certain if Pompia is really a hybrid between lemon and citron, different features seem to confirm some genetic similarities (Chessa et al. 1994). The rind at full maturity is completely yellow and the number of segments is variable among the fruits of the same tree, as occurs in both lemon and citron (Hodgson 1967). The average weight of the fruit is more similar to that of citron than lemon; however, the oblate shape resembles a grapefruit.

We believe that the increasing importance that local traditional products are gaining in Europe justify more attention to this fruit whose use in the local Sardinian food industry can make an important contribution to the local economy. We recommend that further studies for better characterization of the nutritional values of the various components of the fruit (flavedo, albedo, essential oils) be done along with a definitive study of the genetic origin of Pompia based on molecular methods. We believe this can increase the interest in Pompia from either the commercial or the scientific point of view.

Acknowledgments

The authors wish to thank Mr Domenico Mura for his valuable technical assistance. This study was funded by the Ministero delle Politiche Agricole e Forestali (MiPAF) within the framework of 'Liste di orientamento varietale degli agrumi'. The authors contributed equally to this study.

References

Agabbio M, Molinu MG, Mura D, D'Aquino S, Delogu M. 2000. L'arancio "Tardivo di San Vito", cultivar bionda a maturazione tardiva. In: Agabbio M. (ed.), Biodiversità: Germoplasma locale e sua valorizzazione. Atti del 4° Congresso Nazionale, Alghero, 8–11 settembre 1998. p. 625–628.

Barret HC, Rhodes AM. 1976. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. Systematic Botany 1:105–136.

Cherchi Paba F. 1974–77. Evoluzione storica dell'attività agricola, caccia e pesca in Sardegna. Regione Sarda, Assessorato Industria e Commercio, Ed. Fossataro, Cagliari, Italia.

Chessa I, Mulas M, Pala M. 1984. Gli agrumi. In: Patrimonio Genetico di Vecchie Specie Arboree da Frutto: Le vecchie varietà della Sardegna a cura di M. Agabbio. Carlo Delfino Editore, Sassari, Italia.

D'Aquino S, Piga A, Continella G, Agabbio M. 1996. Effect of prestorage high temperature conditioning on fruit quality, respiration rate and ethylene production of "Hass" avocado fruit. Advance in Horticultural Science 10:167–172.

D'Aquino S, Piga A, Agabbio M, Molinu MG. 1998. Decay control of "Femminello Santa Teresa" lemon fruits by prestorage high temperature conditioning. In: Bertolini P, Sijmons PC, Guerzoni ME and Serra F (eds), Non Conventional Methods for the Control of Postharvest Disease and Microbiological Spoilage. COST 914 and COST 915, Joint Workshop. Bologna October 9–11 1997, Bologna, Italy. p. 221–226.

D'Aquino S, Agabbio M, Angioni M, Delogu M, Tedde M. 2002. Evoluzione dei parametri di qualità interna e visivi di limoni (CV "Di Massa") conservati in condizioni di mercato. Atti del Convegno Internazionale "Produzioni alimentari e qualità della vita", 4–8 Settembre 2000, Sassari, Italia. p. 571–578.

Eaks I. 1970. Respiratory response, ethylene production, and response to ethylene of *Citrus* fruit during ontogeny. Plant Physiology 45:334–338.

El-Otmani M, Coggins CW Jr. 1985. Fruit age and growth regulator effects on the quantity and structure of the epicuticular wax of "Washington" navel orange fruit. Journal of the American Society of Horticultural Science 110(3):371–378.

Hodgson RW. 1967. Horticultural varieties of citrus. In: Reuther W, Webber HJ, Batchelor LD, (eds), The citrus industry, Vol. I. Division of Agricultural Science, University of California, Berkeley, USA.

Manca Dell'Arca A. 1780. Agricoltura di Sardegna. Ed. Orsino, Napoli, Italia.

Ting SV, Rouseff RL. 1986. Vitamins. In: Ting SV, Rouseff R. (eds), Citrus Fruits and their Products: Analysis and Technology. Marcel Dekker, New York, USA. p. 121–136.

Tanaka T. 1961. Citrologia. Semicentennial commemoration papers on *Citrus* studies. Citrologia Supporting Foundation, Osaka, Japan.

Swingle WT, Reece PC. 1967. The botany of citrus and its wild relatives. In: Reuther W, Webber HJ, Batchelor LD (eds), The citrus industry, Vol. I. Division of Agricultural Science, University of California, Berkeley, USA.

Conservation of genetic resources of the European black poplar (*Populus nigra* L.) in China

Li Gangtie,[1] Yang Zixiang,[2] Jos Van Slycken[3] and Zhang Weidong[4]✉
[1]Inner Mongolia Agriculture University, Huhhot, P.R. China
[2]Chinese Academy of Forestry (CAF), Beijing, P.R. China
[3]Institute for Forestry and Game Management, Geraardsbergen, Belgium
[4]Project Management Office, PRC-GEF Partnership on Land Degradation in Dryland Ecosystems (OP12), Rm 428, Debao Hotel Bld. 22, Debao Xinyuan, Xicheng District, 100044 Beijing, P.R. China. Email: zhangweidong@gefop12.cn

Summary

Conservation of genetic resources of the European black poplar (*Populus nigra* L.) in China

Populus nigra L. is severely threatened in China. The species has its natural distribution area along the Ertix and Ulungur river systems in the Xinjiang Province (West China). The forests along the two river valleys are being destroyed because of agricultural extension, animal husbandry and an increase of the human population. The area of *P. nigra* is becoming smaller and smaller and the species is actually only present along the two valleys as scattered individuals or in small groups. Trees over 100 years old cannot be found and the remaining trees are generally 40–60 years old. Notwithstanding the large number of seedlings that still germinate on the flooded land, only a few trees can survive animal grazing and drought. In the Ertix River valley, *P. nigra* mixes with P.×*jrtyschensis* C.Y. Yang. In the Ulungur River valley, however, only *P. nigra* is found. Because of its strong resistance to drought and frost and adaptability to poor sites, *P. nigra* is considered as an invaluable parent for breeding and selection work for northern China. In 1998, the Food and Agriculture Organization of the United Nations (FAO) '009' Project was started to conserve the remaining genetic resources of *P. nigra* in *ex situ* collections. *In situ* conservation is also urgently needed.

Key words: conservation, genetic resources, *Populus nigra* L.

Résumé

Conservation des ressources génétiques de peuplier noir (*Populus nigra* L.) en Chine

Populus nigra L. est gravement menacé en Chine. L'aire naturelle de l'espèce coïncide avec les vallées de l'Ertix et de l'Ulungur dans la province du Xinjiang (Ouest de la Chine). Les forêts bordant les deux cours d'eau ont été détruites suite au développement de l'agriculture, de l'élevage et de l'accroissement démographique. L'aire de distribution de *P. nigra* est de plus en plus réduite et, à présent, seuls des individus isolés ou de petits groupes de cette espèce se rencontrent dans les deux vallées. Il n'existe plus d'arbres de plus 100 ans et ceux qui restent sont généralement âgés de 40 à 60 ans. Malgré le grand nombre de plantules qui germent dans les zones inondées, seuls quelques individus survivent au pâturage et à la sécheresse. Dans la vallée de l'Ertix, *P. nigra* est présent en peuplement mixte avec P.×*jrtyschensis* C.Y. Yang. Dans la vallée de l'Ulungur en revanche, seul *P. nigra* est observé. Du fait de sa grande résistance à la sécheresse, au gel et sa capacité d'adaptation en terrain pauvre, *P. nigra* est considéré comme un parent indispensable pour les programmes de sélection dans le Nord de la Chine. En 1998, l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO) a initié le projet « 009 » afin de préserver les ressources génétiques encore existantes de *P. nigra* dans des collections *ex situ*. Il est également indispensable d'organiser la conservation *in situ*.

Resumen

Conservación de los recursos genéticos del chopo europeo (*Populus nigra* L.) en China

En China *Populus nigra* L. está gravemente amenazado. El área natural de distribución de la especie se ubica a lo largo de los sistemas fluviales del Ertix y el Ulungur en la provincia de Xinjiang (China occidental). A lo largo de ambos valles fluviales los bosques están siendo destruidos por el avance de la agricultura, la cría de ganado y el aumento de la población humana. La zona ocupada por *P. nigra* es cada vez más pequeña y en la actualidad en ambos valles la especie está presente solo en forma de individuos aislados o pequeños grupos. No se encuentran ya árboles de más de 100 años y los que quedan tienen por lo general de 40 a 60 años de edad. No obstante el gran número de brinzales que todavía germinan en las tierras anegadas, sólo unos pocos árboles sobreviven al pastoreo animal y a la sequía. En el valle del río Ertix, *P. nigra* se mezcla con *P.×jrtyschensis* C.Y. Yang. En cambio en el valle del río Ulungur sólo se encuentra *P. nigra*. Debido a su notable resistencia a la sequía y las heladas y a su adaptabilidad a los sitios pobres, *P. nigra* es considerado un invalorable progenitor para los programas de mejoramiento y selección en China septentrional. En 1998 comenzó el Proyecto '009' de la Organización de las Naciones Unidas para la Agricutura y la Alimentación (FAO) destinado a conservar en colecciones *ex situ* los recursos genéticos de *P. nigra* que aún quedan. También se necesita urgentemente conservación *in situ*.

Introduction

The GCP/CPR/009/BEL Project on "Afforestation, Forestry Research, Planning and Development in the Three North Region of China" (1991–2002) was jointly financed by the Governments of Belgium and China, with technical assistance by the Food and Agriculture Organization of the United Nations (FAO). The project concentrated its activities on the Korqin Sandy Lands in northeast Inner Mongolia in northeast China, where protective shelterbelts are an essential component of environmental restoration and agriculture. One of the goals of the project was to widen the genetic basis of the poplar clones in order to increase productivity, tolerance to frost and drought, and overall resistance to pests and diseases. Three strategies were implemented to achieve this goal (Sigaud 1996; Villar and Tang 1996; Sigaud 1997; Villar 1997):

1) A long-term poplar breeding and selection programme to continuously produce new material with a wide genetic resource base. Through recurrent selection, the programme relied on intra- and interspecific crosses from a wide collection of native and introduced poplars;

2) Conservation of the remaining genetic resources of native poplar species, concentrating on *P. nigra* L., *P. simonii* Carr. and the related *P. pseudosimonii* Kitag., the only fast-growing indigenous species adapted to the extreme climatic conditions and the poor soils of the region. Whenever possible, this unique genetic material is conserved *in situ*. When this option was not possible, samples of genetic diversity were explored, collected, propagated and stored in *ex situ* collections, genebanks and arboreta;
3) A short-term poplar selection programme to rapidly improve vigour, form and resistance to frost and drought, through collection, testing and evaluation of poplar clones already available in northern China and abroad. The programme quickly provided forest managers with improved but already existing materials.

Populus nigra L. in China

Distribution of P. nigra *in China*

The European black poplar, *P. nigra* L., belonging to the section *Aigeiros* Duby, has a natural distribution area ranging from the south of Europe over central Asia and Siberia up to China.

In China, natural forests of *P. nigra* only occur in the valleys of the Ertix and Ulungur rivers which are situated between the Altay Mountains and the northern edge of the Jungar Basin in the Xinjiang autonomous region. Both rivers rise in the Altay Mountains and are situated about 80 km from each other. The Ulungur River discharges in the Ulungur Lake of Xinjiang, while the Ertix River goes finally to the Arctic via Kazakhstan and the Russian Federation. The catchment areas of both rivers are situated north of Xinjiang. The altitude of both river valleys varies between 450 and 600 m (Figure 1).

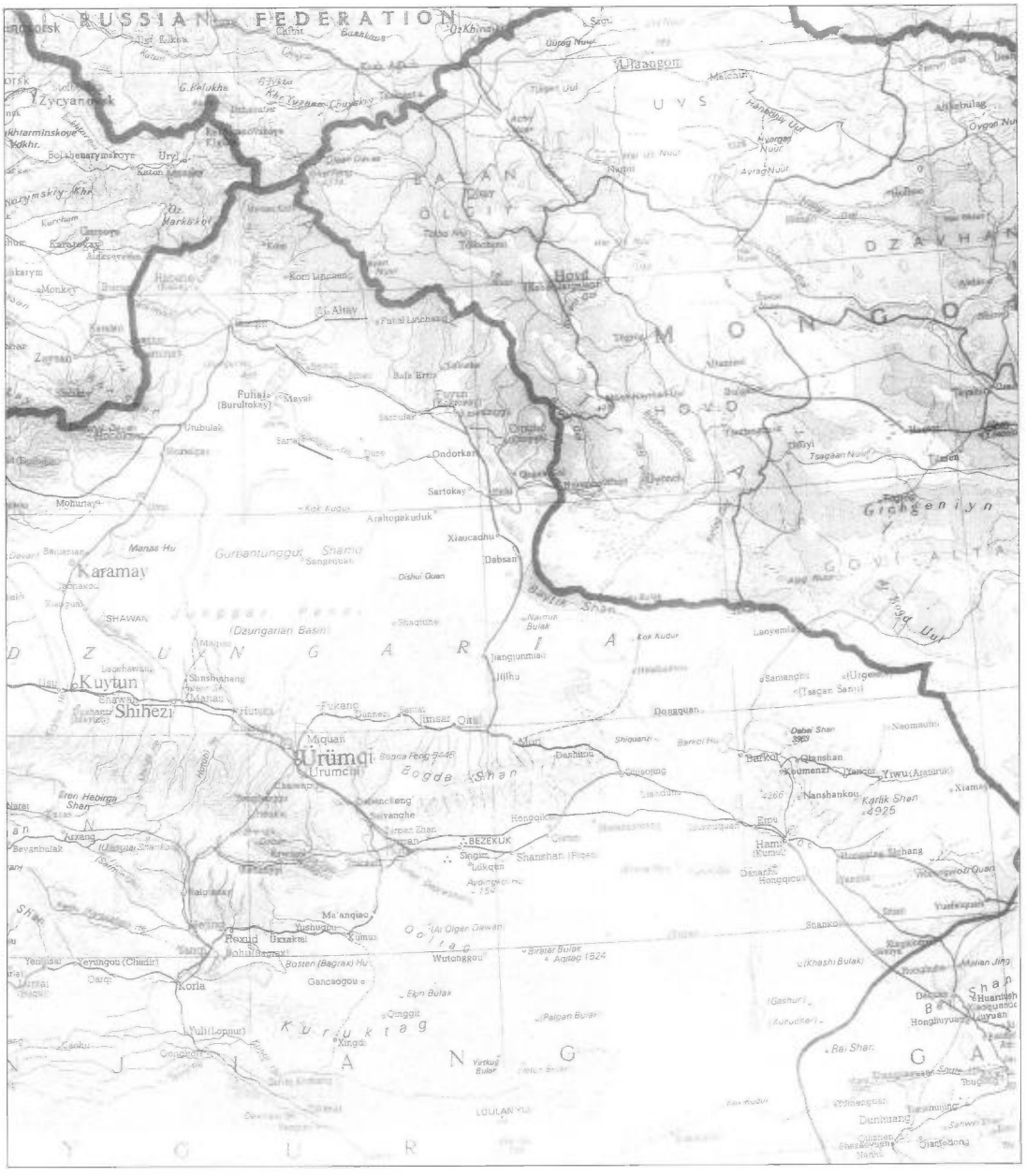


Figure 1. Distribution area of *P. nigra* L. in China.

Climatic characterization of the distribution area

The climate is typical for most northern continental areas. The summers are generally warm and dry with cool nights. The winter season starts by mid-November and is characterized by frost and snowfall, with temperatures remaining below zero until early March. According to the Beitun region weather station (Beitun Town, located near the mid-Ertix River) during the period 1961–1979 the mean annual daily temperature was 4.1°C, the mean daily temperature of the coldest month (January) was –18.8°C, the mean temperature for July was 23.4°C, extremes in temperature were 40.1°C and –46.7°C. The frost-free period varied from 136 to 145 days. The annual precipitation averaged 93.9 mm. Mean annual potential evaporation was estimated as 1661.4 mm.

In spite of the low amount of rainfall, within the river valleys a lot of water is available for plant growth because of seasonal snowmelt in the elevated parts of the catchment areas. The mean annual discharge is 11.29 billion m^3 for the Ertix River and 1.2 billion m^3 for the Ulungur River. Eighty percent of this annual discharge takes place from April to August.

Ecological characterization of the sites

The area is characterized by the presence of several broad-leaved species, mainly *P. nigra* L., *P. tremula* L., *P. alba* L., *P.×canescens* (Aiton) Sm. and *P. laurifolia* Ledeb., but also other species such as *Amygdalus* L. spp., *Elaeagnus* L. spp., *Tamarix* L. spp. do occur.

In the natural forests, *P. nigra* trees occur as scattered individuals or appear in small groups, so-called cohorts, and grow on the riverbanks where a shallow groundwater table is present. Natural regeneration depends entirely on the seeds.

Some stands in both river valleys have been studied in more detail (Figures 2 and 3). The first stand investigated was near Dingshan, located downstream from the Ulungur River. The canopy cover of this stand is 0.5 and is composed

Figure 2. Natural poplar stand in the Ertix River valley (Beitun Forest Farm). The biggest tree in the middle (dbh 2.14 m, height 30 m) is called 'Nigra King' and is more than 100 years old. However, from observations on the leaves of the short and long shoots, the tree has to be considered as *P.×jrtyschensis*.

Figure 3. Stand in the valley of the Wulungu (*P. nigra*).

of *P. nigra* and *P. alba*. The trees are sparse, with most being *P. alba*. *Populus nigra* occurs in valley forests as small cohorts. The *P. nigra* trees are not as tall, are slightly bent and have a thick trunk. Their crown is large and the leaves are typically rhomboid shaped.

The diameter at breast height (dbh) of the biggest tree was 97.5 cm, and the smallest was 35.5 cm. The *P. nigra* trees could be grouped into four age classes: under 30; 30–50; 50–70; and over 70 years. The sex distribution was investigated in a 100×100 m plot; of the 14 trees present, 7 were female.

In the Ertix River valley, *P. nigra* grows together with *P. laurifolia*, *P. alba* and *P.×canescens*. The investigated stand showed a canopy cover of 0.6. The dbh in the stand varied between 20 and 80 cm. Here most of the trees are male with female trees making up less than 20% of the stand.

Typical for this river system is the occurrence of both *P. nigra* and *P. laurifolia*. According to Yang, both species hybridize and can produce valuable clones, so he suggested the hybrid name *P.×jrtyschensis* C.Y. Yang (Yang 1979; Fang, Fang, Zhao and Skvortsov, 2000). Hybrids between *P. laurifolia* and *P. nigra* are also named *P.×berolinensis* Dippel; synonyms are *P.×rasumowskyana* Schr. and *P.×petrowskyana* Shr. (Eckenwalder 1996; Dickmann 2001), but those names represent a very limited number of hybrid clones. The presence and share of hybrids in the natural stands were investigated by the authors of this article. In a one-year-old and two-year-old natural regeneration along the Ertix riverbed, a large variation in leaf form was found. Seedlings with the typical leaf of *P. nigra* and *P. laurifolia* are a minority; more than 90% of the seedlings had to be considered as *P.×jrtyschensis* or introgressed forms of the two species.

Figure 4. Remains of stands without proper protection (valley of the Ertix).

Threats

In this area, environmental degradation and the associated ever-increasing loss of natural resources are the main problems. Formerly, this area was known as a very good forestry and winter herd pasture area, but now a large part of the land has been converted to farmland. Many of the *P. nigra* trees, appreciated for their good stem form, have been cut in such a severe way that the forest area has decreased by two-thirds. Overgrazing by cattle, goats, sheep, camels and horses prevents natural regeneration and restoration of the riparian forest ecosystem. These human-induced pressures result in an increasingly severe land degradation (Figure 4).

Economic potential of P. nigra

Populus nigra shows a good potential for wood production in this region. Table 1 illustrates the potential of the species, which is well adapted to the harsh local climatic conditions, both in plantations and in natural forests (Xu and Chen, 1982), based on measurements of more than 10 trees per plot.

Importance of native P. nigra *in poplar breeding*

The potential of *P. nigra* as a parent in breeding and selection work has been recognized worldwide. In northeastern China, selection and breeding programmes using *P. nigra* as a parent tree started some 50 years ago and resulted in nearly 30 widely used poplar clones. An overview of the used crosses and the resulting clones is given in Appendix 1 (online).

More recently, the '009' Project tested several poplar resources in different locations. Many of the best clones, combining good growth and drought and frost resistance, were related to *P. nigra* and *P. simonii*. This confirms the potential of *P. nigra* for poplar breeding in northern China.

Table 1. Comparison of growth of *P. nigra* and other poplar species (Xu and Chen 1984)

Location	Species	Age (yr)	Diameter (cm)	Height (m)	Volume (m^3/tree)	Volume increment (m^3 $tree^{-1}$ yr^{-1})	Remarks
Manasi Forest Farm	*P. nigra*	22	32.8	19.5	0.5491	0.0250	Plantation forest
	P. nigra 'Italica'	22	22.6	18.6	0.2584	0.0117	
	P. alba	18	25.7	20.5	0.4388	0.0241	
Beitun Forest Farm	*P. nigra*	28	33.0	16.6	0.4600	0.0160	Natural forest
	P.×canenscens	26	14.2	13.3	0.0646	0.0026	
	P. alba	32	24.0	17.0	0.2400	0.0075	
	P.×jrtyschensis	34	15.0	13.5	0.2260	0.0077	

However, it is clear that the genetic basis of the poplars cultivated in the region is still very limited, with most local clones having *P. nigra* var. *thevestina* (Dode) Bean as a mother (Appendix 1[online]). This narrow genetic basis can be a source of many serious problems (Ma 1994, 1995):

- The close relationship of the clones actually used has a potential risk, because they are used on a very large scale;
- The potential of the native *P. nigra* in breeding is not yet explored, but there is a strong need to study the genetic diversity and to characterize its properties using progenies of controlled crosses;
- The establishment of a genebank of the species and *in situ* conservation are essential.

Conclusion

Poplar is the main species used in large-scale plantations in northeastern China. However, the genetic basis of the material used is very small. Untapped native resources of *P. nigra* can provide valuable breeding resources for northern China, where the choice of genera/species for windbreaks is very limited.

Populus nigra is naturally distributed in China only in two river valleys of the Altay region, Xinjiang. These natural stands are threatened by over-cutting, conversion to farmland and overgrazing, causing severe environmental problems. These changes in land use and the land degradation also prevent the natural regeneration of the species. Natural regeneration still occurs on a limited scale, but is further damaged by livestock grazing. The '009' Project has initiated emergency *ex situ* conservation and the use of native *P. nigra* in its poplar breeding programme. However, more urgent action is needed, especially political decisions towards *in situ* conservation. The protection of remaining stands and natural regeneration on the riverbanks should be a priority for the local forest managers and authorities.

Acknowledgments

The authors wish to express their gratitude to P. Sigaud, Forestry Officer (Forest Genetic Resources) of the Forest Resources Development Service, Food and Agriculture Organization of the United Nations (FAO) for his helpful comments on this paper.

References

Dickmann DI. 2001. An overview of the genus *Populus*. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar Culture in North America. Part A, Chapter 1. NRC Research Press, National Research Council of Canada, Ottawa, Canada, pp. 1–42.

Eckenwalder JE. 1996. Systematics and evolution of *Populus*. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinkley TM, editors. Biology of *Populus* and its Implications for Management and Conservation. Part I, Chapter 1. NRC Research Press, National Research Council of Canada, Ottawa, Canada, pp. 7–32.

Fang Z, Fang C-f, Zhao S, Skvortsov AK. 2000. Salicaceae. (http://flora.huh.harvard.edu/china/mss/volume04/SALICACEAE.published.pdf)

Ma C. 1994. Researches on poplar breeding in China to be viewed in the light of the development and achievement of cross breeding of poplars in the world. World Forestry Research 3:23–30.

Ma C. 1995. Current situation and countermeasures for the development of hybrid breeding of poplar in China. Scientia-Silvae-Sinicae 31(1):60–68.

Sigaud P. 1996. Conservation and improvement of poplar resources. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Sigaud P. 1997. Long-range poplar breeding and selection programme. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Villar M. 1997. Poplar breeding programme in North-East China: proposition for 1998 breeding procedures. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Villar M, Tang Q. 1996. Proposition for a poplar breeding programme in North-East China. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Xu W, Chen Z. 1982. Study tour to the Poplar Forests along the Valley of the Ertix River. Xinjiang Forestry Science, Special issue.

Yang C. 1979. New classification of Salicaceae. Xinjiang Plant Sub-Society of China Plant Society, Wulumuqi, Xinjiang, PR China.

Appendix 1. Origin of the main cultivated poplars in North East China is available on http://www.ipgri.cgiar.org/pgrnewsletter/default.asp?id_issue=143

Geographical distribution and cold tolerance at booting stage of the second core collection of rice landraces (*Oryza sativa*) from Yunnan, China

Yawen Zeng,✉ Shenchong Li, Shiquan Shen, Zhongsheng Zhang, Xiaoyin Pu and Liuhua Qi
Biotechnology and Genetic Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650205, Yunnan, P. R. China. Email: zengyw@public.km.yn.cn

Summary

Geographical distribution and cold tolerance at booting stage of the second core collection of rice landraces (*Oryza sativa*) from Yunnan, China

The second core collection of rice landraces (*Oryza sativa* L.) from Yunnan, China consisting of 696 accessions, grouped according to rice-cropping regions and administrative districts, was investigated with respect to the geographical distribution and cold tolerance of accessions at booting stage. The results showed distinct differences in cold tolerance at booting stage of accessions originating from different rice-cropping regions and prefectures. The degree of cold tolerance ranged from highest to lowest in the following order: northwest Yunnan cold highland *japonica* rice region > central Yunnan single-season *japonica–indica* region > northeast Yunnan plateau *japonica* rice region > south Yunnan single/double-season rice region > south marginal paddy–upland rice region. The breeding goals for cold tolerance differed among the various rice-cropping regions. The rice-growing areas of Yunnan can be divided into three categories based on the severity of cold damage experienced, namely serious or severe cold damage; moderate cold damage; and slight cold damage regions. These geographical differences were the dominant factor in producing the range of genetic diversity in cold tolerance found in the rice landraces of Yunnan. Differences in cold tolerance at booting stage result from the fact that the Yunnan rice landraces and the amount of cold damage expected in the different rice-cropping regions have co-evolved over a long period of time.

Key words: Yunnan, rice, landrace, core collection, cold tolerance, booting stage, *Oryza sativa* L.

Résumé

Distribution géographique et tolérance au froid au stade du gonflement dans la deuxième collection de référence de variétés locales de riz (*Oryza sativa*) du Yunnan, Chine

La distribution géographique et la tolérance au froid au stade du gonflement ont été étudiées dans la deuxième collection de référence de variétés locales de riz (*Oryza sativa* L.) du Yunnan, Chine, constituée de 696 accessions, groupées par région de culture et districts administratifs. Les résultats font apparaître de nettes différences dans la tolérance au froid au stade du gonflement parmi les accessions provenant de différentes régions de culture du riz et préfectures du Yunnan. L'échelle de tolérance au froid, de la plus forte à la plus plus faible, est la suivante : riz *japonica* de la région froide d'altitude du Nord-Ouest du Yunnan > riz *japonica–indica* de la région centrale du Yunnan à une seule saison de culture > riz *japonica* du plateau du Nord-Est du Yunnan > riz des régions du Sud du Yunnan à une ou deux saisons de culture > riz de montagne des régions en bordure méridionale. Les objectifs de sélection pour la tolérance au froid diffèrent selon les régions de culture. Les régions de culture du riz du Yunnan peuvent être classées en trois catégories selon la gravité des dommages causés par le froid : dégâts graves ou importants ; dégats modérés ; dégâts légers. Ces différences géographiques constituent le facteur dominant déterminant la diversité génétique de la résistance au froid observée parmi les races de riz du Yunnan. Les différences de résistance au froid au stade du gonflement s'expliquent par le fait que les races locales de riz du Yunnan et l'importance des dégâts du froid prévisibles selon la région de culture ont co-évolué sur une longue période.

Resumen

Distribución geográfica y tolerancia al frío en el estadio de turgencia de la segunda colección testigo de razas locales de arroz (*Oryza sativa*) de Yunnan, China

Se investigaron las 696 accesiones agrupadas con arreglo a las regiones de cultivo del arroz y los distritos administrativos de la segunda colección testigo de razas locales de arroz (*Oryza sativa*) de Yunnan, China, con respecto a su distribución geográfica y tolerancia al frío en el estadio de turgencia. Los resultados mostraron diferencias netas en la tolerancia al frío en el estadio de turgencia de las accesiones provenientes de las diferentes regiones de cultivo de arroz y prefecturas de Yunnan. El grado de tolerancia al frío variaba del más alto al más bajo en el siguiente orden: arroz *japonica* de la región de tierras altas frías de Yunnan nordoccidental > arroz *japonica-indica* monoestación de la región de Yunnan central > arroz *japonica* de la región de meseta de Yunnan nordoriental > arroz de estación simple/doble de la región de Yunnan meridional > arroz cáscara marginal de montaña de la región del sur. Las metas de mejoramiento de la tolerancia al frío diferían con arreglo a las diversas regiones de cultivo del arroz. Las áreas de cultivo arrocero de Yunnan se pueden dividir en tres categorías sobre la base de la gravedad del daño causado por el frío: regiones de daño grave o severo; regiones de daño moderado y regiones de daño leve. Estas diferencias geográficas fueron el factor dominante para compilar la serie de diversidad genética respecto de la tolerancia al frío encontrada en las razas locales de arroz de Yunnan. Las diferencias de tolerancia al frío en el estadio de turgencia se deben a que las razas locales de arroz de Yunnan y la entidad del daño por frío esperado en las diferentes regiones arroceras han evolucionado conjuntamente por largos períodos de tiempo.

Introduction

Rice is a cold-sensitive plant that originated in tropical or subtropical areas. The developmental stages from pollen formation to fertilization are the most vulnerable to low temperature in the life cycle of rice plants (Nishiyama 1984). It has been reported that the young microspore stage in pollen development is the most sensitive to low temperature (Satake

and Hayase 1970). Exposure of rice plants at the tetrad stage to a moderately low temperature (12°C) for 4 days resulted in male sterility in 80% of spikelets (Satake and Hayase 1970; Nishiyama 1984). Spikelet fertility of rice decreases if rice plants are exposed to low temperature at booting stage, due to the failure of microspore development under low temperature conditions (Satake 1989). Low temperatures present a major climatic problem for rice cultivation in 25 countries, including Korea, Japan, the USA, China, Australia and even in some tropical countries such as the Philippines and Thailand (Kaneda and Beachell 1974). Cold damage which results in sterility is a very serious problem because it inevitably leads to yield reduction.

Yunnan Province is a centre of genetic and ecological diversity for cold-tolerant rice in China (Zeng et al. 1999). The area at risk from cold damage comprises about one-half (463 000–533 000 ha) of the total rice-cropping area in Yunnan Province (Zeng et al. 2001a). Cold tolerance at booting stage of Yunnan rice landraces was not only ranked first in importance, but was also closely linked with rice-cropping regions and prefectures through a process of acclimation lasting from hundreds to thousands of years. Rice cold injury is a phenomenon that causes a decrease in seed setting rate under conditions of low temperature, little sunshine and sufficient rainfall, particularly in the rice-cropping regions located at high elevations in Yunnan Province.

A total of 912 (primary), 696 (secondary) and 548 core collection accessions were selected based on 31 morphological traits using 6121 accessions; 41 morphological traits and 12 polymorphic isozyme loci using core collections of 912 accessions; and 20 microsatellite markers using core collections of 696 accessions (Zeng et al. 2002, 2004). The grain yield was most severely reduced by low water temperature (below 20°C) during the reproductive period, as a consequence of low spikelet fertility (Shimono et al. 2002).

So far the relationship between geographical distribution and cold tolerance of the second core collection of rice landraces has not been examined either in China or foreign countries. This research therefore aims to determine the differences in cold tolerance at booting stage among landraces from the whole geographical distribution in Yunnan Province, thereby giving an insight into production, breeding and differentiation of cold tolerance at the booting stage of rice.

Materials and methods

A group of 696 accessions (413 *japonica* and 283 *indica*), referred to as the second core collection, represents more than 90% of the genetic diversity of Yunnan rice landraces (Zeng et al. 2002). These accessions were used for the identification of cold tolerance under low water temperatures (18–19°C). The 696-accession core collection showed serious cold damage at booting stage under natural low temperatures (at Kunming, 1910 m asl, 18–20°C) in 2002. They were sown on 19 March, and the seedlings were transferred into paddy on 8 May 2002. Material from each accession was planted in a row of 20 seedlings, with 10 cm within-row spacing and 17.5 cm between-row spacing. The paddy was fertilized on 14 May and evaluated for cold tolerance using the cold-water irrigation method (18–19°C) from 20 days before heading to maturity (from 1 July to 31 October). The depth of water in the paddy field was about 20 cm. After ripening of the seeds, cold tolerance was evaluated based on the mean seed setting rate of five sampled plants, by measuring numbers of full grains, blighted grains and total grains for every spike and calculating the corresponding seed setting rate. The average value of seed setting rate of five plants was regarded as an evaluation index for cold tolerance at booting stage. The variation in cold tolerance was revealed by standard deviation (SD) and coefficient of variation (CV%) of the second core collection from different rice-cropping regions and prefectures in Yunnan Province.

The rice-cropping area in Yunnan Province is divided into 5 regions spread over 16 prefectures, according to distribution of landraces and climate, as shown in Figure 1 (Zhou 1992).

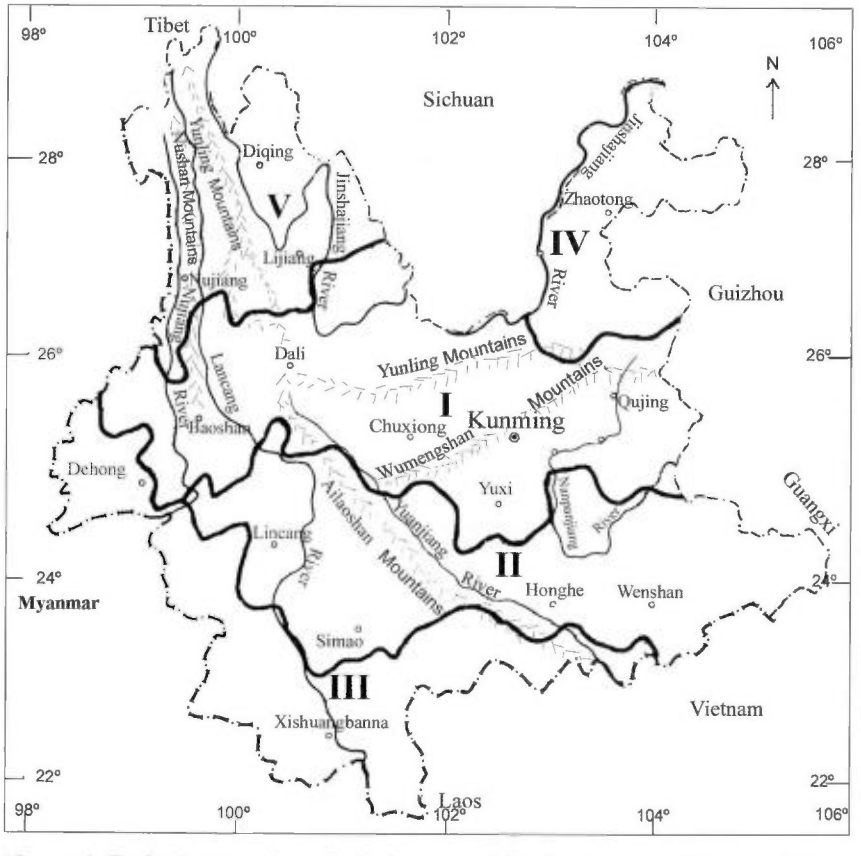


Figure 1. Prefectures and ecological zones of rice landraces in Yunnan, China.

Results

Differences in cold tolerance of rice landraces in the second core collection between the different rice-cropping regions in Yunnan Province

The results showed that there were significant differences in cold tolerance at booting stage of the 696 accessions in the second core collection among the five rice-cropping regions in Yunnan (see Table 1). The average seed setting rates were as follows: northwest Yunnan cold highland *japonica* rice region (region V; seed setting rate 43.79%) > central Yunnan single-season *japonica–indica* region (region I; 27.15%) > northeast Yunnan plateau *japonica* rice region (region IV; 17.22%) > south Yunnan single/double-season rice region (region II; 12.83%) > south marginal paddy–upland rice region (region III; 8.77%). Region IV is located in the northeast at higher altitudes, while region I lies within the central area at a lower altitude; however the seed set of the former was lower than the latter, because the former includes 30 *indica* varieties from the Jinshajiang dry–hot river valley belt (<1500 m asl) in region IV.

In addition, there was a relatively significant standard deviation for seed setting rate from rice-cropping regions in Yunnan: V (36.26) > I (30.71) > IV (25.21) > II (22.07) > III (17.96). CV% was as follows: III (204.79%) > II (172.02%) > IV (146.4%) > I (113.11%) > V (82.8%).

The results showed that accessions from the northwest Yunnan cold highland *japonica* rice region (region V), where cold damage is most prevalent, were extremely resistant to cold damage and had the least variation in cold tolerance. Considering that the 21 *japonica* accessions were predominant and the 4 *indica* accessions were supplementary, the breeding objective should primarily concentrate on breeding for cold tolerance in *japonica* rice. Thus, rice-cropping region V is the ideal breeding basis for cold tolerance at the booting stage of *japonica* rice in China, especially the Lijiang prefecture.

The central Yunnan single-season *japonica–indica* region (region I) is a region where rice-cropping cold damage is relatively pronounced and the cold tolerance variation is relatively small. The numbers of *indica* and *japonica* rice accessions of the second core collection are not large in this region, so the breeding objective of cold tolerance of *indica* rice should be considered first. A tremendous change in rice production, which became necessary due to the lack of a cold-tolerance breeding programme for *indica* in this region, has taken place over the last two decades. In particular, the zone where *indica* and *japonica* rice overlapped has been basically taken over by *japonica* rice alone. Further research has revealed that rice-cropping region I is the ideal basis for breeding cold-tolerant varieties and near-isogenic lines (NILs) with cold tolerance at booting stage in Chinese rice, such as *japonica* in Kunming and *indica* in Yuxi prefecture.

The cold damage was relatively slight but the variation was particularly large in the northeast Yunnan plateau *japonica* rice region (region IV) and the south Yunnan single/double-season rice region (region II). There was a relatively clear differentiation of *indica* and *japonica* rice and great differences in elevation in the two regions. The former is located in the Jinshajiang dry–hot river valley belt and includes Yanjing, Daguan, Weixin, Yiliang, Zhenxiong and Qiaojia counties in the Zhaotong prefecture and Huize county of the Qujing prefecture. The latter includes a region of *indica* and *japonica* rice interlaced and rice-cropping regions of *indica* and *japonica* grown separately.

The cold damage was the slightest but the variation in cold tolerance was the largest in region III (south marginal paddy–upland region). This region is a centre of genetic and gene diversity of Yunnan rice (Zeng et al. 2004). As a result of the humid–hot weather in this region, *indica* and *japonica*, paddy and upland, glutinous and non-glutinous landraces were distributed in this area. Therefore high production, high quality and resistance to diseases should be the main breeding objectives.

Differences in cold tolerance of rice landraces in the second core collection between the different prefectures in Yunnan Province

In Table 2, the relative cold tolerance scores at booting stage of the 696 accessions of the core collection, measured as percentage seed setting rate, are listed in order (highest to

Table 1. Differences in cold tolerance of second core collection accessions in different rice-cropping regions of Yunnan Province, China

Rice cropping region[a]	Elevation (m)	April–October air temperature (°C)	Annual rainfall (mm)	Accessions			Seed setting rate (%)		
				japonica	*indica*	Total	Mean	SD	CV
I	1500–2000	17–20	800–1600	78	55	134	27.15	30.71	113.11
II	800–1600	19–22	800–1600	120	83	204	12.83	22.07	172.02
III	600–1200	20–23	1300–2000	173	111	282	8.77	17.96	204.79
IV[b]	277–2300	17–22	700–1000	21	30	51	17.22	25.21	146.40
V	2000–2700	15–18	600–1300	21	4	25	43.79	36.26	82.80

[a] I = central Yunnan single-season *japonica–indica* region, II = south Yunnan single/double-season rice region, III = south marginal paddy–upland rice region, IV = northeast Yunnan plateau *japonica* rice region, V = northwest Yunnan cold highland *japonica* rice region.

[b] 30 *indica* varieties in region IV are located at elevations of 600–1500 m asl.

Table 2. Differences in cold tolerance of second core collection rice accessions among prefectures of Yunnan Province, China

Prefecture	Elevation (m)	Annual air temperature (°C)	Annual rainfall (mm)	Accessions			Seed setting rate (%)		
				japonica	*indica*	Total	Mean	SD	CV
Kunming	1500–2400	14.5	800–1200	6	1	7	57.33	26.96	47.03
Lijiang	1020–2650	12.6	910–1040	14	6	20	46.33	34.67	74.83
Nujiang	1000–2500	12.8	1100–1185	7	4	11	43.11	39.17	90.86
Qujing	1800–2200	14.2	859–1828	18	3	21	41.94	30.27	72.17
Dali	1500–2200	14.9	800–1000	7	2	9	37.85	29.16	7.04
Chuxiong	800–2000	15.5	615–1000	6	2	8	26.04	31.86	122.35
Yuxi	400–1900	15.7	805–964	19	18	37	22.92	28.91	126.13
Zhaotong	300–2200	16.2	660–1230	21	29	50	16.45	25.40	154.41
Baoshan	550–2000	15.6	700–2100	19	25	44	15.68	25.81	164.60
Wenshan	200–1800	17.4	992–1329	21	19	40	15.55	21.96	141.22
Simao	600–1900	18.2	1155–1878	94	18	142	11.97	21.81	182.21
Lincang	500–2000	17.3	920–1750	83	39	122	10.93	19.66	179.87
Honghe	100–2000	18.5	816–2286	27	16	43	9.51	16.16	169.93
Xishuangbanna	500–1900	22.0	1100–1900	41	36	77	7.87	18.80	238.88
Dehong	300–1800	20.0	1400–1700	27	34	61	6.94	17.46	252.68
Diqing	1500–2700	14.6	600–700	4	0	4	2.10	2.76	131.43

lowest) by prefecture in Yunnan Province. On the basis of these results, accessions from the 16 prefectures can be divided into strong, intermediate, and weak cold-tolerance landraces. The regions from which they originate are correspondingly divided into three categories, namely the serious cold-damage region, moderate cold-damage region and slight cold-damage region.

The serious cold-damage region includes Kunming, Lijiang, Nujiang, Qujing and Dali prefectures (especially those areas at an elevation of 1900–2700 m asl), where there is a risk of serious cold damage. Under Kunming's natural low temperature in 2002, the seed setting rate (%) of accessions from these prefectures ranged from 37.85% to 57.33% and CV% was relatively low (7.04–90.86%). In addition, some regions over 1900 m asl in Zhaotong prefecture and areas over 2000 m asl in Diqing prefecture also belong to the serious cold-damage region.

The moderate cold-damage region mainly includes Chuxiong, Yuxi, Zhaotong, Baoshan and Wenshan prefectures. Under Kunming's natural low temperature in 2002, percentage seed setting rate and CV% of accessions from these prefectures were 15.55–26.04% and 122.35–164.60% respectively. Distribution in these areas is concentrated at 1600–1850 m asl in Chuxiong, Yuxi, Wenshan and Zhaotong prefectures and areas of Tengchong and Longling counties with enough rain in Baoshan prefecture.

The slight cold-damage region mainly includes Simao, Lincang, Honghe, Xishuangbanna, Dehong and five prefectures and the river valley zone in Deqing prefecture. Under Kunming's natural low temperature in 2002, seed setting rate of accessions from these prefectures (except for Diqing) was relatively low, ranging from 6.94% to 11.97%, but CV% was relatively high (169.93–252.68%).

The results of this study revealed that genetic diversity for cold-tolerant rice resources almost exactly corresponded to the centre of genetic diversity for rice germplasm in Yunnan. Furthermore, it was shown that temperature variation was not only the dominant factor resulting in differentiation between *indica* and *japonica* rice but was also the basic reason underlying the difference between six ecological groups of *indica* and *japonica* and the large genetic diversity for rice resources in Yunnan. Strong or weak cold tolerance of the core collection at booting stage reflected the whole pattern of rice-cropping cold damage in these prefectures throughout the year.

Conclusions

There is a relatively large difference in cold tolerance at booting stage among accessions of the second core collection from rice-cropping regions in Yunnan Province, China. Temperatures are highly variable throughout Yunnan Province because of variable altitudes. Accessions from the northwest Yunnan cold highland *japonica* rice region and central Yunnan single-season *japonica–indica* region are the strongest and landraces domesticated in the south marginal paddy–upland rice region are the weakest. Variation in cold tolerance (CV%) showed the opposite pattern. Developing cold-tolerant varieties and suitable cultural practices is of great concern for the future because this will lead to consistently high yields in

cold regions, particularly in rice-cropping regions V (Lijiang and Nujiang prefectures) and I (Kunming, Qujing and Dali prefectures).

Since 1975, about 3000 varieties and breeding lines have been tested, and 25 of the 40 varieties with the strongest cold tolerance have originated from Yunnan Province in China (Javier and Toledo 2001). Therefore, the cold tolerance at booting stage of Yunnan rice germplasm, especially from the northwest Yunnan cold highland *japonica* rice region and central Yunnan single-season *japonica–indica* region, is the most important in the world. Yunnan Province is not only a centre of genetic and ecological diversity for rice resources in China (Zeng et al. 2003), but is also a centre of genetic and ecological diversity for cold-tolerant rice resources worldwide (Zeng et al. 1999).

Sixteen prefectures in Yunnan were grouped into strong, intermediate and weak cold-tolerance regions (i.e. serious, moderate and slight cold-damage regions). The cold tolerance of Yunnan rice landraces at booting stage was closely correlated with the degree of cold tolerance of accessions among rice-cropping regions and prefectures. In addition, the ecological and genetic diversity of Yunnan rice resources could be roughly divided into a central region of diversity, a scattering region of diversity and a lacking region of diversity (Zeng et al. 2001b).

Temperature is the dominant factor controlling the formation and distribution of the two ecological groups, *indica* and *japonica* (Zeng et al. 2000a, 2000b). The differences in cold tolerance at booting stage resulted from Yunnan rice landraces and variations in cold damage between the different rice-cropping regions having co-evolved together over a long period of time. The hybrids domesticated for 2–3 years could be obtained and strengthened for cold tolerance at booting stage (Zeng et al. 2001c). In addition, water temperature can affect various growth processes, and responses to water temperature can differ according to growth stage. The standard screening methods and facilities used need to be reliable in providing the required low air and water temperatures for the whole growing period (as in the cold-water irrigation screening nursery at Kunming). Shimono et al. (2001) confirmed that grain yield was most severely reduced by low water temperature (below 20°C) during the reproductive period, caused by low spikelet fertility, and found that cool water temperatures at any growth period reduced crop growth rate by at least 8% to as much as 74%.

Acknowledgments

This research was supported by the Natural Science Foundation of China (Grant nos. 30160043, 30260060) and a grant from the Yunnan Natural Science Foundation of China (Grant no. 2002C0077M) and Yunnan Introduction and Foster Talent of China (Grant no. 2005PY01-14).

References

Javier EL, Toledo MC. 2001. Finding genetic donors for cold tolerance in the INGER gene pool. In: Fukai S, Basnayake J, editors. Increased Lowland Rice Production in the Mekong Region. ACIAR Proceedings PR101, pp. 147–151.

Kaneda C, Beachell HM. 1974. Breeding rice for cold tolerance. Saturday Seminar Paper 9. IRRI, Los Baños, Philippines.

Nishiyama I. 1984. Climate influence on pollen formation and fertilization. In: Tsunoda S, Takahashi N, editors. Biology of Rice. Elsevier, Amsterdam, The Netherlands, pp. 153–171.

Satake T. 1989. Male sterility caused by cooling treatment at the young microspore stage in rice stage plants. XXIX. The mechanism of enhancement in cool tolerance by raising water temperature before the critical stage. Japanese Journal of Crop Science 58:240–245.

Satake T, Hayase H. 1970. Male sterility caused by cooling treatment at the young microspore stage in rice plants: estimation of pollen developmental stage and most sensitive stage to coolness. Proceedings from the Crop Science Society of Japan 39:468–473.

Shimono H, Hasegawa T, Iwama K. 2001. Response of growth and grain yield to cool water at different growth stages in paddy rice. In: Fukai S, Basnayake J, editors. Increased Lowland Rice Production in Mekong Region. ACIAR Proceedings PR101, pp. 118–128.

Zeng YW, Shen SQ, Xu FR. 1999. Ecological diversity of cold tolerant rice in Yunnan, China. Plant Genetic Resources Newsletter 117:43–47.

Zeng YW, Shen SQ, Lin XH. 2000a. Cold tolerance at booting stage of hybrids of *indica* and *japonica* with strong cold tolerance. Journal of Huazhong Agriculture University 19(5):411–416.

Zeng YW, Shen SQ, Xu FR, Zhang JY, Pu ZG. 2000b. Genetic variation of cold tolerance characters of Yunnan rice landraces at *indica* and *japonica* rice cropping regions. Chinese Journal of Rice Science 14(3):133–138.

Zeng YW, Ye CR, Shen SQ. 2001a. Development of near-isogenic lines and genetic analysis for cold tolerance at booting stage in rice. Progress in Natural Science 11(1):94–96.

Zeng YW, Li ZC, Shen SQ. 2001b. Diversity and good germplasm of indigenous rice varieties in Yunnan Province, China. Chinese Journal of Rice Science 15(3):169–174.

Zeng YW, Shen SQ, Wen GS. 2001c. Genetic studies of cold tolerance characters of the RIL population of Xiaobaigu × Dalizaoxian. Journal of the Southwest China Agricultural University 23(6):494–497.

Zeng YW, Wang XK, Li ZC. 2002. Establishing of core collection bank of Yunnan rice germplasm resources. Chinese Cultivated Science Technology (4):41.

Zeng Y, Shen S, Li Z, Yang Z, Wang X, Zhang H, Wen G. 2003. Ecogeographic and genetic diversity based on morphological characters of indigenous rice (*Oryza sativa* L.) in Yunnan, China. Genetic Resources and Crop Evolution 50:567–577.

Zeng YW, Li ZC, Zhang HL, Shen SQ, Liu X, Wang XK, Sun JL, Wen GS. 2004. Evaluation of genetic diversity in the rice landraces (*Oryza sativa* L.) in Yunnan, China. Breeding Science 54 (in press).

Zhou YH. 1992. Rice Cropping Regional Division in Yunnan Province, China. Yunnan Science and Technology Press, Kunming, China.

Agronomic and yield performance of induced-autotetraploid ensete (*Ensete ventricosum*) clones

Tesfaye Messele
Institute of Biodiversity Conservation and Research, Addis Ababa, Ethiopia. Email: tesfaye2001et@yahoo.co.uk

Summary

Agronomic and yield performance of induced-autotetraploid ensete (*Ensete ventricosum*) clones

Ensete is an important source of food for approximately 20% of the Ethiopian population. Conventional ensete yield improvement requires 3–8 years. Therefore, an efficient alternative technique is essential. It has been suggested that polyploids can be more successful than their diploid progenitors under certain conditions. Inducing polyploidy from the ancestral plant showed far-reaching effects for yield improvement in some plants. However, ensete tetraploids produced by a direct doubling from diploid progenitor were weak. The possibility of improving ensete yields by novel techniques such as cell fusion and selection of agronomically promising clones are believed to be more promising alternative methods than inducing enset polyploids.

Key words: Autotetraploid, colchicine, diploid, ensete, polyploidy, yield

Résumé

Performance agronomique et rendement de clones autotétraploïdes induits chez le bananier d'Abyssinie (*Ensete ventricosum*)

Le bananier d'Abyssinie est une importante ressource alimentaire pour environ 20 % de la population éthiopienne. L'amélioration du rendement nécessite de 3 à 8 ans par les techniques conventionnelles. Il est donc important de disposer d'une autre méthode plus efficace. Il a été suggéré que, dans certaines conditions, il était préférable d'utiliser des polyploïdes plutôt que leurs ascendants diploïdes. L'induction de la polyploïdie à partir des plantes ancestrales produit une amélioration conséquente du rendement chez certaines plantes. Cependant, les tétraploïdes de bananier d'Abyssinie produits par doublement direct du génome à partir de parents diploïdes sont chétifs. La possibilité d'améliorer les rendements du bananier d'Abyssinie par de nouvelles techniques telles que la fusion cellulaire et la sélection de clones intéressants du point de vue agronomique est considérée comme une alternative plus prometteuse que l'induction de polyploïdes.

Resumen

Desempeño agronómico y rendimiento de clones autotetraploides inducidos de ensete (*Ensete ventricosum*)

El enset es una fuente importante de alimentos para un 20% de la población de Etiopía. El mejoramiento convencional del rendimiento del enset lleva de 3 a 8 años, por lo que es esencial contar con una técnica alternativa eficiente. Se ha sugerido que en ciertas condiciones los progenitores poliploides pueden tener más éxito que los diploides. En algunas plantas la poliploicidad inducida a partir de la planta ancestral mostró efectos de largo alcance para el mejoramiento del rendimiento. No obstante los enset tetraploides producidos por duplicación directa del progenitor diploide fueron débiles. Se estima que la posibilidad de mejorar los rendimientos de enset mediante técnicas novedosas como la fusión de células y la selección de clones agronómicamente prometedores son métodos alternativos de mayores perspectivas que los poliploides inducidos de enset.

Introduction

Ensete (*Ensete ventricosum* (Welw.) Cheesman) belongs to the family Musaceae, to which abaca (*Musa textilis*) and banana (*M. paradisica*) also belong (Zeven and de Wet 1982). It is a diploid species with a chromosome number of 2n = 18 (Westphal 1975).

Ensete is an important source of food for approximately 20% of the Ethiopian population (Tsedeke Abate et al. 1996). Almost every part of the plant is economically important and is used for various purposes including feed, as a fibre source and for the starchy food products processed from its pseudostem (sheathing midribs) and corm. The pseudostem of an ensete plant is that part of the plant located between approximately 20 cm above the ground to just below the bases of the very top sheathing leaves. The size of the pseudostem varies, based on the type of clone and environmental growing conditions. The length and number of sheathing midribs determine the yield of an ensete plant. The larger the pseudostem, the higher the yield per unit area of land.

Since the plant flowers late, breeding for yield improvement requires 3–8 years; therefore it is essential to look for efficient alternative techniques to help in improving ensete yield. One of these techniques is inducing polyploidy from the ancestral plant. Polyploidy has far-reaching effects in nature, including the improvement of existing plant species or the generation of new ones (Lewis 1980). It has been suggested that polyploids can be more successful than their diploid progenitors under certain conditions (Tal 1980). In many instances polyploidy causes changes in the shape and texture of organs, by allocating significantly greater dry matter weight than is found in their diploid progenitors (Lewis 1980).

The yield performance of some autotetraploid plants is higher than that of their diploid ancestors in most plants. Comparisons between 2n and 4n varieties of crops using dry matter weight, fresh weight of trifoliate leaves, and DNA content of these leaves have revealed that differences were strongly influenced by increasing the ploidy level of a plant (Zeven 1980). The objective of this study is, therefore, to compare the performance of diploid and autotetraploid ensete plants.

Materials and methods

Two clones of ensete varieties AA1 and IL1 collected from the Addis Ababa and Illubabor areas, respectively, were used. Treating 10-day-old seedlings of ensete plants with 0.2% colchicine solution induced the autotetraploid ensete. The

diploid (2n) progenitor and the induced autotetraploid (4n) ensete plants from both clones were planted in a randomized complete block design in three replicates. Plot size was 10 rows with 2.5 m spacing. Both 2n and 4n ensete were planted in the same field on red clay soil at altitude of 2400 metres above sea level. Ample manuring was achieved by mixing soils in circular band of 10 cm, a technique practised locally as a means of enriching soils.

Five years after planting (i.e. when the plant reached physiological maturity for harvest), comparisons of diploid and autotetraploid ensete plants were made based on various yield parameters. Fifteen plants were randomly collected per clone, and all 60 plants (both diploids and autotetraploids) were analysed. For both 2n and 4n plants of both clones, 11 yield parameters which included leaf length (LL), leaf width (LW), number of leaves per plant (NL/P), pseudostem midrib width (PMW), pseudostem height (PH) and girth (PG), total plant height (TPH), pseudostem fresh weight (PFW), pseudostem dry matter weight (PDMW), total biomass weight (TBMW), total dry matter weight (TDMW) and harvest index (HI) were measured and calculated.

Results and discussion

The performance of two diploid ensete clones and their respective autotetraploid clones was measured and compared (Table 1). It was apparent that the ploidy change from diploid to autotetraploid was accompanied by a striking loss of vigour. Except for midrib width, the diploid plants showed a significantly higher relative value than the autotetraploids for all parameters. The differences ranged from 19% to 98% (Table 1). The pseudostems of the autotetraploids were shorter by 80–88% than those of the diploids. Leaves of the autotetraploids were shorter and thinner than those of the diploids.

The total biomass calculated for the diploid per plant was 38 and 34 kg, while for the autotetraploid per plant it was 22 and 21 kg for AA1 and IL1, respectively. The total biomass and dry matter weight of the diploid plant were higher, due to greater height, larger number of midribs and bigger leaves than the autotetraploids. Consequently, the total yield of the autotetraploid ensete was 15 and 14 kg, while for the diploids it was 32 and 28 kg, for AA1 and IL1, respectively. The difference in harvest index was 19% for AA1 and 20% for IL1 (Table 1).

Food quantity, fibre length and strength are among the most important parameters considered when selecting ensete clones. The larger the pseudostem, the higher the yield per unit area of land (Endale Tabogie et al. 1996). The growth of the autotetraploids was stunted when compared with their diploid progenitors (Figure 1). The autotetraploid ensete plants were 72 cm shorter than their diploid counterparts. Increasing the ploidy level of some plant species, such as potato, sugar beet, ryegrass, red clover, alfalfa, many ornamental plants, banana, caraway (Dijkstra and Speckmann 1980) and apple produced larger dimensions, higher yield or greater tuberization and better adaptability than the diploid progenitors (Zeven 1980; Zeven and de Wet 1982).

Tal (1980) suggested that increasing the cell size or increasing the ploidy level does not necessarily lead to increased size of the plant as a whole or even of its individual organs. This is because, for some plants, the optimal ploidy level in terms of yield seems to be the diploid, while for others it may be the tetraploid. Additional chromosome numbers beyond the optimal number or ploidy level turns out to have a detrimental

Table 1. Yield parameters for comparing diploid and tetraploid ensete

Characters (units)	Means of ensete clones					
	AA1			IL1		
	2n	4n	Difference (%)	2n	4n	Difference (%)
LL (cm)	465a	76c	84	320b	50d	84
LW (cm)	78a	23b	71	70a	20b	71
NL/P	7a	5b	29	7a	5b	29
PMW (cm)	21b	34a	38	18b	29a	38
PH (cm)	280a	33c	80	160b	19d	88
PG (cm)	140a	50c	64	95b	47c	57
TPH (m)	745a	72c	96	480b	46d	95
PFW (kg)	32a	15c	53	28b	14c	50
FBMW (kg/P)	38a	22c	58	34b	21c	62
PDMW (g)	5379a*	58c	98	978b*	32d	97
TDMW (g)	7987a*	194c	98	2128b*	52d	98
HI (%)	84	68	19	82	66	20

* Significant at 5 % level of probability based on Duncan's multiple-range test.
Values in rows with the same letter are not statistically different at the 5% level of Duncan's multiple-range test.
AA1 = collected from Addis Ababa area, central region of Ethiopia.
IL1 = collected from Illubabor region, southwestern region of Ethiopia.
For other abbreviations please refer to text.

Figure. 1. Three year old ensete plant. At the front is the diploid (control); the two at the back right and left of the control are autotetraplods (4n) ensete plants.

effect on the plant (Dewey 1977, 1980). Bingham (1980) suggested that genetic interactions involving more than two alleles in the diallelic duplex state are important determinants of yield. The success of tetraploids (Dewey 1977) can be achieved only if their populations have a broad-based diversity and heterozygosity. In maize (Lewis 1980), tetraploids produced by a direct doubling of inbred diploid lines were weak. In contrast, tetraploids produced by doubling diploids from open-pollinated varieties and hybrid stocks were vigorous. Ensete is a vegetatively propagated crop and is usually harvested before setting flowers. These characteristics will not allow the plant to interchange characters or develop heterozygosity.

It can therefore be concluded that increasing the ploidy level of ensete will not serve as a means for improving vigour and yield of the plant. However, apart from inducing polyploidy, the selection of agronomically promising clones or the possibility of developing heterozygosity by means of novel techniques such as cell fusion are believed to serve as potential alternative methods of improving the yield of the ensete crop.

References

Bingham ET. 1980. Maximizing heterozygosity in autopolyploids. In: Lewis WH, editor. Polyploidy: Biological Relevance. Plenum Press, New York, USA. pp. 471–489.

Dewey DR. 1977. Morphology, cytology, and fertility of F_1 and induced-amphiploid hybrids of *Elymus canadensis* × *Agropyron subsecundum*. Crop Science 17:106–111.

Dewey DR. 1980. Some applications and misapplications of induced polyploidy to plant breeding. In: Lewis, WH, editor. Polyploidy: Biological Relevance. Plenum Press, New York, USA. pp. 445–470.

Dijkstra H, Speckmann GJ. 1980. Autotetraploidy in caraway (*Carum carvi* L) for the increase of the aetheric oil content of the seed. Euphytica 29:89–96.

Endale Tabogie, Mulugeta Diro, Bezuayehu Hailu. 1996. Enset improvement research: past and present. In: Tsedeke Abate, Hiebsch C, Brandt SA, Seifu Gebremariam, editors. Enset-Based Sustainable Agriculture in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia. pp. 228–234.

Lewis WH. 1980. Polyploidy in species populations. In: Lewis WH, editor. Polyploidy: Biological Relevance. Plenum Press, New York, USA. pp. 103–144.

Tal M. 1980. Physiology of polyploids. In: Lewis WH, editor. Polyploidy: Biological Relevance. Plenum Press, New York, USA. pp. 61–76.

Taye Bezuneh. 1996. An overview on enset research and future technological needs for enhancing its production and utilization. In: Tsedeke Abate, Hiebsch C, Brandt SA, Seifu Gebremariam, editors. Enset-Based Sustainable Agriculture in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia. pp. 1–14.

Tsedeke Abate, Hiebsch C, Brandt SA, Seifu Gebremariam. 1996. Introduction. In: Tsedeke Abate, Hiebsch C, Brandt SA, Seifu Gebremariam, editors. Enset-Based Sustainable Agriculture in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia. pp. i–iii.

Westphal E. 1975. Agricultural Systems in Ethiopia. Pudoc, Wageningen, The Netherlands.

Zeven AC. 1980. Polyploidy and domestication: the origin and survival of polyploids in cytotype mixtures. In: Lewis WH, editor. Polyploidy: Biological Relevance. Plenum Press, New York, USA. pp. 385–408.

Zeven AC, de Wet JMJ. 1982. Musaceae. In: Zeven AC, de Wet JJ, editors. Dictionary of Cultivated Plants and Their Region of Diversity. Pudoc. Wageningen, The Netherlands. pp. 140–141.

Assessing genetic diversity in a germplasm collection of kola trees (*Cola nitida* (Vent.) Schott and Endl.) using enzymatic markers

R.S. Sié,[1]✉ J.A.K. N'Goran,[2] C. Montagnon,[3] D.S. Akaffou,[1] C. Cilas[3] and F. Mondeil[4]

[1] UFR-SN, Université d'Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire. Email: sieraoul@yahoo.fr
[2] CNRA, 01 BP 1827 Abidjan 01, Côte d'Ivoire
[3] CIRAD-Montpellier, BP 5035-34032, Montpellier cedex 1, France
[4] UFR Biosciences, Université de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire

Summary

Assessing genetic diversity in a germplasm collection of kola trees (*Cola nitida* (Vent.) Schott and Endl.) using enzymatic markers

The genetic diversity of a germplasm collection of kola trees (*Cola nitida* (Vent.) Schott and Endl.) maintained in Côte d'Ivoire and consisting of accessions originating from Côte d'Ivoire, Guinea and Nigeria, was assessed using 6 enzyme systems and 11 loci. These enzyme systems were GPI with 3 loci and 17 alleles, PGM (2 loci, 4 alleles), IDH (2 loci, 3 alleles), EST (1 locus, 3 alleles), ACP (1 locus, 2 alleles) and MDH (2 loci, 5 alleles). Nine of the 11 loci were polymorphic. A hierarchical ascendant classification (cluster analysis) revealed that the total population was structured into three genetically distinct and diversified groups: A, B and C. The fixation index (F_{st}) and the inbreeding coefficient within subpopulation (F_{is}) were found to be +0.20 and –0.15, respectively. The three groups (A, B and C) differ at allele *f* of the locus *Gpi-2* and the alleles *e*, *f* and *g* of the locus *Gpi-3*. Each group is heterogeneous and contained both Ivorian, Guinean and Nigerian genotypes. The originality of these groups was confirmed by a factorial discriminant analysis. The results led to the conclusion that the three populations studied would have had a common centre of dispersion.

Key words: *Cola nitida*, electrophoresis, enzymes, genetic diversity

Résumé

Évaluation de la diversité génétique dans une collection de matériel génétique de kolatiers (*Cola nitida* (Vent.) Schott et Endl.) en utilisant des marqueurs enzymatiques

La diversité génétique dans une collection de matériel génétique de kolatiers (*Cola nitida* (Vent.) Schott et Endl.) conservée en Côte d'Ivoire et constituée d'accessions originaires de Côte d'Ivoire, Guinée et Nigeria, a été évaluée en utilisant 6 systèmes enzymatiques et 11 loci, dans lesquels GPI représente 3 loci et 17 allèles, PGM (2 loci, 4 allèles), IDH (2 loci, 3 allèles), EST (1 locus, 3 allèles), ACP (1 locus, 2 allèles) et MDH (2 loci, 5 allèles). Neuf des 11 loci sont polymorphes. Une classification hiérarchique ascendante (analyse de clusters) révèle que la population totale est structurée en trois groupes génétiquement distincts et diversifiés : A, B et C. L'indice de fixation (F_{st}) et le coefficient d'endogamie dans les sous-populations (F_{is}) sont de +0,20 et –0,15, respectivement. Les trois groupes (A, B et C) diffèrent au niveau de l'allèle *f* du locus *Gpi-2* et des allèles *e*, *f* et *g* du locus *Gpi-3*. Chaque groupe est hétérogène et contient à la fois des génotypes ivoiriens, guinéens et nigérians. L'originalité de ces groupes est confirmée par une analyse factorielle discriminante. Les résultats permettent de conclure que les trois populations étudiées ont un centre de dispersion commun.

Resumen

Evaluación de la diversidad genética en una colección de germoplasma de árboles de cola (*Cola nitida* (Vent.) Schott y Endl.) empleando marcadores enzimáticos

Se evaluó la diversidad genética de una colección de germoplasma de árboles de cola (*Cola nitida* (Vent.) Schott y Endl.) conservada en Costa de Marfil y que consiste de accesiones provenientes de Costa de Marfil, Guinea y Nigeria, empleando 6 sistemas de enzimas y 11 loci. Estos sistemas de enzimas eran GPI con 3 loci y 17 alelos, PGM (2 loci, 4 alelos), IDH (2 loci, 3 alelos), EST (1 locus, 3 alelos), ACP (1 locus, 2 alelos) y MDH (2 loci, 5 alelos). Nueve de los 11 loci eran polimórficos. Una clasificación jerárquica ascendente (análisis de conglomerados) reveló que toda la población se estructuraba en tres grupos genéticamente distintos y diversificados: A, B y C. Se determinó que el índice de fijación (F_{st}) y el coeficiente de intracruzamiento en la subpoblación (F_{is}) eran de +0,20 y –0,15 respectivamente. Los tres grupos (A, B y C) diferían en el alelo *f* del locus *Gpi-2* y en los alelos *e*, *f* y *g* del locus *Gpi-3*. Cada grupo es heterogéneo y contiene genotipos guineanos, nigerianos y marfilenses. El carácter original de estos grupos quedó confirmado por un análisis discriminante factorial. Los resultados llevan a la conclusión de que las tres poblaciones estudiadas habrían tenido un centro común de dispersión.

Introduction

The kola tree, *Cola nitida* (Vent.) Schott and Endl. is indigenous to West Africa (Bodard 1960). Its fruits contain seeds known as kola nuts. The nuts are consumed for their stimulant properties and their taste and are traditionally used in western and central Africa during weddings, funerals and ritual sacrifices. They are also used in the pharmaceutical and food industries to produce cardiac stimulants, laxatives, sedatives and sodas (Egbe and Oladokun 1987).

Kola cultivation is relatively recent in Côte d'Ivoire and most of the kola nuts sold in the markets come from old and low-yielding wild or semi-wild trees. Because the use of high-yielding genotypes is essential for the development of kola production in Côte d'Ivoire, a kola improvement programme was designed by the national agricultural research institute, Centre National de Recherche Agronomique de Côte d'Ivoire (CNRA), and a germplasm collection of about hundred genotypes of diverse

origins (Côte d'Ivoire, Guinea and Nigeria) was set up. The first step consisted in simple phenotypic assessments of the genotypes and their progenies (Bonsson 1983) and this led to the selection of a few parents whose progenies gave relatively good yields. No prior genetic diversity study had been conducted before the different crosses were carried out, although it was known that such study would have enabled a better choice of the crosses. To assess the genetic diversity of the germplasm established, enzymatic markers that are more stable in relation to environmental effects were used.

Enzymatic markers have been already used to study genetic diversity in many tropical plants including cocoa (Lanaud 1987), coffee (Leroy et al. 1993) and sorghum (Djè et al. 1998). For the species examined, the genetic variability and the structure obtained were useful in establishing appropriate breeding strategies.

This paper describes the organization and the genetic variability of kola germplasm maintained in Côte d'Ivoire and its consequences for kola-tree breeding.

Materials and methods

Plant material

The plant material used consisted of 79 genotypes (41 from Côte d'Ivoire, 9 from Guinea and 29 from Nigeria) collected from primary forests (material from Côte d'Ivoire and Guinea) and from smallholdings set up with local planting material (Nigerian genotypes). The germplasm was maintained at the CNRA station at Bingerville in Côte d'Ivoire. In both Côte d'Ivoire and Guinea, the survey strategy consisted in taking genotypes from several sites spread over the natural range of habitats of the plant. The trees were planted in a design consisting of totally randomized elementary plots of 1–5 replicates per genotype, with a planting density of 312 trees per hectare.

Electrophoresis method

Six enzyme systems with 11 loci were used. These were malate dehydrogenase (EC 1.1.1.37, MDH) with two loci, isocitrate dehydrogenase (EC 1.1.1.42, IDH) with two loci, acid phosphatase (EC 3.1.3.2, ACP) with one locus, glucose phosphate isomerase (EC 5.3.1.9, GPI) with three loci, phosphoglucomutase (EC 5.4.2.2, PGM) with two loci, and esterase (EC 3.1.1.1, EST) with one locus (Sié 1999).

Electrophoresis was carried out on 12.5% starch gel. Two migration systems were used: histidine–citrate pH6 and histidine–citrate pH8. For both systems we used a gel buffer (0.05 M histidine and 0.1 M Tris) and a electrode buffer (0.15 M Tris, 0.04 M citric acid). We adjusted pH with Tris. Enzymes were extracted by crushing pieces of fresh leaves in a small amount of extraction buffer consisting of 0.5 M Tris–HCl, pH7, 0.3 M ascorbic acid, 0.01 M EDTA, 2% mercaptoethanol and 1.5 10^{-5}M of potassium cyanide (KCN) at pH7. Filter paper wicks (Whatman No. 3) were dipped into the leaf extracts. The wicks were then removed, lightly blotted and loaded into a transverse cut in the gels. Information on the genetic determinism (number of loci and number of alleles) of these enzyme systems can be found in Sié (1999).

The first three enzymes (MDH, IDH and ACP) were migrated on a histidine Tris–citrate migration system with a constant current (40 mA for 6–7 h) at pH8. GPI and PGM were migrated on the histidine Tris–citrate migration system with a discontinuous current (20 mA for 1 h 30 min, 30 mA for 1 h 40 min and 40 mA for 4 h) at pH6. For EST, the histidine Tris–citrate migration system was used with a constant current (40 mA for 6 h) at pH6.

Data analysis

Wright's fixation indexes (Wright 1951), F_{st} (fixation index) and F_{is} (inbreeding coefficient within subpopulations), were used to analyse the diversity existing in the overall population of kola trees studied. Thus, F_{is} was used to analyse within-subpopulation diversity and F_{st} between-subpopulation diversity. Each geographical origin was considered as a population.

The statistical method described by Montagnon and Bouharmont (1996) was applied. A factorial correspondence analysis (FCA) was first carried out and each genotype was encoded to each locus as follows: 0 if the allele was absent, 1 if the allele was present in its heterozygous state, and 2 if the allele was present in the homozygous state. Then a hierarchical ascendant classification (HAC) was carried out from the factorial coordinates of the first six FCA axes. The aggregation criterion used was variance. Lastly, a factorial discriminant analysis (FDA) was carried out to confirm the reality of the clusters found with the HAC. The GENEPOP2 statistics program (Raymond and Rousset 1995) was used for the analysis.

Results and discussion

Structure and characterization of kola trees in the collection

The HAC indicated genetic proximity between the genotypes studied (Figure 1). Three genetic groups, A, B and C, were detected, containing 49, 20 and 10 genotypes respectively. The minority group C was the most genetically distant from the others.

The FDA carried out on groups A, B and C confirmed the reality of those groups (100% of correctly classified individuals). Differentiation between these groups occurred primarily for enzyme GPI and allele *f* of locus *Gpi-2* and alleles *e*, *f* and *g* of *Gpi-3*. In fact, these groups differed from each other at the following alleles (Table 1): group A with alleles *Gpi-3g* and *Gpi-2f* at respective frequencies of 0.82 and 0.34, differed from group B which was characterized by allele *Gpi-3f* (0.92), while group C was characterized by allele *Gpi-3e*, which was exclusively present at a frequency of 1.

Glucose phosphate isomerase (GPI) was the most polymorphic enzyme, which revealed all the diversity of the kola trees in the collection. For any future study of the genetic diversity of the kola tree based on enzymes, GPI should be used.

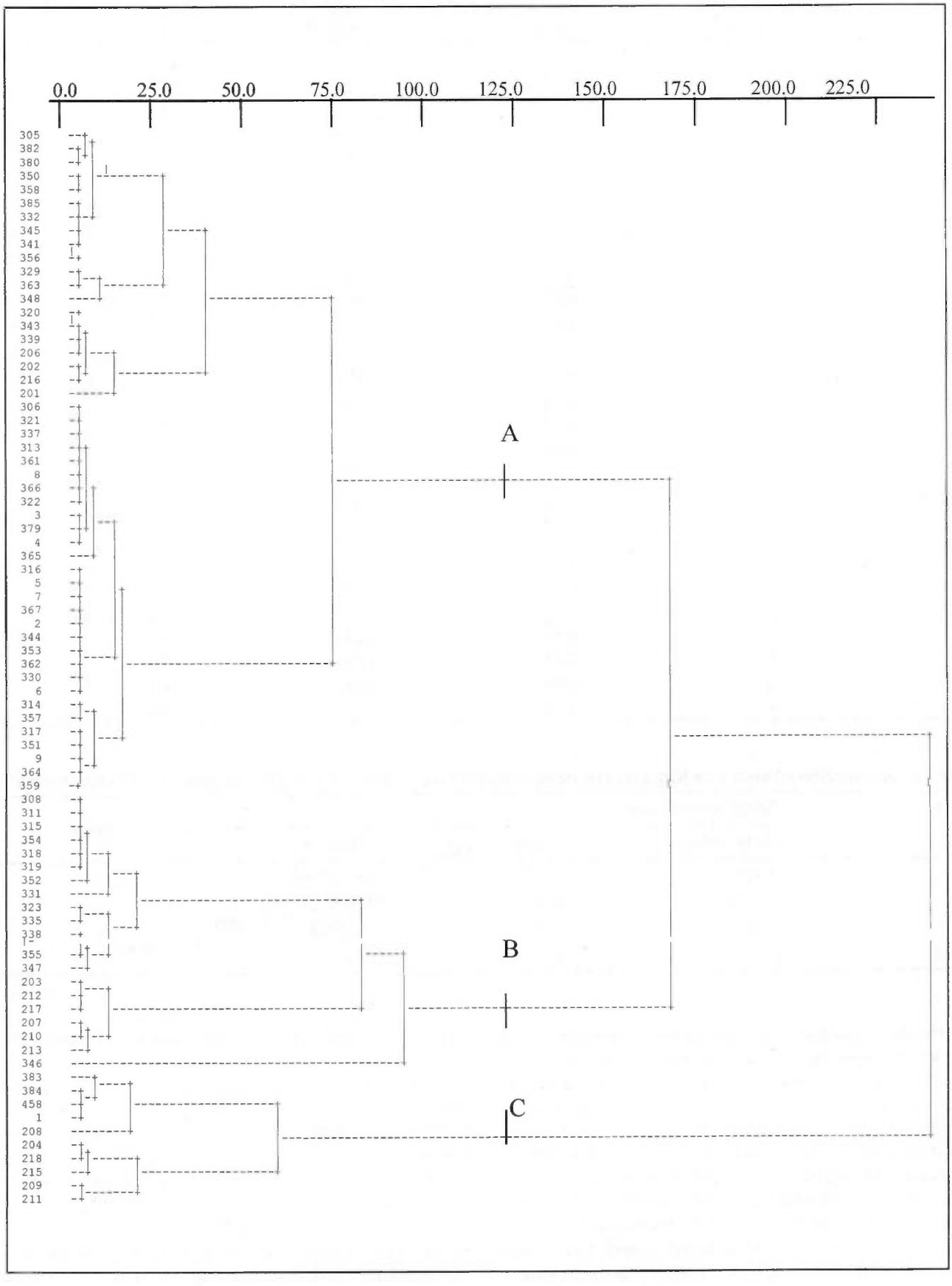


Figure 1. Dendrogram showing kola tree (*Cola nitida*) germplasm diversity.

Table 1. Allelic frequencies per polymorphic locus within the genetic groups of kola trees obtained by HAC

Loci	Alleles	Group A	Group B	Group C
Gpi-2	*a*	0	0.05	0
	b	0.01	0.26	0.28
	c	0.01	0.21	0
	d	0.63	0.45	0.22
	e	0.01	0	0.50
	f	0.34	0.03	0
Gpi-3	*a*	0.01	0	0
	b	0.01	0	0
	c	0	0.03	0
	d	0.07	0	0
	e	0	0	1
	f	0.02	0.92	0
	g	0.82	0.03	0
	h	0.07	0.03	0
Pgm-1	*a*	0.46	0.35	0.50
	b	0.54	0.65	0.50
Pgm-2	*a*	0.46	0.74	0.30
	b	0.54	0.26	0.70
Idh-2	*a*	0.09	0.11	0.06
	b	0.91	0.89	0.94
Mdh-1	*a*	0.32	0.39	0.38
	b	0.68	0.61	0.62
Mdh-2	*a*	0.56	0.58	0.50
	b	0.38	0.35	0.38
	c	0.05	0.07	0.12
Est	*a*	0.60	0.65	0.44
	b	0.18	0.15	0.28
	c	0.21	0.20	0.28
Acp	*a*	0.54	0.63	0.65
	b	0.46	0.37	0.35

Table 2. Distribution of kola tree (*Cola nitida*) geographical origins in the three genetic groups obtained by HAC

Groups	Geographical origin			Total
	Côte d'Ivoire	Guinea	Nigeria	
A	18 (37%)	8 (16%)	23 (47%)	49
B	14 (70%)	0 (0)	6 (30%)	20
C	9 (90%)	1 (10%)	0 (0%)	10
Total	41	9	29	79

The distribution of the genotypes in the three groups was not dependent on their geographical origin (Table 2). In fact, group A contained genotypes from Ivorian, Guinean and Nigerian origins, group B contained Ivorian and Nigerian genotypes, and group C contained Ivorian and Guinean genotypes. This independent distribution of the Ivorian, Guinean and Nigerian genotypes in these three genetic groups indicated that the survey sites were not independent centres of dispersion for this plant. Unlike the kola tree, other perennial plants with the same reproduction system, such as cocoa (Lanaud 1987), and the same natural range, such as coffee *Coffea canephora* (Montagnon et al. 1991), have centres of dispersion that merge with their centres of origin. The lack of allelic differentiation between these different geographical origins reveals the recent human dispersal of kola, from its centre of origin between Liberia and Côte d'Ivoire (Bodard 1960) to the rest of western Africa. According to Dublin (1965) this migration would have taken place in the 19th century.

Axis 1 of the FCA (Figure 2) accounted for 39% of total variability; it was determined by alleles *b*, *e* and *f* of locus *Gpi-2* and alleles *e* and *g* of locus *Gpi-3*. Axis 2 (23.45% of variability) was primarily characterized by *Gpi-3f*. This FCA revealed a distinction between the three groups. Group C was separated from groups A and B on axis 1. Axis 2 separated group B from groups A and C.

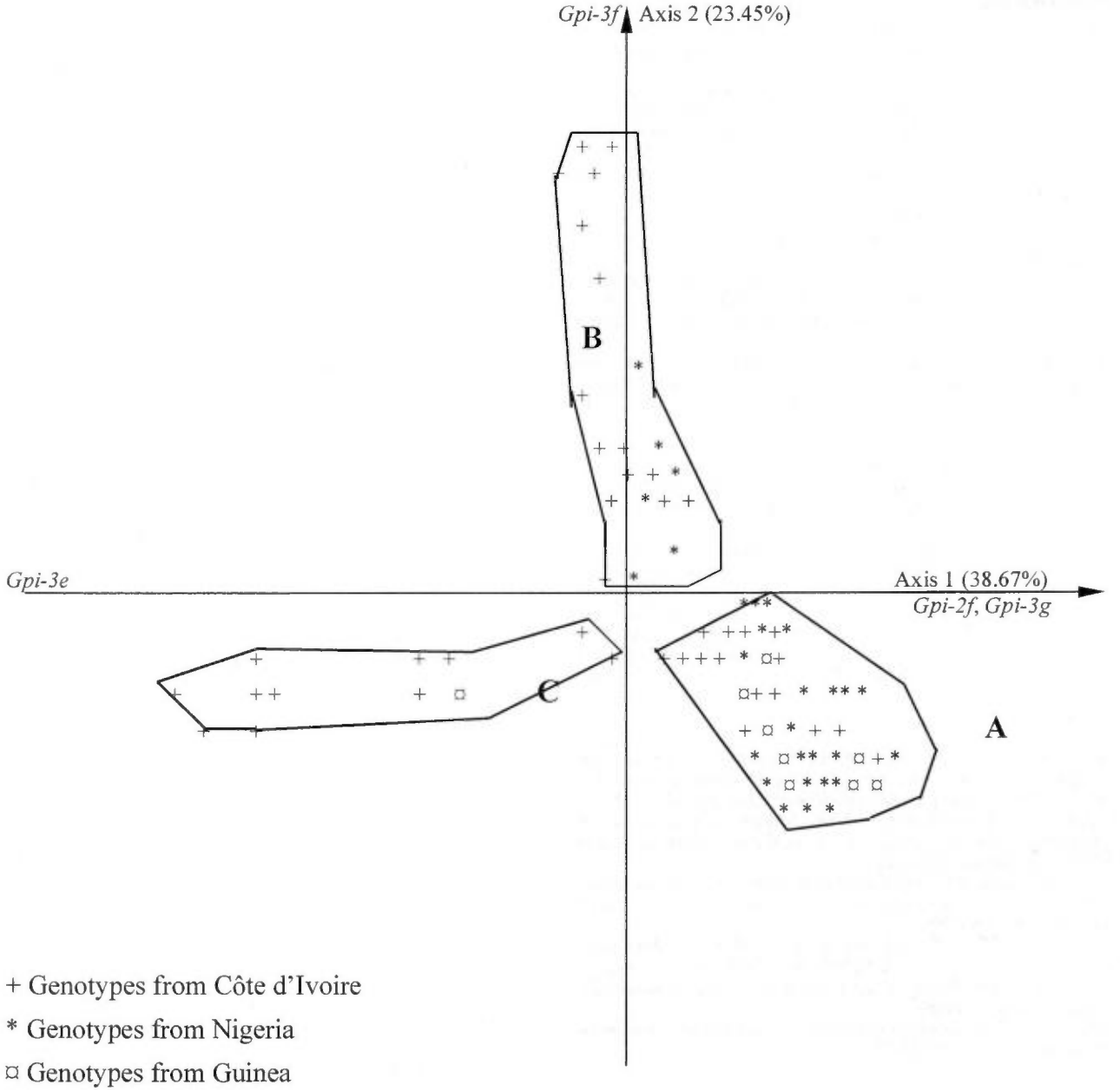


Figure 2. Distribution and clustering of kola genotypes (*Cola nitida*) of different geographical origins by factorial correspondence analysis (FCA).

The F_{st} value (+0.20) calculated from these three groups was quite high and revealed substantial genetic differentiation between the three groups. This considerable genetic diversification between genetic groups A, B and C could be used in a breeding strategy such as recurrent selection (Baudouin et al. 1997).

The relatively high value of F_{is} (–0.15) calculated within the groups revealed the existence of major diversification in each of these groups. In the case of recurrent selection, within-group genetic recombination phases could be introduced at the beginning of each selection cycle. The planting material used as a basis for launching this selection strategy would thus consist of the 79 genotypes used in this research. In comparison, the planting material used as the basis for oil palm selection is composed of 82 genotypes (Gascon and de Berchoux 1964), and of 22 trees for *Hevea* (Besse 1993).

Conclusion

This study revealed a common origin for kola trees from Côte d'Ivoire, Guinea and Nigeria. The diversity existing in these three populations of kola trees is organized in three genetically distant groups. These groups could be used as the basis for setting up a breeding scheme such as recurrent selection. However, it would be interesting to search for morphological and/or production traits specific to each of those groups.

References

Baudouin L, Baril C, Clément-Démange A, Leroy T, Paulin D. 1997. Recurrent selection of tropical tree crops. Euphytica 96:101–114.

Besse P. 1993. Identification des clones cultivés et analyse de la diversité génétique chez *Hevea brasiliensis* par RFLP. Thèse de Doctorat ès-Sciences de l'Université de Paris-Sud, France.

Bodard M. 1960. Contribution à l'étude systématique du genre Cola en Afrique Occidentale. Thèse d'Etat Université de Dakar, Senegal.

Bonsson B. 1983. Amélioration de la production de noix de cola en Côte d'Ivoire—Principes de la sélection. Café Cacao Thé 27 (4): 283–297.

Djè Y, Ater M, Lefèbvre C, Vekemans X. 1998. Patterns of morphological and allozyme variation in *Sorghum* landraces of Northwestern Morocco. Genetic Resources and Crop Evolution 45:541–548.

Dublin P. 1965. Le colatier en République Centrafricaine: culture et amélioration. Première partie. Café Cacao Thé 9(2):97–115.

Egbe NE, Oladokun MAO. 1987. Factors limiting high yields in kola (*Cola nitida*) production in Nigeria. Café Cacao Thé 32(4):303–310.

Gascon JP, de Berchoux C. 1964. Caractéristiques de la production *d'Elaeis guineensis* (Jacq.) de diverses origines et de leurs croisements: application à la sélection du palmier à huile. Oléagineux 19(2):75–84.

Lanaud C. 1987. Nouvelles données sur la biologie du cacaoyer (*Theobroma cacao* L.). Diversité des populations, système d'incompatibilité, haploïdes spontanés. Leurs conséquences pour l'amélioration génétique de cette espèce. Thèse de Doctorat d'Etat, Université de Paris-Sud, Centre d'Orsay, France.

Leroy T, Montagnon C, Charrier A, Eskes AB. 1993. Reciprocal recurrent selection applied to *Coffea canephora* Pierre. I—characterization and evaluation of breeding population and value of intergroup hybrids. Euphytica 67:113–125.

Montagnon C, Leroy T, Yapo A. 1991. Etude complémentaire de la diversité génotypique et phénotypique des caféiers de l'espèce *Coffea canephora* en collection en Côte d'Ivoire. In: ASIC, 14ème Colloque, San Francisco, USA. pp. 444–450.

Montagnon C, Bouharmont P. 1996. Multivariate analysis of phenotypic diversity of *Coffea arabica*. Genetic Resources and Crop Evolution 43:221–227

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Sié RS. 1999. Etude de la diversité et des paramètres génétiques du colatier *Cola nitida* (Vent.) Schott et Endl. de la collection de Côte d'Ivoire. Thèse de Doctorat 3ème cycle, Université de Cocody, Côte d'Ivoire.

Wright S. 1951. The genetical structure of populations. Annals of Human Genetics 15:323–354.

Press Release

Sustainable Wild Collection of Medicinal and Aromatic Plants: Development of an International Standard

Medicinal and aromatic plants are an important resource for mankind. Unsustainable wild collection threatens the survival of many of these species. An *International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants* is being developed by the German Federal Agency for Nature Conservation (BfN), IUCN and WWF/TRAFFIC.

Medicinal and aromatic plants (MAP) are offered in a wide variety of products on the market. An estimated 40 000 to 50 000 plant species are used in traditional and modern medicine throughout the world. The vast majority of MAP species are currently collected from the wild and it seems that this trend is likely to continue over the long-term due to numerous factors including the high costs of domestication and cultivation. Moreover, cultivation is not necessarily the most beneficial production system for some MAP species. Wild collection provides valuable income for rural households, especially in developing countries; it may provide incentives for conservation and sustainable use of important habitats and can strengthen local economies. Approaches to sustainable wild MAP collection that engage local, regional and international collection enterprises and markets are urgently needed to provide specific guidance for industry, collectors and other stakeholders on sustainable sourcing practices.

The German Federal Agency for Nature Conservation (BfN) has provided start-up funding for the development of a project entitled "International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP)". The project is implemented by the Medicinal Plant Specialist Group (MPSG) through IUCN-Canada and by WWF/TRAFFIC Germany. This standard will bridge the gap between already existing but mostly abstract guidelines and management plans developed for specific local conditions. Stakeholders involved will receive an easy-to-handle list of criteria, indicators and verifiers that will enable them to check the sustainability of plant material collected from the wild. ISSC-MAP covers social and economic factors, but clearly focuses on ecological aspects, addressing two important aspects that are often ignored: the need for resource assessments and the question of annual sustained yields.

The development of the ISSC-MAP builds on existing principles, guidelines and standards for sustainable forest practices, organic production and good agricultural practices, fair trade and product quality.

An international advisory group has been established bringing together the medicinal plant and herbal products industry, small-scale collection enterprises, non-governmental organizations (NGOs) and conservation and certification organizations. The advisory group met on the Isle of Vilm, Germany, in December 2004 to revise a first draft of the standard. A revised draft and other documents related to the project are available on the project download website (http://www.floraweb.de/map-pro). The standard will be tested in several field projects beginning in July 2005.

Comments on the current draft of the International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP) are welcome at any time. Please check the current status of the project at our website or by email: MAP-Standards-Criteria@wwf.de

Book Review

Biodiversity and Traditional Knowledge: Equitable Partnerships in Practice

Sarah A. Laird (editor)
2002. Earthscan Publications Ltd ., London UK.; Sterling, VA USA. 504 pp. ISBN 18-5383-914-0

Prior to the Convention on Biodiversity (CBD) that was presented and opened for signature at the United Nations Conference on Environment and Development (UNCED) held in 1992, little consideration was given to the social impact of the extraction, modification, patenting and publishing of materials and information derived from the ethnoecological systems of local communities. Only in the last decade have attempts been made on a global scale to develop and implement general and *sui generis* guidelines for best practice and equitable relationships between parties. In addition, we have seen a growing movement among indigenous and local communities to stop what they consider to be the piracy and exploitation of their biodiversity knowledge from which they receive little or no benefit in return. It is in this context that this timely and useful book has appeared.

Sarah Laird, a noted ethnoecologist with a strong record of involvement in protecting and respecting the rights of local and indigenous communities, has produced a landmark manual that will be of use to many people working at the field level in biodiversity conservation, documentation and management. Her book, Biodiversity and Traditional Knowledge, reviews and analyzes a wide range practices and experiences in the development, acceptance and adherence to equitable research protocols between research institutions, bio-companies and local communities.

Sarah Laird attempts to come to grips with the complexity and conflicting interests between disparate parties and presents models for more equitable partnerships. A fundamental issue that emerges is the different cultural definitions and perceptions of what constitutes equitable relationships and benefit sharing. Add to this the contrasting policies and customs within and between international, national and local governments and it becomes clear that delineating a set of best practices for biodiversity research on traditional knowledge is, at best, an ambitious if not a quixotic task. But as they say, it may not be an easy job but someone has got to do it and in this case we are glad that the author has taken up the challenge.

The book is organized into six sections that explore biodiversity research relationships in protected and non-protected areas; the commercial use of biodiversity and traditional knowledge; and the development and implementation of national access and benefit-sharing policies. It is an excellent primer for those initiating community-based research as well as a rich source of information and recommendations for intermediary institutions, governments, companies, granting agencies, protected area managers and local community groups.

Spliced into every chapter are information-rich and contextualizing case studies and boxes relevant to each section's theme. Some case studies illustrate the problems and benefits that have arisen from research relationships and the varying degrees to which best practice guidelines have been implemented in these situations. Other studies describe initiatives launched by indigenous groups to assert more intellectual and/or property control over their natural and genetic resources. The boxes serve as sources of information for those unfamiliar with the technical, conceptual and historical contexts involved around codes of ethics, written research permits and consent forms and intellectual property rights. They also inform the readers of the myriad of different organizations (from the community to the international level) that are involved in developing 'best practice' guidelines even though these same organizations may show a distinct lack of adherence to such guidelines.

The first section, Biodiversity Research Relationships, deals with the characteristics of biodiversity research and discusses the 'new' obligations and responsibilities of researchers to social and ecological conservation. The authors stress that the academic community needs to consider all the possible effects of their research and needs to communicate these possible effects in a transparent manner to participants. In addition, researchers need to prevent or respond to any negative repercussions caused by their research to the best of their ability. Research relationships also need to assume an equitable benefit-sharing component that must indicate the nature and recipients of compensation.

Section I also describes the processes by which institutions have developed policies to address issues raised by biodiversity research and prospecting, listing specific examples of non-profit research institutions' partnerships with commercial companies and the countries of collection and research. It highlights the 'increasingly blurred' lines between public and private research, the 'increased commodification' of knowledge and genetic resources and the rapidly shifting and culturally dependent perceptions of equitable research relationships that make revised research procedures all the more important.

Section II, Biodiversity Research and Prospecting in Protected Areas, thoroughly defines protected areas, emphasizes their importance for biodiversity and provides case studies of specific protected areas' relationship to researchers' involvement. The section's case studies contextualize research permit processes and the benefits resulting from research collaborations, such as local capacity building,

donation of equipment and acknowledgement of local contributors in publications and reports.

Issues of proprietary rights and the implications related to the documentation, dissemination and appropriation of published information are also examined. The 'publish or perish' regime of so many research institutions, coupled with competitive project funding, can pressure researchers to publish too much too quickly, thereby violating community participants' wishes for intellectual privacy and anonymity. Opening up information flows at the local level can threaten internal/external social stability when knowledge channeled along age, lineage, status or gender lines is abruptly made accessible to all. The publishing of traditional knowledge can also make it difficult for the people who divulged that knowledge to claim rights and/or compensation for any subsequent commercialization of that knowledge.

Community Relationships with Researchers, the theme of the third section, reiterates many of the same themes of equitable relationships in a more detailed and technical manner. Prior informed consent and resource agreements, memorandums of understanding and letters of intent are discussed and specific examples are given of mechanisms to ensure fair conduct. As the book explains, this is not a simple matter, given that the actual process around which such agreements are signed involves extensive cross-cultural communication and contextual cultural appreciation of local power relations and different stakeholders' motives and priorities.

Section IV describes the varying degrees to which industry sectors use genetic resource materials and traditional information for commercial production. Differences in commitment to equitable benefit sharing exist within the industry, but recent trends in public opinion and consumer demand for ecologically and socially responsible products have made some companies more sensitive than they were formerly to the processes by which they extract material and information from community sources. The section examines actual examples and best practices of contractual agreements for biodiversity prospecting and brings to light the practical problems involved in creating equitable benefit sharing between transnational corporations and local communities. Case studies from Surinam, Nigeria and Panama (among others) illustrate the trust fund as a possible mechanism for sharing financial benefits fairly between parties.

Finally, the national policy contexts within which biodiversity research and prospecting take place are examined in section V. Though the CBD has provided an international legal framework for these activities, the ways in which different countries develop and implement their own *sui generis* frameworks for genetic resource access and benefit-sharing under the CBD mandate vary greatly. To balance out inequities in power dynamics between parties, the authors emphasize the need for government regulation of the establishment of minimum terms and contract negotiations.

In summary, the need to create conditions for equitable research relationships entails a vast number of actors and outcomes that no one code can predict or prescribe. While the authors present an array of protocols, case studies and the historical, technical and conceptual contexts relating to research and prospecting, ultimately we are brought back to a very small yet very cumbersome moral pill that everyone must swallow for equitable relationships to work: be honest and fair. The authors touch upon some of the social and economic factors that discourage people from expressing whatever honesty and fairness they possess in their conduct with local communities. They also remind us that the very nature of what constitutes honesty and fairness is context- and culture-dependent. Add to this the imbalances between human resources and natural/genetic resources most commonly split along North and South latitudes and a fertile nesting ground for equitable benefit sharing becomes challenging indeed.

Biodiversity and Traditional Knowledge, which is part of the series, People and Plants, is an essential guide to best-practice biodiversity conservation at the community level for fledgling and experienced researchers alike. However, it runs the risk of being one-sided in its moral preaching. While the authors emphasize the obligation for researchers and bioprospectors to swallow the pill of fairness and honesty, the nature of indigenous communities' ethics and motives goes unexamined. The case studies save the book from seeming to present those indigenous communities studied as passive victims in the unscrupulous hands of science and business. It is important to consider the possible lack of fairness in indigenous institutions and procedures that dictate how knowledge should be divulged and how research relationships with outsiders are to be developed.

Indigenous communities, research communities and corporate communities are all grappling for a piece of the biodiversity- and traditional-knowledge cake. The book provides many excellent suggestions and examples of how that cake might be equitably split up and by whom; the challenge lies in getting everyone to swallow the 'fairness pill' that will facilitate digestion. Certainly for the local communities whose livelihoods depend on their local biodiversity and for the global community concerned to maintain the biological complexity and resources that underpin sustainable development and survival there is no other way but to sit together at the same table and share.

Pablo B. Eyzaguirre
Adriana Woods-Páez

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.

Plant Genetic Resources Newsletter

No. 143, September 2005

Contents

Articles