

Centres of crop diversity and/or origin, genetically modified crops and implications for plant genetic resources conservation

J.M.M. Engels^{1,*}, A.W. Ebert², I. Thormann¹ and M.C. de Vicente³

¹IPGRI, Rome, Italy; ²CATIE, Turrialba, Costa Rica; ³IPGRI, Cali, Colombia; *Author for correspondence (e-mail: j.engels@cgiar.org)

Received 6 December 2004; accepted in revised form 26 June 2005

Key words: Central America and Mexico, plant genetic resources, centres of diversity, centres of agricultural origin, conservation, germplasm management, biotechnology, GMO crops, unintentional introgression, biosafety measures

Abstract

The concept of centres of crop diversity and/or origin of agriculture is briefly reviewed. The conservation status of crop genetic resources, either *ex situ* or *in situ*, cultivated or wild, has been assessed for species of the Central American and Mexican centre, demonstrating that that region is indeed one of the important centres of crop diversity for human kind. Furthermore, biotechnological developments with regard to the creation and spread of genetically modified crops have been analyzed. The likelihood of unintentional introgression of genetically modified traits into conventional seed lots, crops as well as into germplasm collections have been assessed. Related biosafety measures as well as the possible implications of intellectual property rights on transgenic crops and/or genes are being discussed vis-a-vis the possible implications they might have for germplasm management. The Central American crop genetic resources situation has been used as a “case study” to illustrate the potential impact of the spreading of GM varieties on the genetic diversity in genebanks and farmers’ fields and the need for effective and efficient conservation efforts. Conservation management strategies and practices are being proposed of mitigate the potential negative impact of GM crops on the conservation efforts.

Introduction

The concept of centres of origin of our domesticated species was for the first time suggested by de Candolle in the 19th century (de Candolle 1883). Later, after the publication of Vavilov’s ‘On the Origin of Cultivated Plants’ (1926), this concept became eventually and generally accepted for the conservation of plant genetic resources as it established a clear link between genetic diversity, its geographic distribution pattern and the origin of the crop in question. Vavilov defined eight

centres of origin for most of our cultivated plants, each of them harboring significant genetic diversity within and between species. Most of these Vavilovian centres are situated in tropical and subtropical regions of the world, and their locations fall largely within the territories of developing countries. Central America is one of such centres of crop diversity and origin, and its significance is described below.

During the early 1960s, plant breeding activities with a global focus were initiated to produce high yielding varieties of the major food crops such as

wheat, maize and rice. The successes of these breeding efforts resulted, among others, in significant replacement of traditional by high yielding varieties, and, subsequently, to a considerable loss of genetic diversity of these crops. This lost genetic diversity consisted predominantly of landraces in the case of cereals, leguminous crops and other staples, and, this development eventually led to the establishment of the International Board for Plant Genetic Resources (IBPGR) in 1974 as well as of global genebanks for the major food crops and their wild relatives in the international centres of the Consultative Group on International Agricultural Research (CGIAR). Later, many countries also established genebanks and germplasm collections to conserve the threatened genetic resources of the major cultivated crops, resulting in more than 1300 genebanks and collections in 1995 (FAO 1998).

Towards the end of the last century it became generally accepted that people, in particular farmers, play an important role in managing genetic diversity and contribute to their conservation. The recognition that it will never be possible to conserve all plant genetic diversity in genebanks, that readily access to genetic diversity by its users (i.e. farmers, plant breeders as well as researchers) is a key factor in crop development, that conservation activities should be dynamic and allow adaptation processes to take place, led in the early 1990s to a strong focus on *in situ* conservation activities, i.e. keeping the material in its natural habitat or in farmers' fields.

In addition to the above, biotechnological developments took place, especially in molecular genetics. The discovery of the DNA molecule in 1953 provided the foundation for the advances in marker technologies that allowed the identification of variants in the hereditary material. The premise to describe genetic diversity was greatly enhanced, both at the phenotypic level through biochemical markers, and, at the genotypic level through molecular markers. These advances were accompanied by major breakthroughs in information technologies enabling the management and analysis of increasing amounts of data, which altogether meant a significant advance in the area of conservation and use of plant genetic resources.

Developments in tissue culture and from the 1980s in genetic engineering enabled the generation of genetically transformed plants, i.e. plants

modified by the artificial insertion of foreign genes from the same or different gene pools – and, as a result, the commercial production of transgenic varieties. Transgenic varieties are now grown widely and offer new potential for higher yields, better quality traits, reduction in the use of inputs and higher tolerance to biotic and abiotic stresses (Persley 2000).

Despite these significant advances, there are conservation and environmental concerns related to the cultivation of genetically modified (GM) crops as they may result in the transfer of transgenes through gene flow and introgression into wild species or weedy relatives. Such gene transfers might happen through hybridization and backcrossing, potentially resulting in plants with enhanced invasiveness or weediness (Mikkelsen et al. 1996; Halfhill et al. 2002). The possible impact of GM varieties and the legal consequences on conservation efforts in centres of diversity are assessed in this paper and management procedures are being proposed that can help the long-term conservation and sustainable utilization of plant genetic resources.

Centres of origin and crop diversity

Vavilov was able to postulate the existence of eight centres of origin and diversity of our crops. He demonstrated that these centres had important factors in common (e.g. ancient agriculture and an old civilization; distributed in the tropical and subtropical regions; very diverse eco-topographic conditions) and that the distribution of the genetic diversity followed certain patterns (Vavilov 1926). These centres are of critical importance for current and future crop improvement efforts as they harbor major parts of the genetic diversity of a given gene pool, including the domesticated species as well as their wild and weedy relatives. Adaptation and selection processes in the crop gene pools are ongoing in these centres, especially in traditional agricultural production systems, where farmers continue to play an important role in the management and maintenance of this genetic diversity. In many parts of such centres commercial agriculture has not yet been adopted and usually very diverse cultivation practices are followed, frequently based on traditional knowledge that has evolved over centuries and is passed on from one

generation to the next. Most or all of the produced crops are consumed locally and traditional seed systems are an important component of these agricultural practices. However, these traditional systems are vulnerable and specific care is needed to avoid that their built-in strength is undermined through outside interventions.

The traditional agriculture schemes together with the existing genetic and cultural diversity are threatened by many factors and forces. These include, among others, modern agricultural technologies making use of high performance cultivars and genetically very uniform crops. According to Ortega-Paczka (1999) the native diversity of maize is in serious danger of extinction in Mexico due to adoption of improved seed, substitution of maize by other more profitable crops and emigration of peasants to earn their living in other activities and other regions of the country. Only 20% of the local maize varieties reported in 1930 in Mexico are still known (GRAIN 1996). Similar developments have been reported for other crops in other centres of diversity, e.g. of the 10,000 varieties of wheat, that were in use in China in 1949 only approximately 1000 remained in 1970. In the United States, 95% of cabbage, 91% of field maize, 94% of pea, 86% of apple and 81% of tomato varieties of the 19th century have been lost (Arunachalam 1999).

In this paper, the Central American and Mexican centre is being used as a 'case study' to illustrate the potential impact of the spreading of GM varieties on the genetic diversity in genebanks and farmers' fields and the need for effective and efficient conservation efforts. Conservation management strategies are being proposed to mitigate the potential negative impact of GM crops on the conservation efforts.

The Central American centre

According to Zeven and de Wet (1982), 225 domesticated plant species find their origin in the Central American centre of diversity, which represents approximately 9% of the total number of approximately 2500 domesticated species reported worldwide. León (2000) lists a large number of economically important crops domesticated in this region. These include cereals (*Zea mays* L.); grains

(*Amaranthus* spp.), grain legumes (*Phaseolus* spp.); spices and condiments (*Capsicum* spp.; *Vanilla planifolia* Andrews); fibres (*Gossypium hirsutum* L.; *Agave* spp.); vegetables (*Cucurbita* spp.; *Lycopersicon esculentum* Mill.; *Opuntia streptacantha* Lem.; *Physalis philadelphica* Lam.), stimulants (*Theobroma cacao* L.; *Agave tequilana* F.A.C. Weber), root and tubers (*Pachyrhizus erosus* (L.) Urb.), and, fruit trees (*Annona* spp., *Carica papaya* L., *Manilkara zapota* (L.) P. Royen, *Opuntia ficus-indica* (L.) Mill., *Persea* spp., *Pouteria* spp.).

From an economic perspective, *Zea mays* L. and its wild relatives *Z. mays* ssp. *parviglumis* H. H. Iltis et Doebley, *Z. mexicana* (Schrad.) Kuntze, *Z. perennis* (Hitchc.) Reeves et Mangelsd., *Z. diploperennis* H.H. Iltis et al., *Z. luxurians* (Durieu et Asch.) R. M. Bird, and *Tripsacum* spp. possibly form the most important contribution of the Central American centre of diversity to the world (Hernandez 1973; León 2000).

The second economically most important crop gene pool from the region is the genus *Phaseolus*, consisting of the following cultivated species: *P. vulgaris* L., *coccineus* L., *acutifolius* A. Gray, *polyanthus* Greenm. and *lunatus* L., which were domesticated in Central America and the Central Andes (León 2000). The Mesoamerican Centre is the richest of possibly three centres of genetic diversity of *Phaseolus* and is home to about 45 wild *Phaseolus* species and the ancestors of the mentioned five cultivated species (Debouck 1986). The theory of multiple origins is predominant (Gepts et al. 1986). At least two regions of domestication are proposed. According to the theory, small seeded varieties were domesticated from small seeded wild form (*P. vulgaris* var. *aborigineus* (Burkart) Baudet) in Central America, while large seeded varieties were domesticated from large seeded wild form (*P. vulgaris* var. *aborigineus* (Burkart) Baudet) in the Andean region of South America.

Other important genera with significant genetic diversity in Central America include *Capsicum*, *Cucurbita*, and *Lycopersicon*. Cultivated *Capsicum* species include *C. annuum* L., *baccatum* L., *chinense* Jacq., *frutescens* L. and *pubescens* Ruiz et Pav. (León 2000). *Capsicum annuum* L., the best known *Capsicum* species, which spread to every part of the globe, has its centre of diversity in Mexico and northern Central America with a local and more recent distribution in parts of South America

(Eshbaugh 1993). Crossing studies indicate that the wild bird pepper *Capsicum annuum* var. *aviculare* (Dierb.) D'Arcy et Eshbaugh is genetically the most closely related taxon to domesticated *C. annuum* L. (Pickersgill 1971). *Capsicum frutescens* L. was domesticated in Central America, but wild populations can be found from Mexico down to Brazil (León 2000). Cultivated populations of *C. chinense* Jacq. are found in Central America, while wild populations are mainly distributed in the Amazon area. *C. pubescens* Ruiz et Pav. originated from Bolivia and Peru and is cultivated at higher elevations from Mexico to Argentina (León 2000). *C. baccatum* L. has its origin in Southern America and is cultivated from Colombia to Argentina.

The domestication of cucurbits has taken place thousands of years ago in the Americas. Cultivated *Cucurbita* species originating from the Central American region and *C. moschata* Duchesne, *C. pepo* L., *C. ficifolia* Bouché, and *C. argyrosperma* C. Huber. The domestication of the latter started more than 7000 years ago in Southern Mexico. There are two subspecies of *C. argyrosperma* C. Huber: (1) *argyrosperma*, comprising four varieties, which include all cultivated types, and (2) *sororia* (L.H. Bailey) L. Merrick et D.M. Bates, the wild ancestor of this species growing from northeastern Mexico up to Nicaragua (Saade and Montes Hernández 1994; León 2000). *C. moschata* Duchesne is the most important species for the tropics and is closely related with *C. argyrosperma* C. Huber. Archaeological and linguistic evidence indicate two centres of crop diversification of *C. moschata* Duchesne located in Central America and South America (Saade and Montes Hernández 1994). *C. pepo* L. was domesticated in Mexico and the eastern United States. *C. fraterna* L.H. Bailey is the wild progenitor of *C. pepo* L. in northeastern Mexico, from which the Mexican varieties have been derived, which are cultivated in Central America (León 2000). The other wild progenitor, *C. texana* (Scheele) A. Gray is widely distributed in central and northeastern United States. The cultivation of *C. ficifolia* Bouché ranges from northern Mexico to Argentina and Chile. The wild ancestor of this species has still not been discovered and the centre of origin and domestication is still unknown (Saade and Montes Hernández 1994).

Tomatoes are one of the crops from tropical America, which has gained major importance and development outside the tropics. There is historic evidence that tomatoes were cultivated in Mexico before the discovery of the New World (León 2000). *Lycopersicon pimpinellifolium* (L.) Mill. and *L. esculentum* var. *cerasiforme* (Dunal) A. Gray are ancestral forms of tomato. The former has its natural distribution along the dry coastal areas of the Pacific in South America, from Ecuador to Chile, while the latter is found spontaneously in all original tomato growing areas of tropical America (León 2000). The current range of wild tomato relatives extends from the northern tip of Chile on the south, to Ecuador on the north, and reaching inland from the Pacific 100–200 miles, also including the Galapagos Islands http://www.jic.bbsrc.ac.uk/chelsea/crop_origins.htm, accessed March 2005.

For most of the aforementioned crop gene pools significant genetic diversity also exists in South America. An example of a crop that originated in South America and of which significant diversity occurs in Central America is potato. Approximately 55 wild potato species, including *Solanum demissum* Lindl., *S. trifidum* Correll and *S. verrucosum* Schltdl. that have contributed important genes to the domesticated Irish potato *S. tuberosum* L. are found in Central America (Engels 1974).

Two major approaches are being employed to conserve plant genetic resources, i.e. 1. *in situ* conservation, i.e. the maintenance of genetic resources in their natural habitat or on-farm, i.e. in areas where the germplasm obtained its distinctive features; and 2. *ex situ* conservation, i.e. maintaining the collected genetic resources in especially constructed institutions or genebanks. The latter consists of a number of different methods, including seed storage, *in vitro* and cryopreservation, the maintenance of plants in field genebanks and pollen storage. A comprehensive description of the various conservation approaches, with a particular emphasis on management practices leading to effective and efficient results, is given in Engels and Visser (2003). In general, it is important to consider complementary conservation approaches, i.e. to combine the dynamic *in situ* approach with the static but possibly safer *ex situ* conservation approach. A summarized inventory of reported *ex situ* and *in situ* conserved genetic resources and of conservation activities in Central

America are presented (Tables 1, 2 and 3) with the aim of providing a general overview of where currently what kind of germplasm is being conserved.

According to IPGRI's germplasm holdings' database, there are 274,225 (4,7%) accessions conserved *ex situ* in the Central American region out of the globally reported 5,740,000 accessions. The material is being stored in national and institutional genebanks in the countries and in the genebanks of the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) and the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE); see Table 1 for details.

Genetic resources native to the region are also conserved in the 105 botanic gardens registered for the Central American countries in the botanic gardens database held by BGCI (Botanic Gardens Conservation International; <http://www.bgci.org.uk/>, accessed March 2005). Collections reach sizes of many thousand taxa. Most of the Central American gardens (ca. 84%) are located in Mexico.

As shown above, the Central American centre of diversity harbors a significant wealth of economically important species, most of which still exists in nature and only a relatively small portion has been collected and is maintained in genebanks. The conservation of the genetic diversity of wild species and wild relatives of cultivated crops *in situ* in natural protected ecosystems is possibly the only efficient strategy to ensure their survival for the future. On the other hand, landraces can easily be maintained on-farm. Changes in their genetic set-up due to adaptation processes induced by changes in the production environment are totally acceptable and even desirable. Consequently, a complementary conservation approach in which *in situ*, on-farm and *ex situ* methods are being combined will give the best conservation result as has been experienced in the maize-teosinte complex (Garrison Wilkes, personal communication).

Table 3 provides information on nature conservation, and on the management and maintenance of crop genetic resources on-farm in the Central American region. Eight-hundred and three protected areas, including national parks, nature reserves, species management areas and protected landscapes have been established in Central America, covering approximately 8.7% of the total Central American territory.

Table 1. Central American genetic resources stored in local genebanks and the reported existence of genetically modified varieties.

Country	Total no. of accessions conserved	Total no. of species	Total no. of genebanks	No. of species for which GM varieties exist	No. of accessions of species for which GM varieties exist	No. of genebanks holding species for which GM varieties exist
Belize	201	35	2	3	56	2
Costa Rica	20,538	622	15	9	2381 (<i>1002 L. esculentum</i> Mill., 754 <i>Z. mays</i> L., 241 <i>S. tuberosum</i> L., 201 <i>C. pepo</i>) 220	6
El Salvador	1316	91	3	4	601 (<i>524 C. pepo</i> L.)	1
Guatemala	5984	89	7	4	926 (910 <i>Z. mays</i> L.)	2
Honduras	8656	720	17	3	53429 (49048 <i>Z. mays</i> L., 2638 <i>Glycine max</i> (L.) Merr., 608 <i>Gossypium hirsutum</i> L.)	6
Mexico	233,952	567	27	11	177 (150 <i>Z. mays</i> L.) 1064 (750 <i>O. sativa</i> L., 250 <i>L. esculentum</i> Mill.)	11
Nicaragua	1559	38	8	3		2
Panama	2019	94	4	4		2

Note: Holdings reported under Mexico and Costa Rica include the germplasm of Central American origin maintained by CIMMYT and CATIE, respectively.

Table 2. Summary data on accessions that originated in Central America and are being conserved in the CGIAR centres, at AVRDC, CATIE and USA.

Country	CIAT	CIMMYT	CIP	ICARDA	ICRISAT	IITA	ILRI	IRRI	WARDA	AVRDC	CATIE	USA	Totals ^a												
	Acc.	Spp.	Acc.	Spp.	Acc.	Spp.	Acc.	Spp.	Acc.	Spp.	Acc.	Spp.	Acc.	Spp.											
Belize	162	55	7	1	2	2	1	1	170	20	3	1	1	10	5	27	2	82	18	465	79				
Costa Rica	653	57	393	1	41	2	3	2	2	10	5	55	8	7	1	311	14	2073	118	904	97	4471	244		
El Salvador	288	23	105	1	1	1	2	1	25	2	4	1	37	1	4	1	514	12	131	35	759	39	1870	86	
Guatemala	2331	25	596	3	179	9	7	4	7	1	11	1	32	14	2	1	425	17	2185	77	2283	114	8079	197	
Honduras	1107	77	180	1	10	3	70	3	7	2	17	11	1	189	8	281	20	783	63	2645	149				
Mexico	5576	111	10995	8	172	28	300	10	567	8	34	8	216	51	122	2	11	562	23	648	55	6585	253	25788	430
Nicaragua	331	3	89	2	84	9	6	2	2	1	8	4	1	1	1	1	55	6	78	12	306	21	961	47	
Panama	749	92	195	1	48	2	1	1	3	1	16	4	12	2	5	1	86	8	281	41	130	28	1526	152	
Total	11197	186	12560	8	535	37	310	11	657	8	93	13	518	76	216	2	31	2152	41	5704	187	11832	419	45805	

Note: The collections at CIP and ICRISAT are mainly composed of traditional cultivars/landraces (51.0 and 76.1%, respectively) as well as of wild species (39.4% at CIP). On the other hand, 70.4% of the Central American germplasm conserved at CIAT are advanced cultivars, and CIMMYT's collection consists of more than 60% breeding lines and research material. The germplasm maintained by the CGIAR centres and at AVRDC is largely in the public domain and can be obtained for research purposes without many restrictions.

^aThe totals refer to the whole table. The total number of accessions: All accessions held in the 12 genebanks originating from the respective country. Total no. of species: The number of different species of which the collections in the 12 genebanks for the particular country are composed.

Table 3. Protected areas in Central American countries.

Countries	Total area (ha) protected (IUCN categories I–VI and unclassified areas)	% of national territory	No. of protected areas (IUCN categories I–VI)	No. of areas > 100,000 ha
Belize	1,052,000	47.5	59	1
Costa Rica	1,206,000	23.4	158	1
El Salvador	40,000	2.0	62	0
Guatemala	2,775,000	25.3	172	8
Honduras	2,345,000	20.8	73	5
Mexico	9,902,000	5.0	150	20
Nicaragua	2,810,000	21.8	88	6
Panama	1,454,000	19.5	41	6
Total	21,584,000	8.7	803	47

Source: http://earthtrends.wri.org/country_profiles/index.cfm?theme=7, accessed: March 2005. Data reported refer to 2003.

The status of *in situ* conservation of crop wild relatives has recently been reviewed by Meilleur and Hodgkin (2004). Conservation activities are reported for Costa Rica, Guatemala, Mexico and Nicaragua, focusing on teosinte, along with other crops such as beans, chili pepper, squash and coffee. The Sierra de Manantlan reserve in Jalisco, Mexico was established to protect *Zea diploperennis* H.H. Iltis et al. Populations of the wild annual relative *Z. mays* spp. *parviglumis* H. H. Iltis et Doebley, and the traditional races Tabloncilo and Reventador of this area are other specific targets for *in situ* conservation. An *in situ* monitoring programme of Mexican teosinte populations is operated in collaboration between CIMMYT, the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) and the Colegio de Postgraduados in Mexico. In the Balsas River Basin in Mexico, activities of farmers have contributed to an increased conservation of Balsas type teosinte. Opening up land using the slash-and-burn system, planting maize and establishing rotational or alternating pasturing areas have facilitated the establishment of many teosinte populations (Serratos et al. 1997). Other teosinte conservation activities are being carried out in Guatemala and Nicaragua. Studies on the *in situ* conservation of *Phaseolus lunatus* L. have been carried out in Costa Rica (Meurrens et al. 2001). In Guatemala, work is being undertaken on the enhancement of agrobiodiversity in home gardens and the improvement of family nutrition and income (IPGRI 2001).

Biotechnological developments

In 1983 the first reports of genetic transformation were published. They dealt with the construction of tobacco plants resistant to kanamycin and to methotrexate, a drug used to treat cancer and rheumatoid arthritis (Herrera-Estrella et al. 1983), cells of a *Nicotiana* species and *Petunia* plants made resistant to kanamycin (Bevan et al. 1983; Fraley et al. 1983) and a bean gene incorporated into a sunflower plant (Murai et al. 1983). These achievements were just the beginning and represent the first successes of genetic engineering, which enabled the further development of GM varieties. Debates over the transformation technology have been, and still are, in many parts of the world very controversial and address ethical, human and animal health related concerns, food safety and the possible impact on the environment. Therefore, before a new GM variety is introduced for commercial production, a series of tests are performed in order to balance risks against benefits and to determine the contribution the new variety may make to the sustainability of the cropping system in which it will be used (Cook 2000). In the context of this paper we will focus on the possible consequences of releasing and growing GM varieties for germplasm conservation efforts.

In 1995 commercial GM varieties of maize and cotton were released on a larger scale, either based on the Bt (*Bacillus thuringiensis*) insect resistance genes, or possessing tolerance to (specific) herbicides. Since then there has been a dramatic increase both in the acreage and the number of crop

GM varieties. According to James (2003) the estimated global area of commercialized GM crops in 2003 was 67.7 million ha. This was due to as much as 55% of soybean, followed by cotton (21% of global area), canola (16%) and maize (11%). Of this acreage, approximately 30% was in developing countries, especially Argentina, Brazil, China, South Africa and India. Less than 100,000 ha each of GM soybean (25,000 acres) and cotton varieties (62,000 ha) were reported in Mexico (James 2003). Approximately 5000 acres of GM maize in Honduras and a non-specified acreage of GM maize for Mexico were reported. Details on crops stored in Central American genebanks for which GM varieties have been released worldwide are included in Table 2, i.e. papaya, squash, cotton, potato and maize.

The likelihood of introgression of transgenic elements into other varieties and germplasm depends on the reproductive biology, hybrid fertility, seed dispersal and selection pressure. For gene introgression to occur, sexual compatible crops or wild relatives need to be present near the source crop, outcrossing must be possible, the resulting hybrids need to be fertile and the backcrossing between hybrids and target germplasm must render fertile plants. Introgression of genes is highest for out-crossing species, such as maize, lower in self-pollinated crop species like soybean and rice, and minimal in vegetatively propagated crops, such as potato, because their conservation is not based on the botanical seeds. However, even in these cases, transgenes may be unintentionally introduced into accessions through mechanical mixtures with tubers produced from spontaneous seedlings grown out of GM botanical seeds. In experiments with transgenic aspen trees, Fladung et al. (2003) concluded that in risk assessment studies both generative propagation and vegetative dispersal should be taken into consideration. The assessment of 234 root suckers revealed that more than 50% showed the presence of the *rbcS-rolC* gene construct. For more details on transgenic crops see also the following website: <http://www.agbios.com/main.php>

The potential problems that the use of GM varieties in agriculture could cause for conservation activities include:

- Gene flow between GM and traditional varieties of the same crop;

- Gene flow between GM varieties and crop wild relatives and weedy forms of the target crop species;
- Spread of GM seed through commercial and local traditional seed systems, as well as international exchange.

Pollen-mediated gene flow within and between cultivated and wild species, respectively, is an important factor of evolution that significantly contributes to the richness of species as well as within-species diversity. Vertical gene transfer (i.e. the passing of genetic material from mother to daughter cells) is not environmentally damaging, but a rather fundamental mechanism of the development and maintenance of crop evolution (APEC 2003). No negative ecological effect can be expected from transgenic plants, except if a trait associated with one or more genes enters into the genome of a compatible species, is expressed and changes characteristics such as the ecological fitness of the recipient plants (Braun and Ammann 2002). If this introgression results in the increased fitness of the recipients, they could establish a population that out-competes wild relatives and other species in the same community. *A priori* this process seems to be quite unlikely as most 'foreign' genes introduced into crop/native hybrids would in fact decrease their fitness in the wild, leading to a rapid selection of these genes out of the population (Johnson 2000). This is particularly true of genes designed to prevent germination of seed – the so-called terminator gene. But if incoming genes provide resistance to insects, fungi and viruses, then they could increase fitness of resulting hybrids. Thus, potential effects need to be studied experimentally in a case-by-case, trait-dependent approach, both in the laboratory and in the field. These studies, together with aspects related to human consumption, cultural practices or socio-economic features, call for an evaluation of the integrity of landraces and crop wild relatives.

The rapid adoption and cultivation of GM crop varieties world-wide may lead to the unintentional introgression of GM traits into conventional seed lots, which will then spread through commercial and traditional seed systems. Friesen et al. (2003) reported evidence of contamination of pedigreed canola seed lots in western Canada with herbicide resistance traits. Accidental international exchange

of seeds containing GM traits also poses a threat, which has to be taken seriously by germplasm curators. An example of this is the recent detection of GM tomato seeds shipped accidentally to researchers around the world over the last seven years by the University of California, Davis (Lee and Lau 2003). University personnel in charge of the shipments were unaware of the fact that the seeds, received from Petoseed in 1996, contained GM traits. The seed mix-up went undetected for years as the GM tomatoes looked just like the other tomatoes.

Smallholders in many developing countries plant landraces of different crops, which are, in general, well adapted to the local environmental conditions and used to produce food for different cultural purposes. This applies, for example, to the potato and maize varieties grown in the Andean region and Mexico, respectively. The introduction of GM varieties in these circumstances may have similar effects to the introduction of high-yielding crop plants, which caused the replacement of existing landraces. This is a logical consequence of the willingness of farmers to follow market trends if they want to sell their products and also of the consolidation of a global seed market. However, the situation poses indeed a problem for the maintenance of traditional agricultural biodiversity.

The potential risks of GM varieties for conservation purposes call for the existence of testing methods for the presence of transgenic elements in germplasm. In general, these tests are quite straightforward as most of the released GM varieties possess known sequences (e.g. 35S promoter of the Cauliflower Mosaic Virus or of the nopaline synthase terminator), for which kits to test the occurrence of transgenes are commercially available. However, they have to be used with caution as biases (false positives) may exist. It is hoped that further research will develop simpler and more reliable methods. Details on which type of sequences have been used/engineered into the commercial GM variety can be found on the Agbios website (i.e. <http://www.essentialbiosafety.info/dbase.php>).

Germplasm ownership, IPRs and transgene introgression

With the establishment of the International Undertaking in 1983, the concept of ‘common

heritage’ of plant genetic resources got widely accepted. During the negotiation of the Convention on Biological Diversity (CBD) – culminating in 1992 – a shift in emphasis towards ‘national sovereignty’ of states over the genetic resources within their borders was observed, leading to more restrictive conditions to obtain genetic material from countries and, as a consequence, to increased use of material transfer agreements. In 1994, twelve CGIAR Centres placed large parts of the germplasm collections under the auspices of FAO as part of the International Network of *Ex Situ* Germplasm Collections, thus keeping these collections in the public domain (SGRP 2003). The International Treaty on Plant Genetic Resources for Food and Agriculture (IT) entered into force in June 2004. The IT establishes a multilateral system of access and benefit sharing for a list of 35 specified major food crops as well as forages of largely temperate areas.

A close relationship between the aforementioned biotechnological developments and the increased use of Intellectual Property Rights (IPR) protection of research and breeding efforts is observed. According to the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS), specifically Article 27.3 (b), members of the World Trade Organization (WTO) are required to provide patent protection for any inventions, whether products or processes, provided they are new, involve an inventive step and are capable of industrial application (Richer 2000). Members may exclude from patentability *inter alia* plants and animals and essentially biological processes for the production of plants and animals (Leskien and Flitner 1997). They are, however, required to provide for the protection of plant varieties, either by patents or by an ‘effective *sui generis* system, or a combination thereof’.

Contractual agreements are now standard practice between seed companies and farmers in the US and Canada regulating the use of GM crop varieties. These agreements prevent farmers from using saved seeds or supplying seed to other farmers (Yamin 2003). An example of the determination of seed companies to enforce IPRs is the Monsanto lawsuit against a Canadian farmer for infringement of its patent on Roundup Ready® Canola, an herbicide-tolerant canola. In March 2001, the Federal Court Justice ruled that Mr Schmeiser ‘knew or ought to have known’ that he had saved

and planted seed that was Roundup tolerant and had therefore infringed Monsanto's patented technology (AGNET 2002). Since independent tests showed that 416 ha (1030 acres) of Mr Schmeiser's canola were 95–98% tolerant to the Roundup herbicide (AGNET 2002), it is extremely unlikely that the level of contamination might have been caused by pollen or seed blown in from neighbours land as claimed by Mr Schmeiser (Yamin 2003).

Transgene introgression from GM crops into wild relatives is a major concern for historically important crops in their centre of origin. Maize has been the most tested GM crop in Mexico, followed by tomato and cotton (Alvarez-Morales 2000), all three crops having their centre of origin and/or diversity in this country. Around 1997/98 a fierce campaign against GMOs started in Mexico, as a result of transgenic maize being tested in a country, where this crop is deeply immersed in the traditions and culture of the people. In late 2001, Nature published a paper entitled 'Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico'. This paper was later retracted by the editor of Nature, because introgression *per se* was not shown. According to Stewart et al. (2003), F₁ hybridisation between the GM crops and the landraces could have occurred, but not introgression of the transgene, as this requires repeated backcrosses and the stabilization of the transgene in the new host genome.

Having assessed the molecular evidence of crop-to-wild introgression, Stewart et al. (2003) divided the crops into four risk categories for the introgression of transgenes: very low risk, low risk, moderate risk and high risk. The very low risk category comprises soybean (*Glycine max* (L.) Merr.), barley (*Hordeum vulgare* L.), finger millet (*Eleusine coracana* (L.) Gaertn.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), common bean (*Phaseolus vulgaris* L.), peanut (*Arachis hypogaea* L.) and potato (*Solanum tuberosum* L.). While in soybean and peanut introgression from wild to crop species is possible, crop-to-wild introgression has not yet been reported. The low risk category comprises maize (*Zea mays* L.), rice (*Oryza sativa* L.) and cotton (*Gossypium hirsutum* L.). Maize can hybridise with wild relatives, known as teosinte (*Zea mexicana* (Schrud.) Kuntze), but gene flow seems to be mainly uni-directional from teosinte to maize, with insignificant introgression from maize to teosinte. Therefore, as a precautionary measure the release of

transgenic lines of these crops should be restricted to areas in which the wild relatives do not occur.

Alfalfa (*Medicago sativa* L.), sugar beet (*Beta vulgaris* L.), wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) are classified as moderate risk crops (Stewart et al. 2003) with ample evidence of introgression between crops and wild species. The introduction of herbicide tolerance could complicate weed management, while transgenes conferring stress tolerance or resistance to insects and diseases could increase the fitness of the recipients and potentially cause agronomic or ecological problems. Sorghum (*Sorghum bicolor* (L.) Moench) is a high risk crop, as it hybridises easily with the weed Johnson grass (*Sorghum halepense* (L.) Pers.) and other wild populations. GM varieties of sorghum enhancing the fitness of the species through herbicide tolerance or similar traits would have to be handled with great caution.

From the above it can be concluded that the increased production of GM crops poses potential problems to genebanks through gene flow and subsequent introgression of the transgenes into the germplasm and that precautionary measures have to be taken to mitigate the risks.

Biosafety considerations and germplasm conservation

Many countries have already implemented or are in the process of implementing the Cartagena Biosafety Protocol (CBP)¹ of the CBD. At least 50 countries plus the European Union have biosafety rules and/

¹The CBP is an international agreement setting minimum standards that state parties exporting and importing LMOs should follow when those LMOs may have an adverse effect on biodiversity, also taking into account risks to human health. The CBP makes explicit reference to the precautionary approach, i.e. it establishes an Advanced Informed Agreement (AIA) procedure for imports of LMOs intended for introduction into the environment and an alternative procedure for mass movements of LMOs intended for food, feed and for processing (any commodities produced through a GM crop not intended for planting). A permit for trans-boundary movement of plant material will only be issued on the basis of a risk assessment procedure by the national competent authority. In practice, the CBP sets the most onerous standards to be followed by importers of LMOs for the environmental testing process, using the baseline information of the non-modified recipient crop as a reference. All information related to the AIA process will be publicly available through its posting in the Protocol's Clearing House Mechanism under the CBD's website (<http://bch.biodiv.org/>).

or legislation in force that are partially or fully compliant with the CBP. In Central America and Mexico four countries have biosafety rules in place (Mexico, Guatemala, Honduras, Costa Rica), while the others are in the process of their formulation (Belize, El Salvador, Nicaragua, Panama). An Interagency Group composed of IICA (Interamerican Institute for Cooperation on Agriculture), OIRSA (Organismo Internacional Regional de Sanidad Agropecuaria), and CATIE is currently in the process of elaborating a standardized Regulatory Framework for the handling of living modified organisms (LMOs) in Central America (IICA 2003). Also other countries that are not signatories to the CBD and/or the Biosafety Protocol, such as the USA, have adapted their safety regulative framework to accommodate the evaluation, approval, deregulation and release of transgenic crops into the environment. In all these countries, the importation of LMOs in general and their environmental release in particular shall be regulated under a national biosafety or generic environment and food safety framework. Following the risk analysis procedure, according to the CPB countries are allowed to reject GM germplasm of a given crop, especially those countries that are rich in biodiversity and diversity (including wild relatives) of that specific crop, based on a widely accepted interpretation of the precautionary approach.

As the introgression of materials that are the product of biotechnological manipulation transforms the recipient plants into LMOs, the CBP would, in principle, apply to the recipient plants (when they are transferred over international borders between state parties to the Protocol). Consequently, it will be necessary for genebanks that are situated within countries that are parties to the Protocol to comply with the notification procedures set out in the CBP when exporting to countries that are also parties to the Protocol. In this context, yet unanswered legal questions concern (a) the requisite state of knowledge (on the part of genebank curators), and (b) the necessary levels of risk of contamination, for obligations to 'kick-in' pursuant to the Protocol.

Environmental risk mitigation through adequate germplasm management

It is established that centres of diversity and of origin of crop genetic resources are of critical

importance for current and future crop improvement activities due to the (unique) genetic diversity they harbour. As a consequence, these resources need to be adequately conserved. However, the biotechnological developments and their related intellectual property issues cause new potential threats and problems for the conservation of germplasm as well as its use. While collecting germplasm material in nature, in farmers' fields or in markets, the collector should verify if such germplasm could have been exposed to pollen from GM varieties cultivated in neighbouring farmers' fields; subjected to unintentional mechanical mixtures of traditional and GM seed and planting material (in market places or in farmers' fields); or in contact with volunteer populations evolved from fields where GM varieties were previously grown forming unwanted 'weedy' plants in the crops of subsequent years (Stewart et al. 2003). During the preparation of the germplasm material in the genebank for subsequent storage only mechanical mixing could lead to contamination with transgenic material if it was present during manipulation in the genebank facilities.

If germplasm is being obtained directly from a conservation site (i.e. from a regeneration site or from a field genebank), it will be indispensable to gather the information about the presence of current and past neighbouring farmers' fields with GM crops in order to allow an assessment of the likelihood of accessions to contain unintentionally transgenes from GM crops. When a genebank receives germplasm material from another genebank or from any other source for inclusion in its collection, it will be important to get the information relevant to the presence of GM elements in order to allow well-informed decisions to be made with regard to the received material.

The regeneration of samples in the field or greenhouse is another vulnerable step in germplasm management as material may be directly exposed to introgression of GM elements through gene flow, if the known requirements are met. The actual introgression of GM elements will depend on the frequency of regeneration of the respective accessions combined with the likelihood of backcrosses with individuals of the same accession, a process that is far from simple and that occurs in many steps (Stewart et al. 2003).

Several steps should be taken by conservation programmes to mitigate risks caused by GM varieties and/or to comply with existing legislation and policies of a country when exporting germplasm. The following activities may be considered as precautionary measures to minimize legal violations and/or introducing environmental risks along with the germplasm material:

1. Collate and/or obtain comprehensive information if and where in the country GM crops are cultivated;
2. Obtain good information of the reproductive biology of target species, including details on the degree of gene flow under prevailing conditions. Where necessary, targeted research should be undertaken prior to the actual regeneration activities;
3. In case GM varieties of a given crop are being grown in the area where the genebank intends to regenerate germplasm of the same or related species, due consideration should be given to proper isolation methods, including the isolation of individual accessions for a (partially) outbreeding species;
4. Avoid re-growth of volunteer (hybrid and back cross) plants of the same crop from previous years in and around the regeneration plots;
5. Collate baseline information on the areas where landraces and crop wild relatives are being conserved, the material itself as well as on the conservation strategies and methodologies that are being applied;
6. Ensure the use of species specific testing methods for determining the presence of transgenes, giving due attention to genetically non-uniform accessions (i.e. landraces!);
7. Monitor incoming germplasm material on presence of transgenes (important to check country/region of origin) as well as accessions that have been potentially exposed to gene flow/introgression (i.e. after regeneration or when collected from an *in situ* site);
8. Record adequate information on the measures taken to prevent contamination. Consider providing a certificate of testing details undertaken on the germplasm;
9. The complexity of these biosafety considerations warrant a regional or international exchange of information and collaboration between

genebanks to ensure that effective and affordable mitigation measures are put in place.

The Genetic Resources Policy Committee of the CGIAR recently developed guiding principles to address the unintentional presence of transgenes in germplasm collections (GRPC 2004; http://www.ipgri.cgiar.org/Policy/GMOWorkshop/draft_guidelines.htm). To guarantee certain standards on the acceptable levels of transgenes in commercial seed lots, the International Seed Testing Association (ISTA) will establish a new chapter for the detection, identification and quantification of GMO in conventional seed lots in the ISTA Rules and is currently conducting proficiency tests on GMO testing in conventional seed. Each laboratory participating in the tests received a set of 10 maize samples: three negative samples (no GM seeds added) and 7 positive samples. From the seven samples three were spiked with 0.7% GM seeds (MON810) and four with 1.4% GM seeds. Out of 470 samples analysed, 17 samples (3.6%) were not tested correctly (Anonymous 2004). Thus, further efforts are necessary to produce reliable test results.

Conclusions

- Germplasm conservation of crops in their centres of origin or diversity requires special precautionary measures related with the introduction and cultivation of GM crops.
- Awareness needs to be created on the potential risks involved with the release of transgenes into centres of crop origin or diversity among professionals concerned with biosafety regulations for LMOs, as well as those in charge of the conservation and exchange of germplasm material.
- Genebanks should consider routine procedures and affordable methods to test germplasm on the presence of transgenes.
- Concerted efforts at national and international levels are mandatory with regard to legal issues related to the impact of GM plant materials on conservation of germplasm, e.g.

◦ regulations about monitoring protocols and tolerance levels of the presence of transgenes in germplasm;

- responsibilities and liabilities for monitoring and eradication;
- type of certification.

- Methods and procedures for safe germplasm operations, including the exchange of conserved germplasm, should be standardized and agreed upon at a regional or international level to minimize the risks of introgression of transgenes into landraces and wild relatives of crops.
- The authors suggest involving an international body, such as the ISTA, to oversee seed testing and to guarantee the definition and adoption of standards on the acceptable level of transgenes present in conventional seed, such as those set forth by the EU (0.9%).

Acknowledgements

The authors acknowledge with thanks the provision of data on the germplasm holdings from Central American origin at AVRDC by Bingbing Engle. The critical revision of the legal and biosafety paragraphs by Michael Halewood is duly acknowledged as well as the information given by Bert Visser on the molecular genetics aspects. The Genetic Resources Policy Committee of the CGIAR is acknowledged for its permission to include the draft guiding principles to address the unintentional presence of transgenes in germplasm collections.

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