BACKGROUND STUDY PAPER NO. 4 E

November 1994

منظمة الأغذية والزراعة للأمم المتحدة | 联合国粮食及农业组织 | Food and Agriculture Organization of the United Nations | Organisation des Nations Unies pour l'alimentation et l'agriculture | Organización de las Naciones Unidas para la Agricultura y la Alimentación

COMMISSION ON PLANT GENETIC RESOURCES

First Extraordinary Session

Rome, 7 - 11 November 1994

IDENTIFYING GENETIC RESOURCES AND THEIR ORIGIN: THE CAPABILITIES AND LIMITATIONS OF MODERN BIOCHEMICAL AND LEGAL SYSTEMS

by

J.J. Hardon, B. Vosman and Th.J.L. van Hintum

This background study paper is one of a number prepared at the request of the Secretariat of the FAO Commission on Plant Genetic Resources, to provide a theoretical and academic background to economic, technical and legal issues related to the revision of the International Undertaking on Plant Genetic Resources. The study is the responsibility of the authors, and does not necessarily represent the views of the FAO, or its member states.

The authors are members of the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), in Wageningen, the Netherlands.

For reasons of economy, the paper is available only in the language in which it was prepared.

IDENTIFYING GENETIC RESOURCES AND THEIR ORIGIN:

THE CAPABILITIES AND LIMITATIONS OF MODERN BIOCHEMICAL AND LEGAL SYSTEMS

by

J. J. Hardon, B. Vosman and Th. J. L. van Hintum

1. INTRODUCTION

The FAO has for over 25 years highlighted the importance of biodiversity for agriculture and the world's food supply. Awareness has been raised as to the dangers that threaten this important natural resource. Genetic resources were identified as a Heritage of Mankind; the product of countless generations of farmers that domesticated, improved and adapted crops to better suit human needs and a great diversity of environmental conditions. As modern plantbreeding developed, landraces and wild related species provided the basic raw materials for crop improvement the world over. Plantbreeding required increased sources of funding and to stimulate private investment, legal systems were designed to give ownership protection over improved varieties. Initially, both landraces and wild related species were treated as a free resource, referred to as plant genetic resources for agriculture (PGRFA). However in the past decade the value of the contributions farmers have made and still are making in harnessing and conserving genetic diversity have become better understood. The FAO Undertaking on Plant Genetic Resources (FAO 1985) formed a landmark in introducing the concept of Farmers' Rights and its interpretation (FAO 1989) to give expression to such contributions. The Earth Summit, held in 1992 in Rio de Janeiro and the Biodiversity Convention adopted at that conference reaffirmed the concept of Farmers' Rights and emphasized that States have sovereign rights over their natural resources and the authority to regulate access to genetic resources through national legislation. From an essential free resource, genetic diversity has now become a resource on which claims of ownership are developing. This will require new rules and regulations to protect the interests of such owners, either farmers or Nation States.

For the legal enforcement of sovereign rights over plant genetic resources it is important that the identity and origin of PGRFA can be established. Modern techniques involving molecular markers allow for a detailed description of the heritable material of plants and plant populations. Therefor an answer to the following questions can give a basis to the discussion on the issue of ownership of PGRFA:

- What are the capabilities and limitations of "genetic fingerprinting" and related techniques for identifying PGRFA and their origin.
- What are the implications of these techniques for enforcing sovereign rights.
- What is the feasibility of determining the country of origin which provided the PGRFA, considering the different types of PGRFA.
- What legal requirements exist for intellectual property rights (IPR) protection; what types of material can be protected.
- To what extent can legal systems be modified to include a wider range of genetic material.
- What is the significance of various modalities of asserting sovereign rights over PGRFA for the conservation and utilization of PGRFA.

New and complex problems have to be faced in developing workable rules and regulations which, on the one hand honour the rights of farmers and states on their genetic resources, while on the other hand promote conservation and use of such resources in the interest of global food production now and in the future. The present report reviews existing legal systems concerned with biological materials and explores to what extent identity and origin of PGRFA can be established.

2. LEGAL ASPECTS OF OWNERSHIP

Since the dawn of agriculture, farmers have played a major role in domesticating and developing varieties, generally referred to as “landraces”, in a process of natural and human selection. This has led to a multitude of landraces of crops adapted to a wide range of environments far beyond the original range of distribution, in terms of eco-systems, environments and geography. Landraces were treated as a common resource and were generally freely exchanged between farmers and farming communities as crops were introduced to new regions and further developed. In large parts of the world this system is still operating in a continuous process of managing, maintaining and developing genetic diversity as part of agricultural practices and indigenous knowledge systems.

Knowledge of genetics led to a scientific approach to plantbreeding early this century. This occurred more or less simultaneous with the discovery of industrial production of fertilizers and later of chemical control of pests and diseases. These developments fundamentally changed the role and use of genetic diversity in agricultural production systems. In traditional agriculture landraces were and are adapted to different local environments. The main objective is often yield security and sustainability rather than maximizing production. Genetic diversity within and between crops is a major requirement in such systems to allow for adaptation to variation in environmental conditions in time and in space. Hence in such systems genetic diversity is maintained as a natural and essential component. In modern agriculture, production environments can to a considerable extent be adapted to the requirements of the crop through external inputs (fertilizers, irrigation, chemical plant protection) insuring yield security. This reduces the need for adaptation to the vagaries of local environments and thus for genetic diversity within varieties . Hence plantbreeding could concentrate on raising genetic yield potential and does so mainly by developing uniform varieties that make better use of external inputs in the production of harvested product. In this process modern agriculture has become an integral part of the industrial complex.

The commercialisation of plantbreeding in many industrial countries required private investments, and legal ownership of the results of plantbreeding became an issue. This has led to the development of Plant Breeders’ Rights (PBR) legislation. The number of countries adopting some form of PBR has gradually increased over the past 20 years and is being considered by many others as the market for modern varieties increases. However in countries where large numbers of farmers and communities continue to depend on local landraces for both socio-economic and environmental reasons, effective provisions have to be included to protect the role and rights of local innovation. This is especially important since local landraces are an important source of adapted genetic diversity also for modern plantbreeding. Central in this debate is the concept of Farmers’ Right.

A brief overview of PBR and Industrial Patent Systems is given below followed by a discussion on Farmers’ Right.

2.1 Plant Breeders’ Rights

PBR is a right granted by a government to plant breeders to exclude others from producing and selling propagating material of a protected variety for a period of 15 to 30 years. International harmonization of PBR legislation has taken place within the “Union Internationale Pour La Protection Des Obtentions Végétales” (UPOV). In April 1994 there

are 22 member States; Australia, Belgium, Canada, Czech Republic, Denmark, France, Germany , Hungary, Ireland, Israel, Italy, Japan, Netherlands, New Zealand, Poland, Slovak Republic, South Africa, Spain, Sweden, Switzerland, United Kingdom, United States of America.

A central aspect of PBR, relevant to the present report is the unanimous wish of all countries that originally established UPOV to preserve free availability of PGRFA including improved and protected varieties. This is expressed by the Breeders' Exemption, allowing use without restriction of the protected variety in the creation of new varieties and for scientific research. The reasons are obvious. Plant breeding is a step by step process developing better yielding varieties with improved characteristics or adapted to new environments. Current cultivars represent the sum of past achievements going back to the original landrace material. New characteristics identified (f. i. disease resistance genes) need to be bred into varieties throughout the range of distribution of the crop where the disease occurs. Any restriction in the availability of new and useful materials was considered unacceptable in the general interest. It could restrict progress in crop improvement geographically and might stimulate undesirable monopoly situations where industrial companies could decide who would and who would not have access to improvements essentially based on products of nature. Hence PBR was not conceived in its present form because varieties could not meet the requirements of industrial patents. It was specifically designed to provide a careful balance between the interests of the plant breeders and those of producers and consumers (Hardon 1991). The patent requirement of non-obviousness was dropped. New varieties are developed rather than invented. New characters are generally discovered through evaluation of PGRFA and are not new creations. Furthermore the characters in themselves tend to be obvious in the context of crop improvement rather than novel and unexpected.

Recognition under UPOV of the achievements of plant breeders is achieved by providing them with an exclusive right on their new varieties on the basis of uniform and clearly defined principles and conditions. To be eligible for protection varieties must be:

- distinct from existing, commonly known varieties
- sufficiently uniform and homogeneous
- stable under multiplication
- new in the sense that they must not have been commercialised prior to certain dates established by reference to the date of the application for protection

The UPOV Convention was signed in Paris in 1961 and entered into force in 1968. However a number of revisions were realized over the years (1972, 1978, 1991). The 1991 revision has not yet entered into force.

Major issues in these revisions were the rights and privileges of plant breeders and farmers; the former to use protected varieties in breeding, i. e. the previously mentioned Breeders' Exemption, and the latter to produce seeds of protected varieties for own use or use in their communities, i. e. the Farmers' Privilege. There has been growing pressures from privatized plantbreeding to limit both the Breeders' Exemption and Farmers' Privilege. The modification proposed for Breeders' Exemption is to increase the so-called minimal distance between varieties. It is meant to curb cosmetic breeding, only changing one or more characters to satisfy present distinction criteria. In the 1991 revision of the Convention Breeders' Exemption is excluded for *essentially derived* varieties. An essentially derived variety is a variety that retains its original genotype except for inclusion of a specific new characteristic such as for instance resistance against a particular disease introduced through back-crossing or genetic manipulation. The new variety in the

1991 revision can only be marketed with the permission from the holder of the original rights.

Protection is further strengthened by not only covering the use of all reproductive material, but commercial use of all material of the variety. This has potentially far reaching consequences. It allows enforcing PBR protection when harvested product from a specific variety grown in a country not recognizing PBR is imported into a country where the variety is protected. It illustrates increasing control linked to PBR.

A controversial issue is also the Farmers' Privilege allowing farmers to use own harvested seed of protected varieties for next years crop on their own farm. In spite of strong pressures from private plantbreeding, no consensus was obtained on lifting this privilege leaving the decision to individual national governments.

Finally the 1991 UPOV Convention has accepted the possibility of patents on the results of biotechnology and allows member countries choice between both options.

2.2 Patent protection

A patent is a right granted by a government to inventors to exclude others from imitating, manufacturing, using or selling a patented process or product for commercial use for a period usually 17 to 20 years. In return for a patent the inventor discloses how the invention works so that knowledge is available to the public. In order to obtain a patent, the subject matter has to be novel and inventive, i. e. not obvious to a person skilled in the art. Patent law, as PBR contains a provision known as Research Exemption, which however allows others only to study the protected subject matter. Hence, unlike PBR reproducing or multiplying it in any form is not allowed.

Internationally the Paris Convention for the Protection of Industrial Property provides for an overall treaty, establishing equal rights for nationals as well as for residents of its member countries under the national laws regulating intellectual property rights. At present 100 countries are members. A United Nations specialized agency, the World Intellectual Property Organization (WIPO) is charged with the administration.

2.3 Variation in Intellectual Property Protection

In spite of the various international conventions and agreements, there still is considerable variation in procedures of granting and in the interpretation of requirements in both PBR and in industrial patents. Such differences exists specifically between Europe and the US reflecting differences in their legal and political systems. Aspects relevant to PGRFA are briefly reviewed.

Differences in PBR between Europe and the US are mainly procedural. The actual nature of the protection is largely comparable as far as it affects PGRFA.

In patents the situation is more complicated. European patent laws are harmonized through the Strassbourg Convention of 1963 and subsequently by the European Patent Convention (EPC) of 1973. However also within the EPC there still is considerable diversity. The European Patent Bureau can issue patents for one or more of the 14 member countries. However the actual protection provided is still determined by national laws and may differ from country to country. Since not all EC-member states are members

of the EPC, the European Community has drawn up a Community Patent Convention (CPC) providing for unitary patents within the Community.

In essential aspects with regard to patenting biological material there is considerable agreement within Europe. All European patent laws contain provisions by which certain subject matter is explicitly excluded from patent protection. The EPC under Art. 53(b), and thus the patent laws of all EPC member states, exclude from patent protection plant or animal varieties or essential biological processes for the production of plants and animals. US patent law does not contain any exclusions. Here the extent to what is patentable is determined by case law.

In Europe considerable emphasis is placed on the criteria of non-obviousness whereby the invention should be a technical problem plus the solution to that problem, or is a teaching for a technical operation (Bent 1987). In the US a patent may be granted to whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof. The criteria of non-obviousness is less stringently applied and need only be “sufficiently different from the prior art”. In the US patents are granted for parallel cases even when the underlying problem has been solved already. In Europe this is much more difficult. This is a very fundamental difference, specifically with regard to patenting biological material.

However intellectual Property Legislation has now become a global issue as part of the GATT accord and the Biodiversity Convention. Market mechanisms and trade agreements are affecting adoption and scope of patenting in biological materials, widening their interpretation significantly both in Europe and in the U. S. A. Far reaching patent claims have been awarded on genetic modification not only covering varieties but even species. Many of these claims are or are going to be challenged in courts of justice. There still is considerable uncertainty as to what will be the outcome and how patent legislation will affect PBR legislation. Decisions are largely based on legal interpretations of patent legislation not developed for biological materials. The research community is increasingly becoming dependent on contracts from private industry which seriously erodes their critical intellectual and independent role in passing judgement on such matters. In fact many public institutions see patents as a promising way of solving their financial problems and increasingly become motivated by self interest and not by objective standards of the general interest of society.

2.4 Farmers’ Rights

Patenting in plants has come at a time when the whole issue of access to and ownership of PGRFA has become a matter of global debate. Partners in this debate are developing countries, some harbouring still existing centers of diversity of crops and industrial countries requiring such diversity for their crop improvement including biotechnology. Through the FAO Undertaking on Plant Genetic Resources (FAO 1985) developing countries sought recognition for their sovereignty over their natural wealth while accepting the basic principle that PGRFA should be seen as a Heritage of Mankind. Initial misunderstandings over the nature of protection provided in industrial countries by PBR were resolved and balanced by a proposed Farmers’ Right. Farmers’ Right is meant as a recognition of the contributions of many generations of farmers in the development of landraces. Unlike PBR, a Farmers’ Right can however seldom be attributed to a specific farmer or even a farmer community. The notion that specific landraces have evolved exclusively in a particular locality over long periods of time may generally not be realistic.

Landraces tend to be replaced, moved around, introgressed, and so on in a dynamic system of genetic change. It does not provide a stable situation. Hence, while landraces are undoubtedly of great value as a genetic resource for plant breeding, it is difficult to define individual landraces, assign a specific or relative value to them and decide who can be considered the owner. An interpretation of Farmers Right accepted by the FAO Commission on Genetic Resources (FAO 1989) sees it as an expression of the principle of global responsibility to conserve landraces and thereby insure their availability. To give expression to this responsibility and in recognition of the contribution of farmers, both FAO (1989) and Keystone (1991) call for the establishment of a sustained funding facility to strengthen and support community innovation and management of PGRFA. Such a facility should be placed within the United Nations and governed on the basis of one nation - one vote. Contributions to the fund should be made by governments on the basis of some acceptable criteria. At present approximately 125 countries, including most industrial countries are members of the FAO Commission on Genetic Resources while around 90 countries accepted the Undertaking in principle.

The U. N. Conference on Environment and Development (UNCED), held in 1992 in Rio de Janeiro, brought together over 100 Government leaders, representatives from 170 countries and some 30. 000 participants. Amongst its major outcomes were the signing of the Biodiversity Convention and the adoption of Agenda 21 which serves as a blueprint and an action plan for a “new global partnership for sustainable development”. Agenda 21 gives support to the concept of Farmers’ Right as proposed in the FAO Undertaking on Plant Genetic Resources. The Biodiversity Convention stresses national sovereignty over PGRFA. The implication is that insuring conservation and providing adequate rewards to farmers for contributions in development and management of PGRFA would rest with national governments. Governments can regulate the collection of PGRFA within their national borders and enter into agreements on the use of such resources and a share of benefits from products developed from such resources.

2.5 Problems in asserting sovereign rights over Plant Genetic Resources

The Biodiversity Convention still has some unresolved issues regarding agricultural biodiversity. Unlike natural biodiversity, PGRFA for agriculture have been moved around and used widely and are incorporated in many collections around the world. Collections made prior to ratification are excluded from the Convention. Resolution Three of the Nairobi Final Act (22 May, 1992) noted that the status of such collections and Farmers’ Rights need more debate. Such a debate should include the status of the FAO Undertaking *vis a vis* the Convention, and specifically resolve how international responsibility for conservation can be combined with national sovereignty.

Another more difficult issue is how to give formal recognition and realize effective provisions for rewards and compensation to local farmers and communities for their past and continuing contributions in maintaining and enhancing genetic diversity. Such recognition requires reliable and objective genetic definition of the identity of landraces and populations of wild species. The capabilities and limitations of “genetic fingerprinting” and related techniques for identifying PGRFA and their origin are reviewed below.

3. GENETIC ASPECTS CONCERNING IDENTIFICATION

In the present section we will first briefly define the terms that are frequently used in the discussion on genetic identity such as “genotype”, “population”, “landrace” and “stable

variety". This will be followed by a description of the classic and the modern techniques of characterizing plants, a description of origin of genetic resources on an evolutionary scale, and the section will end with a discussion of the feasibility of the identification of the origin of genetic resources.

3.1 Types of genetic material

The genetic material in the plant resides in the DNA, a double chain of linked nucleotides in the nucleus of every cell. A sequence of three nucleotides form a triplet, and specific series of triplets can form a gene. These genes are the fundamental physical and functional units of heredity. The position of a gene on the chromosome is called locus, and each different form of a gene on a specific locus is called an allele (Griffiths *et al.* 1993). Besides the coding regions on the chromosome there is also plenty of DNA that is, as far as we know, not expressed.

The combination of genes of an individual is the genotype. In a given individual with its specific development and environment, not all genes are expressed. The expression of the genotype is called the phenotype, and can be considered to be the result of the interaction between the genotype and the environment in which the individual developed. For example, many genotypic differences between plants in disease resistance will only be expressed if there is an infection pressure for the disease, genotypic drought tolerance can only be expressed under drought stress.

Plants generally occur in groups, called populations. These populations can comprise of many different genotypes. Maintaining heterogeneity is a common strategy of those populations to survive varying conditions. It is not only applied by wild populations but also by landraces. Landraces, also called farmers' varieties, are generally heterogeneous populations of crop plants grown by traditional farmers. The heterogeneity within landraces buffers for the differences in growing conditions within farmers fields and between growing seasons; in some conditions some plants will thrive, while in other conditions others will. As a result these populations are not stable; their composition varies over seasons and farmers.

Commercial varieties resulting from modern plant breeding, are generally genetically homogeneous populations, highly adapted to uniform human controlled growing conditions. This homogeneity is not just the result of extreme selection of the "best" genotype, but also a requirement for obtaining Plant Breeders' Rights, as discussed previously. Thus breeders have to ensure that their varieties are homogeneous. This can be accomplished in several ways:

- By selecting a homozygous genotype, i. e. a genotype that has only one allele per locus, and multiplying it. Homozygous varieties are common, for example, for cereals such as wheat and barley.
- By selecting and vegetatively propagating a genotype. Vegetatively propagated varieties are common for potatoes, tree crops and many ornamentals. Homozygous and vegetatively propagated varieties are stable populations, i. e. the offspring is genetically identical to the parent.

An alternative for producing homogeneous varieties is

- By producing hybrids. A hybrid is a first generation cross product of homozygous genotypes, always having one allele per locus from each parent. Hybrid varieties are common for maize and many vegetables. Since the alleles in the generative offspring of

hybrid varieties will segregate, these varieties are not stable, and need to be reproduced from the original homozygous parental lines.

There are also less strictly homogeneous varieties, for example the varieties of cross pollinating and open pollinated crops, such as rye grass. To register varieties of these crops, which are mixtures of interbreeding genotypes, a minimum level of homogeneity in well defined traits has to be accomplished.

3.2 Classic and modern techniques of characterizing plants

The choice of characteristics is crucial in the description of a plant. The oldest and most commonly used characteristics are morphological and physiological traits such as shape of stem and leaves, presence or absence of hairs, flowering time, disease resistance, etc. These traits are currently used for the registration of cultivars. A disadvantage of using this type of characteristics is the fact that their expression is to varying degrees susceptible to environmental influences. This influence can be avoided by only observing monogenic qualitative morphological traits such as flower colour (like Mendel did in his early pea experiments). These traits more directly show the genotype of the plant, but have the obvious disadvantage that there are only few of them available.

Biochemical markers, such as isozymes and storage proteins, can extend the list of qualitative markers. Not only are they much less influenced by growing conditions as compared to quantitative traits, they also often have the advantage that they can be established in a relatively quick and cheap way on starting materials such as seeds, bulbs and tubers, making them suitable for cultivar identification. However, sometimes biochemical markers do not show sufficient differences between genotypes. In such cases molecular markers can be used.

Molecular markers are determined directly on the genetic material, the DNA itself. Therefor, the results obtained with molecular marker techniques are totally independent of the environment in which the material is grown. The last two decades several molecular techniques have been developed that enable characterisation of genetic material, yielding a wide variety of new markers showing diversity at any level of differentiation. These new powerful techniques deserve a closer examination.

The techniques can be divided in - those that describe a genotype without looking at any trait, - those that describe a specific part of the genome, usually based on markers that detect DNA *linked* to a trait of interest but not detecting the DNA encoding the trait itself, and finally - those that characterize the gene of interest itself.

These techniques are based on three different detection principles: Southern hybridisation, polymerase chain reaction (PCR) and DNA sequencing. All methods have their specific ad- and disadvantages.

3.2.1 Southern hybridisation

Methods based on Southern hybridisation display differences in the length of restriction fragments. Polymorphisms detected are therefore called restriction fragment length polymorphisms or RFLPs. Restriction fragments are obtained by digestion of the DNA with specific endonucleases. These enzymes cut the DNA each time a specific nucleotide sequence, the restriction site, is encountered. After digestion, the DNA fragments obtained are size separated, denatured and transferred to a filter. Since a single plant genome generates approx. one million DNA fragments, a special trick has to be used to visualize individual restriction fragments. For this a labelled DNA fragment, the so-called probe, is allowed to bind to the DNA on the filter. Binding will only occur to complementary DNA fragments. This generates a banding pattern that can be compared between genotypes. Differences between genotypes result from mutations in the restriction site as well as deletions and insertions in the restriction fragment. A probe can either detect a single locus or more then one locus at a time.

The use of RFLP analysis with single locus probes is widespread in plant breeding (Tanksley *et al.* 1989; Miller and Tanksley 1990). The main application at this moment is the construction of molecular maps, which are available now for all major crops. RFLPs can be linked to traits of interest like, for example, genes that confer resistance to pests and diseases. Once linkage is established, the RFLP can be used as an indirect selection marker for that trait (Van der Beek *et al.* 1992). Single locus RFLP probes can also be used for the characterization of a genotype as a whole. In such case a large number (approx. 100) of single locus probes evenly dispersed throughout the genome have to be used. Whether such an approach is feasible depends on the number of polymorphic loci in a particular crop and the amount of money available.

Multilocus probes detect several restriction fragments which may be dispersed throughout the whole genome. A multilocus RFLP pattern is often called a DNA fingerprint. Such type of fingerprint can be obtained by the use of either mini- (Dallas 1988; Nybom *et al.* 1990) or micro-satellite (Weising *et al.* 1991) sequences as probe. Both types of satellite DNA are highly polymorphic (Jeffreys *et al.* 1985; Weising *et al.* 1991). In contrast to single locus RFLP analysis in which marker bands are codominant, the bands in a multilocus RFLP are usually dominant, i. e. a band is either present or absent.

Not every probe works well for each crop. Optimal probe-enzyme combinations have to be determined on a crop to crop basis. Hardly anything is known about the distribution of mini- and microsatellite loci in plant genomes. Linkage of fingerprint bands to traits of interest is purely accidental. Fingerprint patterns obtained with multilocus probes are usually very genotype specific. They can be used for identification purposes, but are of no value for identifying PGRFA, since in breeding practice generally only small portions of a genome will be used to improve cultivars (Van der Beek *et al.* 1992). Besides this, if a fingerprint pattern has to be used for identification of PGRFA, it will have to be compared to the fingerprint pattern of all the potential sources. Whether this is feasible, will depend on the number of sources.

3.2.2 Polymerase chain reaction

The polymerase chain reaction (PCR) is a technique that allows the specific amplification of DNA fragments. Methods based on PCR display differences in the length of amplified DNA fragments. The fragments amplified are determined by the primer(s) used for the amplification process. Depending on primers and amplification protocols used, either a single- or a multilocus amplification pattern is obtained. A multilocus amplification pattern can be obtained without the need for sequence information for designing primers. They are designed randomly. Only a single primer is used and methods that generate such patterns are known by the acronyms RAPD (random amplified polymorphic DNA), AP-PCR (arbitrarily primed - polymerase chain reaction) and DAF (DNA amplification finger-print) (Rafalski and Tingey 1993). Primers are selected that show polymorphisms between genotypes. Also with this method it is possible to link polymorphisms to a trait of interest. In that case they can be used similarly to the single locus RFLP, but they differ by the fact that bands generated by RAPD, AP-PCR and DAF are dominant.

Since the amplification conditions used for producing RAPD, AP-PCR and DAF fingerprints are very sensitive to variation, reproducibility of the fingerprints can be a problem. Factors that can affect reproducibility are: DNA concentration, type of PCR machine used, temperature profile in the machine, use of different batches of primers and polymerase, etc. (Büscher *et al.* 1993; Meunier and Grimont 1993). To overcome this, polymorphic bands produced by RAPD or related methods can be converted into single locus markers (Paran and Michelmore 1993). Such markers are then called SCARs (sequence characterised amplified regions). Amplification protocols used to amplify SCARs are much more robust, and therefore more reliable.

Also by PCR amplification of microsatellites highly polymorphic single locus markers can be obtained (Beckmann and Soller 1990). Acronyms used for this type of marker are: STMS (sequence tagged microsatellite site) and SSR (simple sequence repeat). This type of marker usually is codominant and reliably amplified. Since the microsatellite DNA detected is highly polymorphic, and due to the fact that there are usually more than two alleles, the information content of this type of marker is high (Thomas and Scott 1993).

3.2.3 DNA sequencing

Once a gene is isolated it can be characterized by determination of its nucleotide sequence. DNA sequencing is a well established and reliable technique. Unfortunately, it is not very easy to isolated a gene from a plant genome when nothing is known about the protein it encodes, or when no homologous genes from other sources are available. As a consequence, until now only very few important genes have been cloned and sequenced.

All classical and modern methods described above can detect differences and similarities between genotypes. They look at the DNA at different levels, ranging from a phenotypic expression heavily influenced by environment, to the determination of the nucleotide sequence of a specific gene. The molecular methods are capable of showing small differences between genotypes, making them suited for variety identification purposes and possibly also for assessment of genetic diversity. Showing and/or proving similarity is a different matter, as will be shown below.

3.3 Origin of Plant Genetic Resources

Genetic resources for agriculture (PGRFA) are all sources of genetic material that can be used to breed improved varieties. These sources currently comprise of wild populations of related species, landraces, commercial varieties and research material such as plant breeders' lines, genetic stocks etc. It can be expected that biotechnological techniques will make it possible in the near future to use any genetic material as source for enriching or improving crops, making all DNA, including synthetic DNA, potential PGRFA.

Genetic diversity in crop plants is the result of evolution, including domestication, on-farm improvement and plant breeding. These processes caused increasing differentiation amongst populations, resulting in plants and populations that are to varying extents genetically distinct and have specific traits and adaptations.

The first level of genetic differentiation is that among species. Evolutionary divergence resulted in association of characters that makes it possible to distinguish well defined groups, i. e. species. These groups can be characterised on the basis of a limited number of characteristics defined by a minute part of the total DNA. It can be assumed that due to reproductive isolation a considerable part of the rest of the DNA is also unique to the group, and that differences within a group will be smaller than those between groups.

The second level of genetic differentiation results from infraspecific divergence as a result of domestication and/or on-farm crop development. The processes causing genetic change under domestication are basically the same as those acting under evolution in nature, i. e. mutation, natural selection, genetic drift and migration. Only human selection, partly conscious and partly unconscious, is added, and the rate of change is much higher as compared to natural evolution (Pickersgill 1984). Domestication generally causes a reduction of genetic diversity as compared to the wild diversity in the species due to selection and the founder effect; only a small part of the wild gene pool is taken into cultivation (Ladizinsky 1985). Natural introgression of additional "wild" diversity into crops is limited due to the generally recessive nature of cultivated characteristics (Ladizinsky 1985, Lester 1989). The results of introgression from wild populations will show mainly wild characteristics, and will thus often have a selective disadvantage in the human environment.

Within domesticated species landraces developed. Landraces generally show some form of adaptation to the local practices and environment of a certain region. Since exchange of heritable material between landraces grown within a region will be more frequent than between regions, they can be assumed to share a common genetic background including the adaptations to the general growing conditions.

In the geographical distribution of domesticated diversity it is possible to identify spatial scales (Zimmerer and Douches 1991). The largest or macro-geographic scale was first described by Vavilov (1926) in his centres of origin, and subsequently enlarged by Zhukovskys mega-centres of diversity (1975, Zeven and de Wet 1982). Several studies indicated that these often subcontinental centres divide into smaller sub-centres, the meso-geographical scale (Simmonds 1976). Micro-geographical centres of crop diversity are often related to geographical distinct areas such as coasts and mountainous areas (e. g. Weltzien 1989). Besides this genetic differentiation in geographical regions, within a region often landrace groups (Zeven 1986) can be distinguished on the basis of a complex of name, morphology or usage (e. g. Elings 1991, Zimmerer and Douches 1991).

Modern plant breeding brings the processes of evolution under human control and changes the rate radically, resulting in commercial varieties.

3.4 Identification of the origin of Plant Genetic Resources

Given our present knowledge of differentiating processes and our ability to describe individual genotypes, it is sometimes assumed that it should be possible to identify the origin of genes, genotypes or populations. There are many complicating factors.

Using large number of markers, as made available by techniques such as RAPD, it is possible to characterise an original PGRFA population to such an extent that it can be traced to an region of origin if all candidate regions of origin have also been characterized in a similar manner. “Region of origin “ has to be defined usually on a meso-geographical scale and will only rarely be confined to one country.

If the combination of genes and genotypes is altered by selection and recombination with material from other sources, and the original material thus becomes a component of the new population or genotype the situation becomes more complex. In traditional breeding, cultivars can be improved by introgression of new genes from unrelated germplasm. These breeding programs are very time consuming due to large number of backcrosses that are needed to eliminate the undesired genetic material from the unrelated parent. To circumvent this, breeders are starting to use molecular genetics to clone the gene of interest prior to introduction into the already high yielding cultivar. In addition, an isolated gene can be transferred beyond species boundaries. In such an extreme case in which only one gene is considered, it becomes impossible to trace the origin of the gene. Even if it can be shown that the DNA sequence of the gene, and the genetic material that was transferred with it, is equal to that of the expected origin, this might be explained by homology, i. e. resemblance due to inheritance from a common ancestry. The gene might have originated early in the domestication, or even before domestication. It might always have been present in considerable frequencies in the expected origin, but simultaneously in low frequencies in other sources, thus making it impossible to prove the sources of the gene on the basis of genetic makeup.

An added complication is that when the sequence of a useful gene is known it is relatively easy to change that sequence without affecting the function or amino acid composition of the protein encoded by the gene. This is due to degeneracy in the genetic code, i. e. different triplets can code for the same amino acid. It is sometimes even necessary to change the nucleotide composition of a coding region, or to add or change the promoter- or regulatory region to obtain sufficient or tissue specific expression when transferred to another genetic background. Therefore, many changes in the gene sequence can be expected on a regular basis once genes are used to transform plants.

In summary, the characterization of genotypes has improved considerably with the development of modern biochemical techniques. This allows us to prove that for example two plants are identical, or that a hybrid is derived from two given parental strains. This gives these techniques a high potential for PBR systems. If original PGRFA are considered, such as a landrace or a wild population, it is possible, if sufficient data are available, to locate the most probable of all candidate origin regions of such a PGRFA. This probable origin region will often not be confined to one country. If a single gene is considered, it is not possible to prove its origin, since any gene might occur in different places due to homology.

4. SUMMARY DISCUSSION AND CONCLUSION

National sovereignty over natural resources has been reaffirmed in many international agreements, declarations and resolutions amongst which is the UNESCO Convention for the World Cultural and Natural Heritage. Also the Biodiversity Convention affirms that right and authority to regulate access to PGRFA through national legislation. There is however an emerging concept of "common concern" of human kind that requires consideration in sovereignty over natural resources (Hunter *et al.* 1994). PGRFA obviously qualify for such consideration since they are essential to global food production while no country is self-sufficient for the genetic diversity needed for crop improvement. Hence national governments should be encouraged to cooperate in their own interest in the conservation and sustainable use of these resources. This is a basic principle in the FAO Undertaking on Plant Genetic Resources. However the issue of common concern seems to have been weakened in the Biodiversity Convention, probably because its specific requirements were not well understood. This applies specifically to the exclusion of genebank and botanic garden material collected prior to the coming into force of the Biodiversity Convention. The implication is, that unless agreed interpretations are going to be negotiated, holders of such collections, both governments and private corporations hold full authority over use and access to such collections regardless of their origin.

Regardless of the outcome of such negotiations in which the planned FAO Technical conference on Plant Genetic Resources could play an important role, reliable identification of specific genetic entities (varieties, landraces, wild populations and specific genes) and their origin are an important issue.

4.1 Varieties

Reliable and exclusive identification of modern commercial varieties has been and is subject of considerable research to allow PBR protection to be enforced. It is important to realize that this did not only involve the development of appropriate methods of identification, but also and in conjunction set rules for the genetic requirements varieties had to satisfy to fit such identification. Morphological characterization is now, through biotechnological research being supplemented by characterisation at the biochemical and molecular level. Refining techniques of identification allows PBR protection to be enforced between morphological and genetically more closely related cultivars. PBR legislation is continually adapted and modified to satisfy changing economic situations and to better satisfy the requirements of plantbreeding. It should be remembered that PBR is a national legislation only harmonized by UPOV. Hence any country is free to establish a PBR system that is appropriate to its particular circumstances. However, as it stands methodologies developed in the context of PBR assume a level of genetic uniformity not matched by landraces and wild populations. In addition, restricting exchange of landraces between farmers within countries by some form of ownership or regulatory framework may well reduce the effectiveness of this informal breeding system and may favour the stronger farmers and the richer communities. This matter requires very careful consideration.

An interesting proposal to recognize Farmers' Rights in PBR legislation is provided by India. The M. S. Swaminathan Research Foundation (Swaminathan and Vineeta Hoon 1994) in India proposes inclusion of Farmers' Rights in a special article in PBR legislation. It states that "the identification of donors of the genes contributing to the success of a new variety will be based on the recommendations of the National Institute for Plant Variety

Testing and Evaluation which will analyze the pedigrees and other relevant details relating to the parentage of varieties submitted for release and identify to the extent possible the locations from which the useful parent material originated". Where it is not possible to identify precise origin of parent materials, agreed royalties will be credited to a National Community Gene Fund. This formulation avoids the need for precise genetic identification of landraces as is a condition for modern varieties protected by PBR. It is specifically stated that this regulation can only be enforced within the territory of India. It is interesting to note, that in this proposal protection of landraces (understandably) concerns their use as a genetic resource in further breeding, which is excluded for varieties in the UPOV-PBR legislation, but does not restrict exchange between farmers.

4.2 Landraces and wild populations

Sovereign rights over landraces and specific wild populations of plants assume identifiable uniqueness of such entities to specific countries. There are examples of species evolved in and/or adapted to very rare and geographically limited environments. These include species endemic to island-states. If used in any form of development, it might be possible to trace their origin with the present techniques. However theses are rare and so far have not yielded material of wider economic importance. The common situation is that species and their different populations transcend over national boundaries. As was shown above, it might be theoretically possible to trace the origin of samples of such genetic entities to their original site/population/landrace origin with the present range of techniques available for characterisation. It however requires the actual sample to be a true representation of its original source, which seldom will be the case and requires characterisation of all possible source populations which is generally physically and economically impossible. The problem of tracing the origin becomes even more intractable if the combinations of genes and genotypes in a sample have been altered by selection and recombination and/or regeneration (for instance in an environment that differs from that of the original source population).

Even if the original site of origin or source population is known through passport data, assigning ownership remains a problem. The original farmer from whose field a specific accession was collected, may have obtained his material from other farmers or communities and so on in a long process of migration. The same applies to wild materials and natural migration. Similar material may have been collected elsewhere reducing the uniqueness of any landrace material or wild populations to a specific site. Hence biology and biochemistry does not as yet provide the tools to establish or support specific ownership of biological materials even if the principle of "beyond reasonable doubt" could be applied. The controversial principle of "reversal of proof" suggested for protection of patents of individual genes in a new genotypic background realised through biotechnological manipulation would not solve this problem. There is generally no difficulty in establishing differences of sub-samples even with the original source population. All that is possible given the present state of technology is to assign a probable geographic origin to certain accession. This however rarely means a country, much less a farming community.

4.3 Genes

Specific genes are of major interest to plantbreeding. For instance genes that provide resistances to pests and diseases or certain other qualitative improvements. Such genes are routinely screened for in collections of PGRFA. Location of such genes in specific

accessions however does not mean to say that they are unique to the source screened. The gene might have originated early in the evolution and/or domestication and be present in many other sources if looked for. An added problem is that even if the actual DNA-sequence of the gene is known, which is rare, the sequences in such genes can be changed relatively easy without affecting their expression and thus blurring their origin. There are some examples of specific resistances that so far only have been found in material from restricted regions. An example is resistance to yellow dwarf virus in barley originating so far exclusively from Ethiopia. These examples are however extremely rare and can not be used to establish general rules and regulations.

The conclusion is evident. It is impossible, even at the present state of increased knowledge, to earmark genetic entities “beyond reasonable doubt” to any specific genetic source-materials. In fact it is doubtful whether it will ever be possible. Genetic diversity results from random events of mutation that may occur at any time and in any population. Through selection the frequency of specific genes or gene-combinations may be increased or decreased, but not its actual occurrence. Uniqueness as a principle therefor makes in fact no biological sense. Are there any alternative forms of protection?

4.4 Alternative options for enforcing sovereign rights

As stated before, the principle of sovereign rights on biodiversity as present in the Biodiversity Convention concerns primarily access to natural biodiversity and other plant genetic resources grown in a country. National governments can regulate access to such diversity and state the terms on their use. The Biodiversity Convention by implication suggests that such access between countries could or should be regulated by bilateral agreements. In the context of PGRFA this may have some far reaching consequences. The economically stronger and larger countries will be in a much better position to negotiate such agreements than smaller and poorer ones. This will negatively affect access to genetic diversity and options for improvement of specifically countries and farmers in the poorer regions, leaving aside the ethics of ownership and control over use and access to essentially products of nature. This however only applies to original PGRFA. Once such diversity is used in further crop improvement, it will be extremely difficult if not impossible to reliably trace their origin.

Within countries, the use of landraces could be regulated as suggested by an Indian proposal to include Farmers’ Right in PBR legislation. Specifically the avoidance of any restrictions on the use and exchange of landraces between farmers and farming communities is essential. These community systems will remain important for some time to come for those regions to which modern varieties are not adapted. Free access and exchange is an essential requirement for such systems.

All in all it is clear that the only innovations that are not legally protected within present intellectual property (IP) regimes are those of indigenous populations. This was also concluded the Keystone International Dialogue on Plant Genetic Resources (1989-1991). This dialogue suggested that given the complications of assigning individual or even country ownership to specific PGRFA, the most practical way of recognizing Farmers’Rights would be a mandatory fund, such as the fund currently existing within FAO, which supports genetic conservation and utilization programmes particularly, but not exclusively, in the Third World. The logic is that such a fund would benefit farmers and farm communities in general, and would compensate them for their past and present contributions.

In a follow-up study reported in the book People, Plants and Patents (IDRC 1994) by the so-called “Crucible Group”, various alternative *sui generis* possibilities are suggested to provide more specific recognition of Farmers’ Rights and National Sovereignty. Essentially three approaches are suggested; (i) to propose mechanisms that will give protection to the intellectual achievements of rural communities within the IP system; (ii) to develop a *sui generis* system of “protection” that will meet the letter, if not the spirit, of the GATT proposal; (iii) or to propose an alternative *sui generis* system of intellectual recognition that may be outside of IP protection. Various options are discussed, including the possibility of Compulsory Licencing within IP Regimes, Material Transfer Agreements, adaptation of the WIPO-Unesco Model Provision for Folklore and others. Without going into details, the Crucible Group was unable to propose a single and straightforward solution.

It is evident that the present policy vacuum in the framework of the Biodiversity Convention around issues regarding enforcement of national sovereignty and Farmers Rights on PGRFA need to be addressed at both national and international levels. It is suggested that it should be a major topic at the FAO’s Fourth International Technical Conference on Plant Genetic Resources planned to be held in Berlin in 1996.

5. REFERENCES

Beckmann, J.S., and M. Soller, 1990. Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. Bio/technology 8: 930-932.

Bent, S.A., 1987. Intellectual property rights in biotechnology worldwide. New York Stockton Press, New York.

Büscher, N., E. Zyprian and R. Blaich, 1993. Identification of grapevine cultivars by DNA analyses: Pitfalls of random amplified polymorphic DNA techniques using 10mer primers. Vitis 32: 187-188.

Dallas, J.F., 1988. Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite DNA probe. Proceedings of the National Academy of Science (USA) 85: 6831-6835.

Elings, A., 1991. Durum wheat landraces from Syria. II Patterns of variation. Euphytica 54: 231-243.

FAO, 1985. Report of the first session of the FAO Commission on Plant Genetic Resources. FAO, Rome, CPGR/85/Rep., 12p.

FAO, 1989. Agreed interpretation of the International Undertaking. Resolutions 4/89 and 5/89 of the Twenty-fifth Session of the FAO Conference, Rome, Italy, November 11-29, 1989.

Griffiths, A.J.F., J.H. Miller, D.T. Suzuki, R.C. Lewontin and W.M. Gelbart, 1993. An introduction to genetic analysis, fifth edition. Freeman and Company, New York. 840p.

Hardon, J.J., 1991. Intellectual property protection and genetic resources. IBPGR International Crop Network Series 4, Rome: 29-36.

Hunter, D., J. Sommer and S. Vaughan, 1994. Concepts and Principles of International Environmental Law: An Introduction. United Nations Environ
ment Programme; Environment and Trade Series 2. 51p.

IDRC, 1994. People, Plants and Patents, the impact of intellectual property on biodiversity, conservation, trade and rural society. IDRC, Ottawa, Canada. 112p.

Jeffreys, A.J., V. Wilson and S.L. Thein, 1985. Hypervariable 'minisatellite' regions in human DNA. Nature 314: 67-73.

Keystone, 1991. Keystone International Dialogue Series on Plant Genetic Resources, 1991. Oslo Plenary Session. The Keystone Center. 42p.

Ladizinsky, G., 1985. Founder effect in crop-plant evolution. Economic Botany 39: 191-199.

Lester, R.N., 1989. Evolution under domestication involving disturbance of genetic balance. Euphytica 44: 125-132.

Meunier, J.-R. and P.A.D. Grimont, 1993. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res Microbiol 144: 373-379.

Miller, J.C. and S.D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theoretical and Applied Genetics 80: 437-448.

Nybom, H., S.H. Rogstad and B.A. Schaal, 1990. Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus*, *Prunus*, and *Rubus* (Rosaceae). Theoretical and Applied Genetics 79: 153-156.

Paran, I. and R.W. Michelmore, 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theoretical and Applied Genetics 85: 985-993.

Pickersgill, B., 1984. Evolution of hierarchical variation patterns under domestication and their taxonomic treatment. in: B.T. Styles (ed.), Infraspecific classification of wild and cultivated plants. Clarendon, Oxford, UK. pp 191-209.

Rafalski, J.A. and S.V. Tingey, 1993. Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends in Genetics 9: 275-280.

Simmonds, N.W. (ed.) 1976. Evolution of crop plants. London, UK: Longman Group Ltd. 339p.

Sugiura, M., 1989. The chloroplast chromosomes in land plants. Annual Review of Cell Biology 5: 51-70.

Swaminathan, M.S. and Vineeta Hoon, 1994. Methodologies for recognizing the role of informal Innovation in the conservation and utilization of plant genetic resources. Proceedings No. 9, CRSARD, Madras. 46p.

Tanksley, S.D., N.D. Young, A.H. Paterson and M.W. Bonierbale, 1989. RFLP mapping in plant breeding: new tools for an old science. Bio/technology 7: 257-264.

Thomas, M.R. and N.S. Scott, 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theoretical and Applied Genetics 86: 885-990.

Van der Beek, H., R. Verkerk, P. Zabel and P. Lindhout, 1992. Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: CF9 (resistance to *Cladosporium fulvum*) on chromosome 1. Theoretical and Applied Genetics 84: 106-112.

Vavilov, N.I., 1926. Studies on the origin of cultivated plants. Bulletin of Applied Botany 26. Leningrad, USSR: Institute of Applied Botany and Plantbreeding. 248p.

Weising, K., B. Beyermann, J. Ramser and G. Kahl, 1991. Plant DNA-fingerprinting with radioactive and digoxigenated oligonucleotide probes complementary to simple repetitive DNA sequences. Electrophoresis 12: 159-169.

Weltzien, E., 1989. Differentiation among barley landrace populations from the Near East. Euphytica 43: 29-39.

Zeven, A.C., 1986. Landrace groups of bread wheat (*Triticum aestivum* L. em. Thell.). Acta Horiculturae 182: 365-376.

Zeven, A.C., and J.M.J. de Wet, 1982. Dictionary of cultivated plants and their regions of diversity, excluding most ornamentals, forest trees and lower plants. Wageningen, The Netherlands: PUDOC. 263p.

Zhukovsky, P.M., 1975. World gene pools for plant breeding: mega-genecentres and endemic micro-genecentres. Leningrad, USSR: USSR Academy of Sciences.

Zimmerer, K.S., and D.S. Douches, 1991. Geographical approaches to crop conservation: the partitioning of genetic diversity in Andean potatoes. Economic Botany 45: 176-189.