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Agriculture germplasm resources: A tool of conserving diversity

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Three major physical resources in the world comprise land, water and the biological diversity. Agricultural biodiversity is an important component of biodiversity, which has a more direct link to the well being and livelihood of mankind than other forms of biodiversity. In fact, it is one of our most fundamental and essential resources, one that has enabled farming systems to evolve since the birth of agriculture about 10,000 years ago. Food plant and animal species have been collected, used, domesticated and improved through traditional systems of selection over many generations. The resulting diversity of genetic resources developed by early farmers now forms the basis on which modern high yielding and disease resistant varieties have been produced to feed the growing human population, expected to reach 9.1 billion by 2050. According to the Convention on Biological Diversity (CBD), "agricultural biodiversity includes all components of biological diversity of relevance to food and agriculture, and all components of biological biodiversity that constitute agro-ecosystems: the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agricultural ecosystem, its structure and processes". The effective conservation and use of agricultural biodiversity is very important in ensuring sustainable increases in the productivity and production of healthy food by and for mankind as well as contributing to increased resilience of agricultural ecosystems.

Key words: Agricultural biodiversity, ecosystem, Convention on Biological Diversity (CBD), domestication, human population, variability.

INTRODUCTION

There are many threats or drivers of changes on biodiversity that have been recognized and intensified in recent years (Millennium Ecosystem Assessment, 2005). With regard to agriculture the most important ones include changes in land use, replacement of traditional

varieties by modern cultivars, agricultural intensification, increased population, poverty, land degradation and environmental change (including climate change) (FAO, 2010). It is predicted that climate change will have a significant impact on agriculture with temperatures

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rising on average by 2 to 4°C over the next 50 years, causing significant changes in regional and seasonal patterns of precipitation (Burke et al., 2014). Climate change will also impact agricultural biodiversity in a major way. Model projections carried out by Lane and Jarvis (2007) based on global distribution of suitable cultivated areas of 43 crops, highlight that more than 50% may decrease in extent. Evidence based on bioclimatic modelling suggests that climate change could cause a marked contraction in the distribution ranges of CWR. In the case of wild populations of peanut (*Arachis* spp.), potato (*Solanum* spp.) and cowpea (*Vigna* spp.), studies suggest that 16 to 22% of these species may go extinct by 2055, with most species possibly losing 50% of their range size (Jarvis et al., 2008). These threats or drivers of change are leading to large scale degradation and loss of agricultural biodiversity and consequently its genetic variability (Millennium Ecosystem Assessment, 2005; van de Wouw et al., 2009). Information regarding the threat and rate of genetic erosion among various components of agricultural biodiversity is important, yet very little work has been carried out to quantify the magnitude of any trends. The availability of large gene pools, including CWR, is becoming even more important as farmers will need to adapt to changing conditions that result from these pressures. It is likely that many of the genetic traits which will be necessary to adapt our crops to changing climate will be found in CWR. Hence, it is widely urged that such strategies be adopted which may be used to get maximum crop stand and economic returns from adverse environments. Major strategies which may be used to overcome the adverse effects of such stressful environments may include screening and selection of well adopted existing germplasm of potential crops (Ahmad et al., 2014).

There are two main strategies for conserving agricultural biodiversity, namely *ex situ* and *in situ* conservation, both of which are equally important and should be regarded as complementary (Thormann et al., 2006; Engelmann and Engels, 2002). *Ex situ* conservation is the conservation of components of biodiversity outside their natural habitats. It is generally used to safeguard populations that are at present or potentially under threat and need to be collected and conserved in genebanks in the form of seeds, live plants, tissues, cells and/or DNA materials. Article 2 of the CBD defines *in situ* conservation as “the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties” (UNCED, 1992). It thus refers to the maintenance of a species in its natural habitat. This can be either on farm, requiring the maintenance of the agro-ecosystem along with the cultivation and selection processes on local varieties and landraces, or in the wild, which involves the maintenance of the ecological

functions that allow species to evolve under natural conditions.

STATUS AND TRENDS OF AGRICULTURAL BIODIVERSITY

Little is known about the global status of agricultural biodiversity. Although the CBD recognize genetic diversity as one of the fundamental levels of biodiversity, actions to protect genetic diversity are lacking (Laikre et al., 2010). Policy makers and scientists require a better understanding of how the intraspecific diversity is changing over time and space in order to make informed decisions for their conservation. However, there is no routine global scale monitoring of genetic diversity over time (Frankham, 2010; Laikre et al., 2010), except for a few target species at national level (Laikre et al., 2008). A major challenge remains to develop simple inexpensive means to monitor genetic diversity at a global scale (Frankham, 2010). Several efforts under the 2010 Biodiversity Indicators Partnership (<http://www.twentyten.net>) have been made to identify indicators useful to detect changes in species and ecosystem diversity, but there are only two initiatives that are explicitly working on developing indicators that deals with genetic variation for agricultural biodiversity (Laikre, 2010; Walpole et al., 2009).

The only authoritative account of agricultural biodiversity status at the global level is represented by the First and Second reports on the *State of the World's Plant Genetic Resources for Food and Agriculture* published by the Food and Agriculture Organization of the United Nations (FAO, 1998, 2010). The Second Report mention that there are about 7.4 million accessions conserved in over 1750 gene banks around the world in either seed banks, field collections, and *in vitro* and cryopreservation conditions (FAO, 2010). This represents an increase of more than 1.4 million accessions added to *ex situ* collection since publication of the first report on the *State of the World's Plant Genetic Resources for Food and Agriculture*. Although reportedly over-represented, a large part of the genetic diversity of major food crops is stored in *ex situ* collections. The exact proportion is still uncertain, but estimates suggest that more than 70% of the genetic diversity of some 200 to 300 crops is already conserved in gene banks (SBSTTA, 2010). In addition there are over 2,500 botanic gardens maintaining samples of some 80,000 plant species (FAO, 2010). However, regeneration of gene bank accessions remains a major problem, threatening collections (FAO, 1998). In the past decade there have been significant advances made in regenerating collections at risk, in part due to efforts made by the Global Crop Diversity Trust (CGDT) in supporting regeneration programmes of globally important priority gene bank collections for 22 priority crops for which crop specific regeneration guidelines

have recently been produced (Dulloo et al., 2013). Another major achievement has been the creation of the Svalbard Global Seed Vault (SGSV) in 2008, established to serve as the ultimate safety net for seeds samples from the world's most important collections (GCDT, 2010).

Great efforts for the conservation of many CWRs and wild species have been made by the Millennium Seedbank (MSB) at Wakehurst Place, Royal Botanic Gardens, Kew, UK which aims to house up to 10% of the world's seed-bearing flora, principally from arid zones by 2010. Genetic erosion has also been prevented by the significant amount of crop genetic diversity in the form of traditional varieties and neglected and underutilized species (NUS) that continues to be maintained on-farm. Yet, in spite of these advances, important reservoirs of adaptive variation such as CWR, landraces and NUS, which are increasingly recognized by the global scientific community as key resources for the maintenance of agro biodiversity, remain under-represented (FAO, 2010). CWR in particular, which have avoided the genetic bottleneck of domestication, contain greater genetic variation than their cultivated relatives and represent an important reservoir of genetic resources for breeders (Maxted and Kell, 2009). Yet to retain the genetic characteristics that make them so valuable for crop improvement, it is now widely recognized that populations of CWR are best conserved *in situ*, in their wild habitats, where they can continue to adapt and evolve along with their natural surroundings, thus ensuring new variation is generated in the gene pool and the continued supply of the novel genetic material critical for future crop improvement. The underpinning of the conservation strategy of most countries is a protected areas system and this is reflected in the CBD, where the main thrust of biodiversity conservation is *in situ*, through the development of such protected systems. Populations of many CWR occur in existing protected areas, but this alone does not in many cases represent effective *in situ* conservation without some degree of management or intervention targeted at the populations of the particular target species, particularly if the species is threatened. Despite protected areas being in existence for many years we still have not been able to undertake significant actions to conserve the CWR they contain, except a few cases.

Despite this, the *in situ* conservation of CWR has gained increasing attention in many countries, as demonstrated by their inclusion in the many national reports drafted for the Second report on the *State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 2010). Unfortunately, little quantitative data were provided by countries on the changing status of CWR, but several reports indicated that specific measures had been taken to promote their conservation. The Second report also mentions that surveys and inventories of CWR were carried out in at least 28 countries and many

new priority sites for conserving CWR *in situ* have been identified over the last decade. There is also evidence that public awareness of the importance of CWR, and neglected and under-utilized species such as traditional vegetables and fruits, is growing both in developing and developed countries (FAO, 2010). This has been furthered by a number of global initiatives aimed at conserving CWR, such as the proposed establishment of a global network for the *in situ* conservation of CWR (Maxted and Kell, 2009), and more concretely by the creation of web-based international platforms for the exchange of CWR information and data. These include the European platform "An Integrated European *In Situ* Management Work Plan: Implementing Genetic Reserves and On Farm Concepts" (AEGRO) and the CWR Global Portal, developed as part of the UNEP/GEF Crop Wild Relative Project, that provides access to CWR information and data at the global level (Thormann et al., 2012). The significant increase in number of scientific articles published on CWR and on specific actions targeting their conservation is also a testimony to the renewed interest in CWR, however, to the best of our knowledge few of the recommendations have been implemented, largely due to a lack of funds and capacity.

Over the last decade, the number and coverage of protected areas has increased by approximately 30% (United Nations, 2010), yet limited efforts have been made to target CWR, whose conservation remains unplanned and largely an indirect effect of protecting flagship species or threatened habitats. For example, despite the increase in isolated activities targeting CWR conservation, the formal recognition and/or the adoption of appropriate management regimes to protect CWR are largely lacking. Furthermore, considering that national parks and other conservation areas cover only 12 to 13% of the earth's surface, it is clear that these areas alone will not be able to ensure the continued existence of CWR species, the majority of which occur in marginal lands outside protected areas, where no form of legal protection is offered. If protected areas are to ensure the long-term survival of CWR they will need to become more flexible in size and scale and a connected network of habitats will need to be established to allow species to migrate and adjust their ranges in response to global change and anthropogenic disturbances, along with the development of effective management strategies targeting their conservation (that is, off-reserve management). The success of this strategy will depend largely on promoting more biodiversity-sensitive management of ecosystems outside protected areas, and successfully engaging private landowners and local communities living around protected areas in the conservation process. Finally, more effective policies, legislation and regulations that take into account the impacts of global changes on future species distribution and that govern the *in situ* conservation of CWR, both inside and outside of protected areas, are needed, along

with closer collaboration and coordination between the agriculture and environment sectors.

Formidability of genetic resources

Wild plants have often played an important role in many diets due to their higher nutritional value than cultivated species. These are, at the same time, hardy and resilient. Crop varieties are improved by the suitable recombination of genes from the wild, made more productive, resistance to biotic stresses, tolerant to abiotic stresses, and better nutritional and keeping quality. Such characteristics, needed to improve crop varieties, may be found in a range of cultivated as well as wild plants. This broad variability provides essential link in the food chain, which, in turn, provides the basic for world food and nutritional security. Plant genetic resources essentially constitute the prime components of the food chain ever since the dawn of agriculture. In the history of some 12,000 years, nearly 30,000 edible plant species have been utilized as a source of food. However, merely a hundred odd plant species out of these have been propagated to provide about 90% of the world food and, further, only three species among these, namely, rice, wheat and maize produced the two-third. An assessment of the contribution of different plant sources towards the dietary energy supply at the global level shows predominance of only two crops, that is, rice (26%) and wheat (23%) (FAO, 1996). The search for new diversity is, therefore, important.

In the developing and the economically weaker parts of the world, the discovery of wild species for food may have coincided with the hunger season, such as, those preceding the crop harvest particularly when drought or flood situations occurred. Mother Nature provided food for people at such junctures when they badly needed it and the resultant discovery of plant species or their diversity became the automatic human choices for further propagation. Even today, though agriculture has advanced so much, humans still gather many wild and semi-wild plants or plant parts like fruit, leaf root, seed, nut, wood etc. for use. About 80,000 species of plants have been used to meet the routine needs by the human beings. Of these, 30,000 species so far have been identified as edible and about 7,000 species have been cultivated and/or collected for food at one time or the other. Presently only 20 to 30 crops, such as cereals (wheat, rice, maize, millets, sorghum), root/tuber crops (potato, sweet potato, cassava), legumes (pea, beans, peanut, soybean) and sugarcane, sugar beet, coconut and banana are mainly used to feed the world (NAS, 1975).

Synoptic view of plant diversity

There are 425,000 species in living plants in the plant

kingdom from unicellular algae to the highly evolved flowering plants. The flowering plants are a diverse group which are seed producing plants that have evolved in synchronization with the evolution of insects which help the plants in cross-pollination assuring heterozygous population. Hence, this group of flowering plants (about 250,000 species) have developed great plasticity for adaptation for different climatic regimes. They consist of a variety of life forms from the minute *Wolffia* (1 mm long) to the largest *Eucalyptus* regnant growing to height of 100 m. This spectrum of flowering plants includes humble herbaceous species, beautiful orchids, parasitic *Rafflesia arnoldii* having largest flowers (1 m across), plants of medicinal value, trees of timber importance, food plants, fodder species, gums and resin producing plants. The 250,000 flowering plant species are packed in about 17,000 genera and 300 to 400 families. Some of the economically important families which hold life supporting food sustenance species are Gramineae, Legumonosae, Criciferae, Cucurbitaceae, Rosaceae, Brassicaceae and Rutaceae. The drug yielding families cover a spectrum of alkaloids producing crops such as, Apocynaceae, Papavaraceae, Asteraceae, Cannabinaceae, Piperaceae, Zingiberaceae and Rubiaceae. Gums and resins occur in several families as Eurphorbiceae, Dipterocarpaceae, Mimosaceae and Sapotaceae. The families vary in size from monotypic ones to large families having 25,000 to 35,000 species. The family Orchidaceae has about 25,000 to 35,000 species, Compositae has about 20,000 species, Legumionceae has about 14,00 species and Gramineae has about 8,000 species, while there are about 35 families which has only one species.

Plant diversity in India

Indian subcontinent has a rich and varied heritage of biodiversity, encompassing a wide spectrum of habitats from tropical rainforests to alpine vegetation and from temperate forests to coastal wetlands. It is one of the eight centres of origin (Vavilov, 1951) and is one of the 12 mega gene centres of the world; possess 11.9% of world's flora. About 33% of the country's recorded flora are endemic to the region and are concentrated mainly in the North-East, Western Ghats, North-West Himalays and Andaman and Nicobar islands, nurish one third of the human population on this earth (Damania, 2002; Mayres et al., 2000) have brought out an updated list of 25 global hotspots of diversity out of which 8 hotspots are in figured in India. Indian sub-continent is a centre of domestication and diversification of several economically useful wild plant species comprising about 3,000 plants of edible value, 4,000 species having known reputed medicinal value, 700 plants of traditional and social significance, 500 fibre yielding species, 400 fodder species, 40 species having insectivorous uses, 300 gum and dye yielding plants, 100 aromatic and essential oil yielding

species (MoEF, 1994; Chowdary and Murti, 2000). Indian diversity comprises of 49,219 higher plant species, out of which, 5,725 are endemic and belonging to 141 genera under 47 families. Of these endemic species, 3,500 are found in Himalayas and adjoining regions and 1,600 in the Western Ghats alone.

India is a homeland of 167 cultivated species and 329 wild relatives of crop plants (Arora, 1991). It has around 30,000 to 50,000 landraces of rice, wheat, Pigeonpea, mango, turmeric, ginger, sugarcane, etc. and ranks 7th in terms of contribution to world agriculture. Further, around 1,000 wild edible plant species are exploited by native tribes. These include 145 species of roots and tubers, 521 of leafy vegetables/greens, 101 of buds and flowers, 647 of fruits and 118 of seeds and nuts (Arora and Pandey, 1996). In addition, nearly 9,500 plant species of ethano-botanical uses are reported from the country of which around 7,500 are of ethano-medical importance and 3,900 are multipurpose edible species.

The endemic plant wealth of the country has also been supplemented with the species/forms that had been introduced from abroad. These species got naturalized over time and have undergone the process of domestication on being isolated climatically and spatially. Prominent among these are apple, pear, peach, apricot, grape, almond, date palm, maize, potato, sweet potato, tomato, bean, onion, garlic, chilli, lentil, clove, coriander, cumin, fennel, coffee, cocoa, cashew nut, litchi, cinchona, strawberry, blueberry, tea, rubber and pineapple.

Biodiversity in Jammu and Kashmir

The State of Jammu and Kashmir has been regarded as heaven on earth, and is also called the bio-mass state of India. This area, located in the far north of the Indian republic, is a mountainous zone in the north-west Himalayas that shares international boundaries with Pakistan in the west, Chinese autonomous region of Xinjiang in the north and Tibet in the north-east. The North-western-Himalayan region being the rich repository of biological heritage, particularly in respect of agri-horticultural crops and was recognized that collection and maintenance of germplasm is essential to provide genetic diversity within a crop and to reduce chances of genetic vulnerability. Exploration and collection of native biodiversity, particularly in agri-horticultural crops of the region, including the wild relatives, rare/endangered plants together with the documentation of related ethano-botanic information for exercising to concomitant with regeneration and preliminary evaluation of the collected genetic resources to ensure their long term conservation as well as use in crop breeding programmes recognizing the fact that improvement and sustenance of cultivated crop species requires variability.

A total of 1911 germplasm accessions comprising local cultivars that were in cultivation before introduction of

improved cultivars, old varieties, land races, wild crop relatives and under-utilized crops of agri-horticultural significance were collected in respect of various field, vegetable, and horticultural crops as well as medicinal and aromatic plants. The collected biodiversity included 742 accessions in cereals, 38 in pseudo cereals, 28 in millets, 71 in oilseeds, 358 in pulses, 377 in vegetable crops, 21 in spices and condiments, 13 in fodder crops, 204 in medicinal and aromatic plants, 55 in fruits crops and 4 in others. The collection of agro-biodiversity in different crops has not only helped in ensuring their conservation on a long term basis but their use may also increase productivity, food security and economic returns. The valuable biological resources will make the farming systems more stable and sustainable. By establishing suitable linkages with user scientists in the university and sister institutions in the region a total of 382 accessions in cereals, 135 pulses, 78 vegetables, 26 horticultural crops, 110 in medicinal and aromatic plants were made available for use in respective crop improvement programmes. Their eventual use in the development of varieties with high yield potential and improved quality characteristics may diversify production and income opportunities for the end user.

Conservation of germplasm

Global concern about loss of valuable genetic resources prompted international action. Programs for conservation of plant genetic resources for food and agriculture were thus initiated and gene banks established in many countries. The main objective was to collect and maintain the genetic diversity in order to ensure its continued availability to meet the needs of different users. The concept of germplasm conservation demands that collection methods initially capture maximum variation and subsequently, conservation and regeneration techniques minimize losses through time. To this effect, plant genetic resources (PGR) conservation activities comprise collecting, conservation and management, identification of potentially valuable material by characterization, and evaluation for subsequent use.

There are two approaches for conservation of plant genetic resources, namely *in situ* and *ex situ*. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of the traditional agricultural systems. *Ex situ* conservation on the other hand, involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Approaches to *ex situ* conservation include methods like seed storage, field gene banks and botanical gardens. DNA and pollen storage also contribute indirectly to *ex situ* conservation of PGR.

Ex situ conservation approach

Ex situ conservation refers to the conservation of germplasm away from its natural habitat. This complementary approach for conservation had begun on a wide scale about three decades ago and is now practised, to some extent, in almost all countries as a means to conserve crop species diversity for posterity. This strategy is particularly important for crop gene pools, and can be achieved by propagating/ maintaining the plants in genetic resource centre, botanical gardens, tissue culture repositories or in seed gene banks. The Second Report mention that there are about 7.4 million accessions conserved in over 1750 genebanks around the world in either seed banks, field collections, and *in vitro* and cryopreservation conditions, (FAO, 2010).

Various approaches are employed for the *ex situ* conservation depending upon the mode of reproduction and nature of plants to be conserved. *Ex situ* conservation approach generally comprises the following methods: seed storage, field gene banks, *in vitro* storage, pollen storage, DNA storage and botanical gardens.

Seed storage

In the past, many collections were maintained without the help of storage facilities which would affect the viability of seeds. Due to this, the conserved accessions had to be regenerated very frequently leading to loss of genetic diversity in gene banks (Frankel and Hawkes, 1975). In maintaining genetic purity of the conserved accessions, problems arise due to differential survival in storage, selection during regeneration, out-crossing with other entries and genetic drift (Allard, 1970). Good storage conditions coupled with proper grow-outs are expected to reduce the effects of such problems (Rao, 1980).

Storing orthodox seeds at low moisture content and at subzero temperature is the most convenient and widely used method of genetic conservation. Orthodox seeds are the seeds which can withstand dehydration without damage. This type of seeds can be stored in the dry state on long term basis (indefinite period) which can be prolonged by decreasing their moisture content and storage temperature (at sub zero temperature).

The number of seed storage facilities has increased dramatically over the last two decades. Today, according to the WIEW – World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture – databases of the FAO, there are 1320 national, regional and international germplasm collections in the seed form, 397 of which are maintained under long- or medium-term storage conditions. Over 6.1 million accessions have been conserved as seeds.

As opposed to common orthodox seeds, there are a number of species whose seeds are unable to withstand desiccation, that is, cannot be dried to low levels for

optimum storage. Such seeds are referred to as 'recalcitrant' seeds (Roberts and King, 1986). Mainly these seeds originate from the plants grown in tropical and sub-tropical regions. These seeds can be stored for short duration (up to several months) by imbibed storage (at higher levels of seed moisture/hydrated state) and relatively warm conditions (well above zero temperatures) because they are often chilling sensitive. e.g., rubber, cocoa, coconut. Seeds such as oil palm and coffee, showing intermediate storage behaviour (Ellis et al., 1990, 1991) were grouped as recalcitrant until recently. Careful adjustment of the desiccation level and storage conditions allowed their storage for increased period (1 to 2 years).

Very low temperature storage using liquid nitrogen, called cryo-preservation, appears to be promising, with a more extended life span, described as long-term storage (-20°C). Another area in which considerable work is required is on storage of ultra dry seeds (dried to seed moisture content of 2 to 5%) at room temperature conditions and in hermetically sealed containers (Zhou et al., 1995). However, more research will be necessary before ultra dry seed technology can be adopted (Zheng et al., 1998). Prior to embarking on any seed conservation programme, a decision is to be made on how long it will be necessary to maintain the germination capacity of the seed lots, because longer storage requires more exacting storage conditions. This shall be determined by the objective of the conservation which could be research, introduction, breeding, etc.

Field gene bank conservation

Many important varieties of field, horticultural and forestry species are either difficult or impossible to conserve as seeds (that is, no seeds are formed or if formed, the seeds are recalcitrant) or reproduce vegetatively. Hence they are conserved in field gene banks (FGB). FGBs provide easy and ready access to conserved material for research as well as for use. It is one of the options of a complementary strategy for the conservation of germplasm of many plant species.

The conservation of germplasm in field gene bank involves the collecting of materials and planting in the orchard or field in another location. Field gene bank has traditionally been used for perennial plants, including:

- i) Species producing recalcitrant seeds;
- ii) Species producing little or no seeds or sterile seeds;
- iii) Species that are preferably stored as clonal material;
- iv) Species that have a long life cycle to generate breeding and/or planting material.

Field gene banks are commonly used for such species as cocoa, rubber, coconut, coffee, sugarcane, banana, cassava, sweet potato, yam, tropical and temperate

fruits, vegetatively propagated crops (e.g. wild onion and garlic) and forage grasses (e.g. sterile hybrids or shy seed producers). This is the traditional method of conservation to keep the germplasm in plantations as mature trees. It provides mature material for vegetative propagation, hybridization and characterization. The site for a field gene bank should have a suitable climate and soil for the species and should have an adequate water supply. The site should be chosen in a location with little or no threat of pests, diseases, bush fire and vandalism. To avoid loss of vigour as well as to prevent the incidences of attack by pests the plants have to be replanted routinely, and this adds to the cost further.

Botanical gardens

There are about 2500 botanic gardens and arboreta worldwide. It is estimated that these gardens maintaining samples of some 80,000 plant of threatened species in botanical gardens and arboreta. The objectives of most of the gardens include:

- (a) Maintaining essential ecological processes and life support systems,
- (b) Preserving genetic diversity, and
- (c) Ensuring sustainable utilization of species and ecosystem.

However, the botanical gardens may play a limited role in the context of conservation and propagation and probably a greater role in public awareness and education. Botanical gardens may mainly be used to display a great number of different and exotic species. As the number that can be maintained in this manner is limited, it cannot reflect or conserve genetic diversity. There is a possibility that a few well-managed gardens can emphasise on conservation of certain groups of species as living collections (that is, field gene banks). Often botanical gardens focus their conservation efforts on wild, ornamental, rare and endangered species. Indeed botanical garden conservation could be considered as field gene bank and/or seed gene bank, depending on the conservation method being used. The living plant collections in botanic gardens and arboreta may be considered as field collections, but the original purpose of the gardens and arboreta is not for germplasm conservation. Most of the germplasm conserved in botanical gardens do not belong to the PGRFA.

In vitro storage

Research on finding solutions to better conserve these difficult-to-store seeds has focused on the use of biotechnology (Engelmann and Engels, 2002). *In vitro* slow-growth conservation methods, involving culturing

different parts of the plant (meristem, tissues, cells) into pathogen-free sterile culture in a synthetic medium with growth retardants have been cited as good ways of complementing and providing backup to field collections. It has long been known that *in vitro* slow growth method suffers high risks of somaclonal variation (Withers, 1993) and also from the need to develop individual maintenance protocols for the majority of species (Thormann et al., 2006).

Slow growth

Slow growth procedures allow clonal plant material to be held for 1 to 15 years under tissue culture conditions with periodic sub-culturing, depending on species. There are several methods by which slow growth can be maintained. In most cases, a low temperature often in combination with low light intensity or even darkness is used to limit growth. Temperatures in the range of 0 to 5°C are employed with cold tolerant species, but for tropical species which are generally sensitive to cold, temperatures between 15° and 20°C are used. It is also possible to limit growth by modifying the culture medium, mainly by reducing the sugar and/or mineral elements concentration and reduction of oxygen level available to cultures by covering explants with a layer of liquid medium or mineral oil (Withers and Engelmann, 1993). Although slow growth procedures have been developed for a wide range of species, they are routinely used for conservation of genetic resources of only a few species including *Musa* spp., potato, sweet potato, cassava, yam, *Allium* spp. and temperate tree species. About 37,600 accessions are reportedly conserved by *in vitro* techniques in gene banks, worldwide (FAO, 1996).

Cryopreservation

Cryopreservation, the process in which living tissues are conserved at very low temperatures (-196°C) in liquid nitrogen (LN) or in vapour phase (-150°C) to arrest mitotic and metabolic activities, provides a more promising option (Thormann et al., 2006). Significant progress has been made in cryopreservation research over the past twenty years and much of that research has been focusing on understanding the desiccation sensitivity of recalcitrant seeds and on the underlying mechanism of desiccation tolerance (Engelmann and Panis, 2009). The techniques for cryopreservation currently in use are quite varied and include the older classical techniques based on freeze-induced dehydration of cells as well as newer techniques based on vitrification (Engelmann, 2000). In classical techniques, tissues are cooled slowly at a controlled rate (usually 0.1-4°C/min) down to about -40°C, followed by rapid immersion of samples in liquid nitrogen. Slow

freezing is carried out using a programmable freezing apparatus. Cryoprotectants are added to the freezing mixtures to maintain membrane integrity and increase osmotic potential of the external medium. Classical cryopreservation procedures have been successfully applied to undifferentiated culture systems such as cell suspensions and calluses (Kartha and Engelmann, 1994). However, in case of differentiated structures, they have been employed for storage of apices or embryonic axes of only cold-tolerant species (Reed and Chang, 1997), and their utilization for tropical species has been limited (Escobar et al., 1997). Vitrification-based procedures involve removal of most or all free able water by physical or osmotic dehydration of explants, followed by ultra-rapid freezing which results in vitrification of intracellular solutes, that is, formation of an amorphous glassy structure without occurrence of ice crystals which are detrimental to cellular structural integrity. These techniques are more appropriate for complex organs like embryos and shoot apices; they are also less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic facilities for tissue culture.

DNA storage

With the rapid development in the field of molecular genetics and genomics, DNA material is becoming more and more in demand for molecular studies and is one of the most requested materials from gene banks (Anderson, 2006). The establishing of a DNA storage facility as a complementary “back-up” to traditional *ex situ* collections has been suggested (Dulloo et al., 2013), but little effort has been made to collect and conserve DNA as a genetic resource. Some efforts have been made to establish DNA banks for endangered animals (Ryder et al., 2000) and a few plant DNA banks including Missouri Botanic Garden, Royal Botanic Gardens - Kew, Australian Plant DNA Bank and Trinity College Dublin (TCD) (Rice et al., 2006; Hodkinson et al., 2007). The Global Biodiversity Information Facility (GBIF) in Germany has established a DNA Bank Network in 2007, last accessed 22 September 2010, and offers a worldwide central web portal, providing DNA samples of complementary collections (microorganisms, protists, plants, algae, fungi and animals). The GBIF Germany DNA network would provide a good mechanism to link both to the scientific community conserving genotypes in genebanks and to breeders and molecular biologists that use the resources for genetic improvement.

Pollen storage

Pollen storage was mainly developed as a tool for controlled pollination of asynchronous flowering genotypes, especially in fruit tree. Even if it may not be considered to be a viable method for meaningful genetic

conservation of genotypes, cryopreservation is likely to be more successful than other storage techniques routinely employed for pollen. Pollen can be easily collected and cryopreserved in large quantities in a relatively small space. In addition, exchange of germplasm through pollen poses fewer quarantine problems compared with seed or other propagules.

The pollen longevity of different species varies between minutes and years depending on the taxonomic status of the plant and on abiotic environmental conditions. For some crops, the storage of pollen grains is possible in appropriate conditions, allowing their subsequent use for crossing with living plant material. It is also possible to regenerate haploid plants from pollen culture for some crops. By controlling the storage temperature and relative humidity (0 to 10°C, 10 to 30% RH, depending on species), pollens of *Citrus* spp., *Cocos nucifera*, *Fragaria* sp., *Olea europea*, *Pinus silvestris*, *Pistachio altantica*, *Pyrus malus* and *Vitis vinifera* could maintain their viability for more than 1 year.

For long-term conservation, cryopreservation seems to be the most efficient method. For example, maize pollen could be dried to 50% of its original water content in an air current for 1 h and then stored at -196°C in liquid nitrogen. Deep-frozen maize pollen can be used for fertilization after 10 years storage. Successful cryopreservation of pollen from various 24 crops has been reported (Barnabas and Kovacs, 1997).

In situ conservation

In situ conservation refers to conservation of genetic resources within their ecosystem and natural habitats. These techniques involve maintenance of genetic variation at location where it is encountered, either in wild or traditional farming systems.

Genetic reserves: in this type of conservation location, management, and monitoring of genetic diversity is carried in natural wild populations within defined areas designated for active, long-term conservation.

On-farm conservation: This refers to the sustainable management of genetic diversity of locally developed traditional crop varieties with associated wild and weedy species or forms by farmers within traditional agricultural, horticultural or agrisilvicultural cultivation systems.

Home gardens: This type of conservation is similar to on-farm conservation, involves smaller scale but more species-diverse genetic conservation in home, kitchen, backyard or door-yard gardens.

Complementary conservation

For *ex situ* conservation of PGR in a crop or crop group, a gene pool approach has to be followed for safe and

effective conservation. Following this approach, it is very likely that a range of *ex situ* conservation methods would be applicable to satisfy the needs of a gene pool. For example, the rice gene pool consists of self-pollinated cultivars and a range of wild *Oryza* spp. habitat to range of climatic conditions with breeding ranging from obligate vegetative to facultative and obligate self-pollination. In a situation, it is quite logical to have an approach, which is appropriate and has balanced application of both *in situ* and *ex situ* conservation methods. This will lead to the adoption of a more "holistic" approach to conservation. Even with *ex situ*, a balance has to be struck as per the need. For example, in case of wild *Oryza* species, it has to be assessed, whether they would be best conserved in a field gene bank or *in vitro* as cell, tissue, organ, pollen or perhaps as DNA or in combination thereof. Therefore, a network of complementary and comprehensive strategy is needed to ensure effective conservation and sustainable use of PGR for food and agriculture by present and future generations.

Svalbard global seed vault

A major achievement for the conservation of the germplasm have been the creation of the of the Svalbard Global Seed Vault (SGSV) in 2008, established to serve as the ultimate safety net for seed samples from the world's most important collections (GCDT, 2010). The Svalbard Global Seed Vault is a secure seedbank located on the Norwegian Island of Spitsbergen near the town of Longyearbyen in the remote Arctic Svalbard archipelago, about 1,300 km (810 miles) from the North Pole. The facility preserves a wide variety of plant seeds in an underground cavern. The seeds are duplicate samples, or "spare" copies, of seeds held in genebanks worldwide. The seed vault will provide insurance against the loss of seeds in genebanks, as well as a refuge for seeds in the case of large scale regional or global crises. The seed vault is managed under terms spelled out in a tripartite agreement between the Norwegian government, the Global Crop Diversity Trust (GCDT) and the Nordic Genetic Resource Center (also known as NordGen and previously named the Nordic Gene Bank, a cooperative effort of the Nordic countries under the Nordic Council of Ministers). The Prime Ministers of Norway, Sweden, Finland, Denmark, and Iceland participated in a ceremonial "laying of the first stone" on 19 June, 2006. The Svalbard Global Seed Vault opened officially on February 26, 2008. The first seeds arrived in January 2008. Five percent of the seeds in the Vault, about 18,000 samples with 500 seeds each, come from the Centre for Genetic Resources of the Netherlands (CGN), part of Wageningen University, Netherlands.

Construction of SGSV

The seedbank is constructed 120 m (390 ft) inside a

sandstone mountain at Svalbard on Spitsbergen Island. The bank employs a number of robust security systems. Seeds are packaged in special four-ply packets and heat sealed to exclude moisture. The facility is managed by the Nordic Genetic Resource Center, though there is no permanent staff on-site.

Spitsbergen was considered ideal due to its lack of tectonic activity and its permafrost, which will aid preservation. The location 130 m (430 ft) above sea level will ensure that the site remains dry even if the icecaps melt. Locally mined coal provides power for refrigeration units that further cool the seeds to the internationally recommended standard -18°C (0°F). Even if the equipment fails, at least several weeks will elapse before the temperature rises to the -3°C (27°F) of the surrounding sandstone bedrock. Prior to construction, a feasibility study determined that the vault could preserve seeds from most major food crops for hundreds of years. Some seeds, including those of important grains, could survive far longer, possibly thousands of years.

Mission and seed storage

The Svalbard Global Seed Vault's mission is to provide a safety net against accidental loss of diversity in traditional genebanks. While the popular press has emphasized its possible utility in the event of a major regional or global catastrophe, it will certainly be more frequently accessed when gene banks lose samples due to mismanagement, accident, equipment failures, funding cuts and natural disasters. Such events occur with some regularity. In recent years, some national genebanks have also been destroyed by war and civil strife. There are some 1,400 *crop diversity collections* around the world, but many are in politically unstable or environmentally threatened nations. The seeds are stored in four-ply sealed envelopes, then placed into plastic tote containers on metal shelving racks. The storage rooms are kept at -18°C (-0°F). The low temperature and limited access to oxygen will ensure low metabolic activity and delay seed aging. The permafrost surrounding the facility will help maintain the low temperature of the seeds if the electricity supply should fail. Approximately 1.5 million distinct seed samples of agricultural crops are thought to exist. The variety and volume of seeds stored will depend on the number of countries participating – the facility has a capacity to conserve 4.5 million.

Gene bank standards

Research on seed storage has indicated that the potential of seeds to store, that is, retaining genetic integrity and seed viability, is influenced by storage seed moisture content and temperature. Germplasm is generally conserved as a base collection or an active collection. Base collections are those that are being

conserved on a long-term basis for posterity. These are unique accessions that are closest to the original samples and are not to be disturbed except for regeneration of active collections. Active or working collections are those that are immediately available for multiplication and distribution for use in research and crop improvement. To minimise the alteration in genetic structure and loss of viability in germplasm accessions during storage, the seed genebanks (that are part of the national network) preferably follow the genebank standards as recommended by FAO/IPGRI (Anonymous, 2001) in relation to various factors important to the good maintenance of active and base collections. The base collections are being stored in modules maintained at -20°C . Such a low temperature minimises metabolic activities and is expected to enable the seed to retain viability for 50 to 100 years without any change in genetic structure. Active collections are stored in modules maintained at 4°C and 35 to 40% relative humidity, under which seeds are expected to remain viable for 15 to 50 years without substantial change in viability and genetic integrity. For both types of collections, seed is processed after validating physical and genetic purity of seed, assessment of seed viability and seed moisture content. In most crops, seed samples with more than 85% seed viability are conserved. However, recognising inherent problems, such as indeterminate nature, which limits the harvest of physiologically mature seed of the same age in certain crops like cotton, several forages and vegetable crop species, the initial viability standards have been lowered down to between 50 to 75%. For long-term storage, the seed moisture content is brought down to 3 to 7%, while for medium-term storage the seed moisture content is brought down to 8 to 10%. For base collections to be put under long-term conservation, the preferable size of accession is 2,000 seeds in the case of self-pollinated and 4,000 in the case of cross-pollinated crops. However, in many cases, such as groundnut and castor, because of large seed size and low multiplication rates, the sample-size of the accessions has been reduced to between 1,000 to 1,500 seeds. The base and active collections are regularly monitored for seed viability, seed quantity, seed health, etc., at recommended intervals of 10 and 5 years, respectively. However, the monitoring of accessions at the National Seed Genebank (NSGB) in the Germplasm Conservation Division, NBPGR has generated valuable information on storability in a number of crop species, such as wheat, minor millets, cotton, grain legumes etc. (Anonymous, 2001). These results suggest a revision of the exact period of monitoring intervals. This information will be useful in revising the seed genebank standards in relation to other components and make seed conservation more cost effective. Seed storage problems are more common in India, because a large part of the country has a predominantly hot and humid, tropical and sub-tropical climate with great variation in temperature, rainfall and relative humidity

across the year.

National network on conservation of PGR

Efficient conservation of PGR in a country of the size and dimension of India, one of the 12 mega-centres of plant biodiversity and where 384 crop plants are reported to be cultivated (of which 168 species were earlier reported under the Hindustani centre, one of the eight Vavilovian centres of origin and diversity (Paroda et al., 1999), essentially requires a network approach. Network facilitates short-, medium-, and long-term conservation requirements, the division of responsibilities, application of complementary conservation strategies, and access for the use of these genetic resources in crop improvement programmes. The national network consists of the NSGB at NBPGR headquarters, New Delhi, 11 NBPGR Regional Stations situated in different agro-climatic zones of the country, and 40 crop-based National Active Germplasm Sites (NAGS), located generally at various ICAR institutes. The network is linked with the All India Co-ordinated Crop Improvement Projects, various research institutes (crop-based institutes, project directorates and national research centres; multi-crop based institutes) in the ICAR, SAUs, etc. All network components operate in close collaboration to ensure the efficient conservation and sustainable use of germplasm in crop improvement, in which the National Seed Genebank plays a pivotal role in conservation.

The National Seed Genebank

The NSGB is responsible for conservation of seeds of unique accessions on a long-term basis, as base collections for posterity. In addition, it provides technical support to the network in the planning, development and operation of medium-term genebank facilities, in human resource development, and in provision of accessions for the regeneration of active collections. The Indian NSGB has 12 modules with a capacity to hold around 1 million accessions.

NBPGR Regional Stations

The NBPGR has 11 regional stations/base centres/satellite stations located in different agroecological and phytogeographical zones of the country. They are responsible for the collection, characterisation, evaluation and/or conservation of germplasm in the region. The regional stations also coordinate various PGR activities in the region with other partners. Seven of the regional stations have medium-term seed storage modules for the conservation of active collections to meet the requirement of the region for

various crops. The regional stations hold around 98,498 active collections. In addition, plant quarantine is looked after at the NBPGR headquarters, New Delhi and at the NBPGR regional station, Hyderabad.

National Active Germplasm Sites (NAGS)

The NAGS are based at ICAR institutes, at All India Co-ordinated Crop Improvement Projects and at SAUs. They are entrusted with the responsibility of crop specific collection, multiplication, evaluation, maintenance and conservation of active collections and their distribution to users at a national level. Large multiplications of active collections are preferred to reduce the number of regeneration cycles that can cause possible genetic changes and to meet the demand of seed distribution. The NAGS have a multidisciplinary team of scientists to study all the aspects of crop improvement, production and management. Therefore, the NAGS, in addition to their conservation role, are well equipped for the evaluation of germplasm and the generation of information on the potential value of accessions. This information forms the basis for use of accessions in research and crop improvement. Eleven of the NAGS have been provided with medium-term seed storage modules, to facilitate the use of active collections in research and breeding programmes.

Safety duplicates of crop species

There is a built-in duplicity of accessions in the system, wherein the accessions conserved at NAGS and the crop-based institutes as active collection are conserved as base collection in the National Genebank. The active collections are used in research and crop improvement and the National Genebank helps in restoration of lost accessions to the active sites. This also serves as safety mechanism. There exists medium to high capability for research and use of improved methodologies for *ex situ* conservation. Nevertheless, strengthening of technical and infrastructure capabilities is required in some cases.

The capacity building in genebank management and information systems has been carried out satisfactorily, though there is a need for extension of medium-term facilities to more crop based institutes to cover larger number of crops. In last ten years 196,745 accessions were collected under 166 projects involving 599 professional and of these 104 084 accessions have been conserved. The maximum number of accessions conserved in *ex situ* is in the category of traditional cultivars and landraces. A significant number of collections belonging to wild and weedy relatives and advanced and improved cultivars developed using various genetic resources is also being conserved.

Major achievements through germplasm conservation

Plant Genetic Resources for Food and Agriculture (PGRFA) are vital to the development and welfare of human society. They contribute enormously towards achieving the global objectives of food security and poverty alleviation, environment protection and sustainable development. The local communities and farmers in India have sustained and enriched the diversity of these resources which they domesticated, used, conserved and made available to meet the ever increasing needs of the present and future generations. Characterization and evaluation of germplasm is required to know its worth or usefulness and availability of information on characterization and evaluation of conserved genetic resources is the key to utilization. Plant breeding provides many examples of the use of genetic resources for the improvement of the varieties of crop plants. There are examples that range from highly specific improvement to one major factor such as susceptibility to a pest or disease to all round improvement in yield, agronomical traits, disease resistance and to changes in the form and structure of the plant type.

FUTURE THRUST

- i) Endangered germplasm from the threatened areas of diversity to be salvaged and conserved for future use.
- ii) Morphological and molecular characterization of germplasm to enhance their utilization in crop improvement.
- iii) Conservation, management and protection of bio-resources especially plant resources, through the participation of the people.
- iv) Conservation and use of diversity needed to be addressed in a holistic manner and to meet the demands of the users of germplasm.
- v) Research on core and mini-core collections and identification of new diverse sources.
- vi) Public awareness of the importance of CWR and neglected and underutilized species.
- vii) Need to maximize synergy through appropriate collaboration between various national, sub-regional and international levels.

Conflict of Interest

The authors have not declared any conflict of interest.

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