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The Ramiah Gene Bank : A Step-Forward in Agro-Biodiversity Conservation at TNAU

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India is one of the richest countries for plant diversity with approximately 16,000 vascular plants, 5000 endemic species and 140 endemic genera. However, an estimated 3000-4000 plant species are being threatened to be on the verge of extinction, fixing India, one of the highest priorities for plant biodiversity conservation. In recent decades, India has increasingly recognized the importance of plant diversity in efforts to conserve and sustainably use its plant diversity. Conservation requires a sound scientific and technical basis and there are two methods *viz*., *ex situ* and *in situ* conservation which are equally important and should be regarded as complementary. In the process of *ex situ* conservation, the National Bureau of Plant Genetic Resources (NBPGR) possess 3,85,645 germplasm accessions of various agri-horticultural crops. Realising the need of ago biodiversity conservation, TNAU has created a gene bank facility named after the legendary rice breeder Dr. K. Ramiah, with the capacity to conserve more than 1,00,000 germplasm accession under medium term storage (MTS) and long term storage (LTS) conditions. Presently a total of 13,567 accessions of more than 21 species are deposited in the gene bank. These provide an important reserve of plant resources for sustainable economic and social development. Thus, TNAU's strategic and vision for conservation of plant diversity and sustainable use of plant resources in the 21st century is of far-reaching significance for sustainable development of our economy and society.

Key words: Agro-biodiversity, ex situ conservation, in situ conservation, Plant Genetic Resources, Medium Term Storage, Long term Storage

Indian gene centre is one of the 12 mega biodiversity centres of crop plants with three hot spot areas *viz*., Himalayas, Indo-Myanmar region and Western Ghats with approximately 17,000 of higher plant species. However, the plant diversity in India is increasingly at risk, with an estimated 3000-4000 plant species being threatened or on the verge of extinction (Nayar and Sastry, 1987), making India, proportionally, one of the highest priorities for global plant biodiversity conservation. Coming in the face of the current ecological crisis, it is timely that India has initiated National Biosphere Reserve Programme during 1986 and so for 15 biosphere reserves have been designated all over the country (Mishra et al., 2006). These natural reserves play important roles in plant conservation.

Plant diversity is the basis for bio-resources and sustainable utilization. Crop improvement, particularly for climate resilience, pests and diseases, nutritional and food security depends on the genetic diversity of our plant genetic resources, which are arguably inadequately conserved and poorly used. It is widely recognized that that the Convention on Biological Diversity's 2010 target for reducing the loss of biodiversity has not been met.

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Biodiversity is at risk from multiple threats, and the genetic diversity contained within plant genetic resources, particularly of species that are wild relatives of our crops, faces similar threats; but it is essential to respond to the new threats to agricultural ecosystem resulting from changing weather condition. It is important to consider the genetic value of the crop diversity, how they may be conserved and what new technologies can be implemented to enhance their use.

The general trend of the past decades has been the development and cultivation of improved cultivars of many major and minor crop species. These cultivars tended to be uniform and are usually derived from a limited number of elite lines resulting in an increasingly narrow genetic base for the crop. This, together with large-scale cultivation of such genetically uniform cultivars, has increased the genetic vulnerability of many major agricultural crop species, often with disastrous consequences. An often quoted-example is the Irish potato famine of 1840s, when the potato crop in Ireland was virtually wiped out as the potato varieties grown then had no resistance to the leaf blight disease. Similarly the outbreak of rice brown spot disease in Bengal area in 1943, aggravated by typhoons, contributed to

serious famine in India (Council, 1972). In 1970, as a result of southern corn leaf blight, corn production decreased by about 25% in the southern states of the USA (Anon, 1973). However, in many of these cases, public and private plant breeders had access to genetic diversity and were able to produce resistant material within a relatively short time. Vulnerability due to increasing uniformity continues and several potential disasters may be brewing right now. For example, several important traditional crops of Oceania are highly threatened due to their narrow genetic base (Lebot, 1992). In 1993, taro leaf blight destroyed about 95% of the taro crop in Samoa, where it is the major staple food. The vineyards in California were being invaded by new biotypes of phylloxera, the aphid relative that affects the root system of vines. Since more than 70% of wine grapes in Napa and Sonata counties were grafted on susceptible rootstock, the grape crop was seriously threatened (Granett *et al.,* 1991). Given this situation, it is necessary to broaden the genetic base of crop plants requiring access to a large amount of genetic diversity. Although, the results of some surveys (Brown, 1988; Chang, 1994; Smale, 1997) indicate that the genetic base of several important crops has begun to increase over the years, breeding programmes of many important crops continue to include only a small part of genetic diversity available and the introduction of new and improved cultivars continues to replace indigenous varieties containing potentially useful germplasm. In fact, genetic diversity can be seen as a defense against problems caused by genetic vulnerability. Traditional farmers built this defense into the genetic structure of landraces through selection over many generations and it may be necessary to introgress such defense mechanisms into modern cultivars to make them sustainable (Martin *et al.,* 1991; Chang, 1994; Kannenberg and Falk, 1995).

Effective conservation of plant genetic resources requires a sound scientific and technical basis. An understanding is needed of the conservation methods that can be used and the ways in which they can be deployed. Central to any effective conservation programme must be a clear understanding of the extant genetic diversity in the species of concern - its structure and distribution in nature and in the material conserved, either *ex situ* or *in situ* (Allard, 1988; Hamrick and Godt, 1990; Hamrick, 1993; Hamrick and Godt, 1997).

Conservation of Plant Genetic Resources

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.*, 2010; Engelmann and Engels, 2002; Dulloo *et al.*, 1998; Maxted *et al.*, 1997). Article 2 of the Convention on Bio-Diversity (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

Ex situ conservation is the conservation of components of biodiversity outside their natural habitats (CBD definition, UNCED, 1992). 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 (Ramanatha Rao and Hodgkin 2002).

Ex situ conservation and sustainable use of Plant Genetic Resources

Ex situ conservation plays a key role in securing the conservation of plant diversity, not only as an insurance policy for the future, but also as a basis for restoration and reintroduction of threatened and endangered plants back to natural habitats. The most effective *ex situ* conservation measures are the conservation of living plants in botanical gardens (and arboreta) and field germplasm repositories and seed gene banks (Hawkins, *et al*., 2008).

Seed Gene Banks

It is a facility for maintaining crop diversity, usually in the form of seeds stored and conserved in a frozen state. Some genebanks use normal household freezers for this purpose. The ideal temperature is between -10°C and -20°C. The seeds are stored in containers, such as bottle, can, or a sealed tri-laminated aluminium foil package. The Food and Agriculture Organization of the UN lists about 1400 gene banks, ranging in storage capacity from a few hundred samples to the U.S. collection with 4,64,000 different samples. The National Bureau of Plant Genetic Resources (NBPGR) in India possesses 2,57,432 accessions of more than 200 species. Other major genebanks include those in China, Russia, Japan, S. Korea, Germany and Canada as well as those operated by Centers of the Consultative Group on International Agricultural Research (CGIAR). Seeds stored in genebanks can be maintained for indefinite period of time. Seeds of some crops, such as paddy, sorghum, small millets etc., may survive for a period more than 30 years, whereas, soybean, sunflower, cotton etc., may survive only for 10-20 years in a genebank. For continuous maintenance of seed gene banks, periodic monitoring of the viability of the stored seeds is essential and the deteriorating seeds have to be identified. A few seeds cab be taken from the

stored sample and planted. Fresh, new seed is then harvested and placed in the genebank. This way the original variety can be perpetuated forever.

Tamil Nadu Agricultural University 's initiative in Plant Genetic Resources conservation

Realising the need to conserve crop biodiversity to meet the future challenges, Tamil Nadu Agricultural University (TNAU), a pioneer institution of the country in agricultural research, technology development and education has established a Gene Bank facility, which was inaugurated by Prof. Dr. P. Murugesa Boopathy, Vice-Chancellor, TNAU on 23rd April 2010. The Gene bank facility is located in the premises of the newly incepted Department of Plant Genetic Resources at the Centre for Plant Breeding and Genetics. The gene bank is named after the legendary rice breeder Dr. K. Ramiah. It has an estimated 5000 cubic feet of cold storage space for medium and long term storage. The cold rooms in Ramiah Gene Bank have been commissioned with the state of art technology for maximum storage efficiency, energy conservation and eco-friendliness. The genebak is designed to store up to 1,00,000 germplasm entries including land races, wild species and commercially cultivated varieties. The seeds of the germplasm accessions intended for storage in the Ramiah gene Bank are processed adopting scientific principles of seed storage in a precision controlled packaged dehumidified chamber. The seeds are hermetically sealed with tri-laminated aluminium pouches before depositing in the cold room. The estimated storage life of seeds is likely to be 5 to 20 years depending upon the nature of seed. Thus by depositing seeds in the gene bank plant breeders can save resources, reduce the frequency of rejuvenation and reduce risk of genetic deterioration due to out-crossing and physical admixtures which are commonly encountered while raising crops in the fields.

Facilities in Ramaiah Genebank

The Facilities Available at Ramaiah Genebank include a medium-term cold storage unit capable of storing seeds of 50,000 germplasm accessions at 5°C and a long-term cold storage unit for conservation of 50,000 entries at -20°C. The cold storage units are equipped with stainless steel seed storage cabinets. The cold storage units are supported by a power generator for uninterrupted power supply. Besides, the gene bank has a walk-in precision controlled packaged dehumidifier (PCPD) unit for drying seeds, an imaging and documentation system for effective storage and retrieval of passport and characterization data, a vacuum sealing and packaging unit, a geographical positioning system and a rapid electronic moisture meter. The genebank also possesses a finger printing laboratory with facilities for molecular and biochemical characterization of germplasm entries. At present, Ramaiah Genebank is well equipped for conserving plant Genetic Resources through seeds. Experiments for conservation of vegetative propagules are underway and in the near future facilities to process and conserve vegetative propagules will be added.

Seed Processing Procedures

Seeds are received either from our own collection missions or from the plant breeders of TNAU. As soon as the seeds are received, they are transferred for temporary storage in PCPD unit where the temperature is maintained at 15°C and relative humidity at 45%.

For seed conservation in the gene bank we adopt the following principles.

1. The seeds meant for storage in the gene bank should not be treated with any chemicals like fungicides and pesticides as they may act as mutagens during long term storage.
2. Good quality seeds with a minimum viability of 80% or above will be accepted.
3. A minimum of 500 seeds in self-fertilized crops and 1000 counts in cross-fertilized species will be used for storage.
4. Seeds are dried up to 8% moisture content for medium and up to 6% for long-term storage.
5. A temperature of -20°C for long and 4°C for medium term storage will be adopted.
6. Monitoring of viability will be done once in every 5 years in medium and 10 years in long term storage.

Testing Seed Viability

Before processing for storage, the seeds are subjected to viability test in the viability monitoring unit through germination counts. A random sample seeds of 10% of the accessions received are subjected to viability Testing. The seed lots that record a minimum of 80% viability alone will be subjected to further processing.

Registration of germplasm entries

The seed lots that possess the required viability will be subjected to the registration process in the documentation unit. The registration is done through an in house database "TNGRID"(Tamil Nadu (Plant) Genetic Resources Integrated Database) using a high end computer server provided with barcoding facilities.

The registration process

Step 1 : Assigning a genebank ID

The genebank ID is assigned to each accession in the form of a thirteen character alpha-numeric string. The first four characters are alphabets "TNAU" which stands for Tamil Nadu Agricultural University. Fifth character is an alphabet to indicate the crop

code, which may be either 'F' for a Field crop or 'H - for a Horticulture crop, or 'T' for a tree crop and 'M' - for miscellaneous category. The next three characters will be numerals to accommodate '999' crop species under each crop code. The remaining five characters will also be numerals to indicate the accession ID of each entry. The entire process of assigning gene bank ID will be guided by a menu driven procedure of TNGRID.

Step 2: Passport data entry

The second step in registration process is to feed the passport data of the accessions received. This is also accomplished by a user friendly menu driven data entry form in the front end of TNGRID.

Step 3: Assigning barcodes

The third step of registration process involves the assignment of a unique barcode for each accession through a software "barcode tender". The documentation system is integrated with a barcode scanner and printer to facilitate this process. The barcode labels pertaining to each accession are printed out in duplicates from which one of the labels will be pasted on the aluminum pouch assigned for storage and the second label will be pasted on a printed out document that holds the information on the corresponding accession ID and the vernacular ID of an accession. This barcoding and documentation procedure will help quick retrieval of the seed packs physically from the gene bank as well as retrieval of data records from the database.

Seed storage

The two basic parameters that have to be controlled for enhancing the shelf life of seeds include storage temperature and moisture content of the seed. The storage temperature control is easily accomplished through the fully automated refrigeration systems of the gene bank. But, the control over the moisture content of seeds is still a challenging process that involves a lot of manual steps. At the Ramaiah gene bank we have standardized a simple and cost effective silica gel based rapid desiccation system for germplasm seed drying. As a first step in the drying process we estimate the initial moisture content in the seeds of 10% of the total number of accessions received chosen on a random basis with a rapid electronic moisture meter. The seeds are then transferred along with labels into cloth bags, which are then piled up in a plastic box containing a layer of approximately 1 inch thick desiccated silica gel. The plastic box is then closed with an air tight lid and the seeds are allowed to dry for specified period that depends on the drying properties of the seeds. We have standardized drying procedures for 30 different crop species and these procedures can be used to dry the seeds to the required level of 6-8%. The greatest advantage of the silica gel desiccation system is that silica is nontoxic and hydrated silica gel can be recycled indefinitely through microwave heating.

Vacuum Packing and Sealing

The seeds dried to the required level of moisture content are transferred from the desiccation unit to the vacuum packing unit. In this unit, the seeds from the cloth bags are transferred to pre-barcoded trilaminated aluminium pouches assigned to specific accessions. The aluminium pouches containing seeds are then subjected to vacuum sealing and packing process. This process is accomplished through an 'AudionVac' automated vacuum sealing and packing machine. It involves two steps and in the first step, the aluminium pouches are filled with a specified quantity of soft filtered air to maintain a uniform pressure throughout the pouches. In the second step, the pouches are vaccuumised to Zero Bar pressure and simultaneously subjected to a bilayered sealing. The entire process of vacuumization and sealing is guided by preset programmes which vary with seed properties like size and density.

Germplasm Deposition

The vacuum packed and sealed seeds in the aluminium pouches will be stacked in the barcode order inside a stainless steel basket. The stainless steel baskets with the stacked pouches will be placed in a specified stainless steel cabinet drawer to aid the process of easy location of the stored pouches. We assign a physical position number which is an alpha-numeric string of six digits. For instance, the physical position number C1-D1-B1, indicates that a particular pouch is placed in cabinet 1, drawer 1 and basket 1 in the genebank. This help in easy location and speedy retrieval of the germplasm seed materials.

Viability Monitoring Process

To monitor the viability of the stored accessions the users are requested to supply 2000 counts of seeds of at least one accession for each germplasm lot. This reference accession should have been harvested from the same field, in the same season as that of the other accessions of the particular lot. Two thousand seeds of the reference accession will be processed and packed in similar way and deposited in the same conditions as that of the other accessions of the lot. This pack of 2000 seeds is referred as the "monitoring pack" and will be used for viability monitoring through germination counts in periodic cycles. As and when the percentage of germination goes below 50% in the monitoring pack, seeds of 10% accessions of the lot, drawn on a random basis will be subjected to viability test. If the viability of the tested accessions also record germination count below 50%, then the seed packs of all the accessions of the lot will be sent back to the breeders for rejuvenation.

Germplasm under storage at Ramiah Gene Bank

In the first year of establishment, seeds of the following germplasm in various crops have been deposited in the Ramiah Gene Bank.

Crop	Germplasm under storage (Nos.)
Rice	2400
Maize	848
Sorghum	2429
Pearl millet	1200
Finger millet	2219
Foxtail millet	774
Barnyard millet	232
Little millet	108
Pigeon pea	230
Mungbean	195
Urd bean	851
Chick pea	334
Soybean	400
Groundnut	171
Sesame	344
Sunflower	255
Cotton	352
Guinea grass	100
Fodder bajra	50
Jatropha	35
Okra	40
Total	13567

Plant breeders and researchers are the major users of genebanks. The diversity stored in genebanks is the raw material for plant breeding and for a great deal of basic biological research.

The germplasm may have several unique characteristics, not all of which are visible to the eye - genetic traits that provide disease resistance, adaptability, nutritional quality etc. If these potentially unique and sometimes hidden traits found in germplasms needed to be used for crop improvement, then these germplasms should be conserved.

Plant Genetic Resources for a sustainable future

Plants are the core component of biodiversity on earth, the primary source of material for human living and sustainable economic productivity and the ultimate guarantee of the environment upon which human survival depends. They are the fundamental sources for provision of human necessities, including food, timber, oil and medicine, and a source of spiritual needs. So far, we have only explored and utilized a small proportion of all plant species, and the potential of the vast majority of wild plants has yet to be understood and is still beyond human knowledge, despite their great economic and social values. Although we depend on plants for our livelihood and socio-economic development, the damage to plants by human activities has reached an unprecedented level, gravely threatening human survival. The impact of human destructive activities is taking its toll on nature and the current extinction rate of plant species is 2000 times faster than would normally occur in nature (Brooks et al., 2006). One of the major challenges facing humanity in the 21st century is how to resolve the paradox of the increasing demand on plant resources (food, timber, natural medicines, energy, oil, fruit and vegetables, flowers, etc.) against their sustainability in the future. The convergence of our knowledge with the intention of solving this paradox is hopefully managed by developing the revolutionary bio-industry and bio-economy. 'An important species can affect a country's economy and a special gene can affect a country's prosperity' (Zhang and Huang, 2009). Botanic research, knowledge and technology innovation and sustainable utilization of plants in a country can be a reflection of its overall development and strength. In the 21st century, biological resources may be one of the most crucial resources for the globalized economical and societal sustainability. Whoever possesses rich plant resources, innovative knowledge and new technologies for plant conservation and sustainable utilization will have a distinct advantage.

Considering the current global scenario and policies related to the conservation and sustainable use of Plant Genetic Resources, the initiative taken by Tamil Nadu Agricultural University by commissioning the Ramiah Gene Bank is timely in the lines of Convention of Biodiversity. This gene bank will act as a state repository for the conservation of germplasm that will aid in the efforts on characterization, documentation and sustainable use by the Department of Plant Genetic Resources. Intensive efforts are underway for collection and conservation of land races of native crops like rice, sorghum, finger millets, other small millets and pulse crops, so that these valuable genetic resources will be available for the future generations which are otherwise likely to face extinction.

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