

CONSERVATION OF INDONESIA PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE IN ICABIOGRAD GENE BANK

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ABSTRACT

Diversity of plant genetic resources for food and agriculture (PGRFA) represents a critical resource to achieve and maintain global food security. Indonesia rich on agro-ecosystem, and thus the agro-biodiversity. These genetic reservoirs is useful for crops and food diversification, farming methods, and plant improvement. Despite of its priceless, there are various factors threaten PGRFA. Ex-situ conservation in genebank is crucial to save PGRFA from extinction and to ensure the availability for utilization. The ultimate goal of genebank management is on maintaining availability, genetic integrity, and quality of the collections. The objective of this study is to review management practiced in ICABIOGRAD genebank. The genebank management practices is reviewed based on FAO standard. In ICABIOGRAD genebank, PGRFA are conserved and managed through serial activities, starting from germplasm acquisition, management, characterization, evaluation, documentation, and distribution for utilization. Currently, around 10.848 accession of PGRFA are conserved, comprise of 30 species which mainly are food crop. The germplasm are collected through explorations and donations. Germplasm are conserved as seed, plants, and tissue culture, depending on the biological and physiological characters of the plants. Twenty crop species with orthodox seed are stored in low temperature and low humidity to maximize its longevity as active and base collection. Ten tuberous crops species are conserved in the field and part of these collections are conserved in-vitro. Germplasm distributions to users are conducted as part of gene bank function to promote germplasm utilization. Sharing germplasm collection to another gene banks were conducted for safety duplication.

Keywords: ICABIOGRAD Gene Bank, Indonesia PGRFA, ex-situ conservation.

INTRODUCTION

Indonesia possesses 17,000 islands, and is considered as the world's largest archipelago. Supported with its specific geographical position which located under the hemisphere and spread throughout two continents and oceans and with geological and climatic condition, Indonesia possesses diverse agricultural ecosystems (agro-ecosystem), including terrestrial dryland such as moor, yard/garden, and upland; wetland such as irrigated wetland, rainfed, tidal and pet swamp; and aquaculture such as freshwater culture (pond) and marine culture (sea bank) (Elizabeth *et al.*, 2014; Pusdatin, 2015). With this diversity of

agro-ecosystem, Indonesia is also possessing diversity in agricultural genetic resources. However, the genetic resource is threatened by global warming and climate change, dwindling land and water resources, and environmental degradation. Most of crop's varietal diversity has been lost through genetic erosion. FAO estimated that since 1900 approximately 75% of genetic diversity in agricultural crops has been lost (Mohamad & Zakri, 2001). The continuing loss of plant genetic diversity for food and agriculture greatly reduces our options for adapting the resources those environmental changes in order to ensuring food security.

Attached with its essential role as the basis of food security, the need to conserve and sustainably use the PGRFA is very critical. The best conventional approach for the conservation of PGRFA is an ex-situ conservation in Gene Bank, where samples stored off site away from the environments in which they naturally grow. In gene bank, genetic material stored and maintained in controlled condition. And from gene bank, people can use the material for breeding program and other research activities (Mohamad & Zakri, 2001).

The ultimate goal of genebank management is on the maintaining the existence, the genetic integrity, and the quality of the collections. Some underlying principles as basic of management practices are: identity of the accessions, maintenance of the physiological quality, genetic integrity, physical security collections, availability of information and use of germplasm, and proactive management (FAO, 2014).

Sustainable conservation of genetic resources depends on effective and efficient management of gene banks through the application of standards and procedures that ensure the continued survival and availability of plant genetic resources. Proactive management is critical for ensuring that germplasm is efficiently conserved and made timely available in adequate quantity and quality for further utilization. According to FAO (2014) standard, the management should emphasizes the importance of securing and sharing material as well as the related information, and sets in place a functional strategy for management of human and financial resources for a rational system. This includes a risk management strategy and encourages collaborations with third parties in providing services to gene banks in the efforts to conserve biodiversity. Included in the efforts are the development of complementary collections *in vitro* or in cryopreservation, adherence to the legal and regulatory frameworks, long-term and continuous commitment with regard to the availability of human and financial resources, and encouragement on application of practical experiences and knowledge and seeks to apply the Genebank Standards to the extent possible under locally prevailing conditions.

In Indonesian Agency for Agricultural Research and Development (IAARD), PGRFA is conserved in Implementing Units. Some perennial PGRFA are conserved on-farm under monitory of Assessment Institute for Agriculture Technology (AIAT). Ex-situ conservation in gene banks is conducted in ICABIOGRAD and other sub gene banks in commodities research centers, along with their mandate. In Research Institutes, the conservation more focus on the working collection in short – term conservation. Whereas, the ICABIOGRAD Gene Bank is facilitated with seed storage facilities, in-vitro conservation, and field conservation.

Objective

The objective of this study is to review management practiced in ICABIOGRAD genebank. Reviewing activities on management practices will result in how well the genebank management practices have done according to the International standard. Furthermore, this benchmarking technique will give us an idea on what and how to revise and to improve the management practices to be more efficiently in order to support the gene bank function for safe conservation and promoting utilization.

MATERIAL AND METHOD

The study was conducted by reviewing management practices implemented in ICABIOGRAD genebank. Gene bank management practices include series of activities starting from germplasm acquisition, conservation (which is including monitoring and regeneration), characterization and evaluation, documentation, and utilization. There is standard on operating each activity. However, since implementation of international standard demands some requirements, local gene banks is generally conducted some modification on the management practices to be well suited to the national condition, availability of supporting facilities, and other considerations. In this paper, implementation of standard in each activity was observed

RESULT AND DISCUSSION

Conservation of PGRFA in ICABIOGRAD Genebank

Currently, ICABIOGRAD gene bank conserve PGRFA of at least 10848 accession numbers, comprise of 30 species which mainly are food crop (Table 1). These crops are conserved in the form of seed, plants, and tissue culture. The PGRFA management includes series of activities starting from germplasm acquisition, conservation (which is including monitoring and regeneration), characterization and evaluation, documentation, and utilization.

Table 1. Seed collection at ICABIOGRAD Seed Bank

Crop species	Number of accession
Rice and the wild relatives (<i>Oryza</i> sp.)	3400
Maize (<i>Zea mays</i>)	1279
Wheat (<i>Triticum aestivum</i>)	80
Other cereals (<i>Sorghum bicolor</i> , etc)	276
Soybean (<i>Glycine max</i>)	888
Groundnut (<i>Arachis hypogea</i>)	804
Mungbean (<i>Vigna radiata</i>)	1058
Other legumes (<i>V. suterranea</i> ; <i>Cajanus cajan</i> ; <i>Lablab purpureus</i> ; <i>Mucuna pruriens</i> ; <i>Canavalia ensiformis</i> ; <i>V unguiculata</i>)	388
Others (Eeg-plant/ <i>Solanum tuberosum</i> ; wijen)	239

Germplasm Acquisition and Registration

In ICABIOGRAD genebank, the collections were obtained through collection mission, introduction, and donation, and exchange through research collaboration. During last five years, an intensive collection mission was conducted in IAARD through collaboration between ICABIOGRAD and Assessment Institute for Agriculture Technology (AIAT). From this collaboration, big number of germplasm accessions came to genebank. In genebank, those new materials are undergoing series of checking, observation, and confirmation steps before the registration.

Some sample received are not met standard requirements. They were not accompanied with an adequate passport data, or received in poor condition, or not meet the quantity requirement. Samples obtained from collection mission are generally had a better quality and adequate data passport. Whereas, some samples received from other institution are majority not feasible due to improper handling as well as inadequate passport data. Accession that met the requirements are then assigned as genebank collection and given accession number.

Standard for new coming clonal material for field bank is the need of land preparation, including selecting for suitable site appropriate are the soil type, agro-ecological, associated natural and manmade disasters, availability of water resources, secure long-term land tenure, and accessibility. In ICABIOGRAD, those preparation steps are not carried out in ICABIOGRAD. Plantation of new material is conducted in the same site with the existing collections.

Germplasm Management

Genebank collection is being managed properly to keep their availability, quantity and quality. For germplasm management collected in gene banks, FAO publish standard for seed banks, field gene banks and *in vitro*/cryopreservation gene banks. The seed bank standards deals with the conservation of the desiccation-tolerant orthodox seeds, whereas for field genebank and *in vitro* conservation/cryopreservation gene banks dealing with particular method of conservation at low temperature and humidity for recalcitrant and vegetatively propagated plants.

Seed Bank Management Practices

Standard for seed storage is interlinked with standard for seed drying. Seed sample for base collection should be dried to equilibrium and packed in suitable airtight container before store them at -18 ± 3 °C and relative humidity of 15 ± 3 percent. Whereas, active collection could be packed in non-airtight container and stored for medium term at $5-10 \pm$ °C and relative humidity of 15 ± 3 percent (FAO, 2014). The base collection should intend for collection with distinct of genetic integrity. The most original sample (MOS) must be stored as base collection.

In ICABIOGRAD Gene Bank, twenty of the generatively reproduce PGRFA conserved as seed that are conserved as active collection and as base collection. Active collections are provided for multiplication and distribution (utilization), thus this collection will be accessed more frequently. Two categories of storage facilities are available i.e. Long-term storage conditions at -20 °C for base

collections and short-term condition at 18°C with 40% relative humidity for active collections.

Seed that will be stored in the genebank should be of highest quality. In order to maximize seed longevity, prior to the storage, the seed are prepared through series of processing steps including seed cleaning, seed drying, seed testing, and seed packaging.

Seed drying among of performed to reduce seed moisture content (SMC), the most important factor determining the rate at which seeds longevity. Small changes in moisture content have large effects on storage life (Rao *et al.*, 2006). Several methods are available for drying seeds. Methods that are safe and not detrimental to seed viability (minimize loss) such as dehumidified drying and silica gel drying is recommended. The general drying method to reach equilibrium is in controlled condition 5 – 20 °C and 10 – 25% relative humidity. But this is depending upon species (FAO, 2014). In ICABIOGRAD, seed is dried in several steps. Fast drying under sun for several hours is performed once after harvesting. Some seed that are arrive at genebank as grain are processed with fast drying in drying cabinet and slow drying in dehumidified room. The best methods and the duration needed to meet the required SMC level is not yet found.

Seed testing is performed to observe the initial quality of seed. The testing includes seed viability and observation of seed health. Seed storage requires at least 80% seed viability and below this level, seed need to be regenerated to produce higher seed quality. However due to budget limitation, some seed are stored even when the quality not met the standard. Seed that showed insect and disease is treated with insecticide or fungicide. Before storing, qualified sample is packaged into labeled container. Active collections were packaged in sealed aluminum foil. However, this type of packaging is expensive and laborious. To manage the collection more efficiently, starting from 2016 the active collections are stored in plastic bottle, whereas base collection for long-term is still packaged in aluminum foil.

Seed viability declines with period of storage. Therefore, it is necessary to assess viability periodically in order to detect loss in viability during storage. Monitoring should be in place to check the viability status of stored samples at appropriate intervals depending on expected seed longevity, before viability has fallen below the threshold for regeneration. International standard put the threshold for PGRFA at 85%. The monitoring test intervals should be set at one-third of the time predicted for viability to fall to 85 percent of initial viability. Seed with viability less than 85% should be regenerated.

In ICABIOGRAD seed monitoring was conducted as part of seed preparation for regeneration. There were found seed with viability below threshold due to inadequately monitoring system. Regeneration is prioritized for the very urgent accessions that are needed to be saved. The instability of electric plant and the initial quality that not met requirement reduce seed longevity. Seed deterioration goes faster and thus the regeneration needed more frequent. The initial quality of seed, along with the facilities determines the seed longevity.



Fig. 1. Rice seed multiplication in green house

Seed regeneration is conducted in field in an optimum condition. To minimize plant and seed diseases, the plantation is conducted in the early of dry season or in the end of wet season. With this plantation schedule, the phase of seed maturation will fall in the dry season, such that insect and diseases can be prevented. Furthermore, seed harvesting in the dry season will minimize the loss.

Maintaining genetic integrity conducted by rogueing. The high possibility of seed contamination (mixing) occurs during seed production and seed processing after harvesting. For open pollinated crop such as maize, special treatment is performed to prevent contamination and maintaining seed purity. Majority of seed collection in ICABIOGRAD seed bank are of self-pollinated crops. Rogueing is conducted in vegetative and generative phase of seed plantation. During seed processing, seed are treated as new sample. Seed have to undergo series step of processing before storage. During seed cleaning seed is checked to ensure the genetic integrity by confirm it to seed reference or the initial seed stock.

Field Management of Tuberous Crops

FAO standard for establishment field collections include requirement of sufficient number of plants to capture the genetic diversity within the accession and to ensure the safety of the accession; a proper planned layout and field plan to enhance efficiency of space use and management of the collection; and an appropriate cultivation practices with consideration on micro-environment, planting time, rootstock, watering regime, pest, disease and weed control. During maintenance of the plantation, monitoring should be conducted for plant and soil and genetic identity of each accession, and daily care should be conducted as part of appropriate cultivation practices. Regeneration/propagation should be conducted when the vigor and/or plant numbers have declined to critical levels. Thus, information regarding plant regeneration cycles and procedures should be properly documented and included in the genebank information system.

In ICABIOGRAD Gene Bank, there are 2441 accession of tubers, comprise of 10 species (Table 2). These field conservation are established in Pacet (Cipanas), Citayam, and Cikeumeuh, Bogor (Fig. 2). Pacet (1,100 m asl) is assigned for sweetpotato originated from highland, whereas Citayam and Cikeumeuh (250 m asl) are designated for all tubers collections.



Fig 2. Core collection plot of sweet potato at Cikeumeuh, Bogor.

The conservation sites are facilitated with water resources, however, the water supply is limited during dry season and extra effort is required to keep the plant survive. Some other problems are related to controlling weed and preventing disease. Periodical soil monitoring was not carried out. Since initial soil status was not observed, diseases attack cannot be avoided and once disease attack occurred, it will spread throughout accession. Regeneration of the collections are scheduled and adjusted to the annual financial settlement. This sometime resulted in the improper planting season.

Table 2. Tuberous crops collected in ICABIOGRAD Genebank

Crop species	Number of accession	Field site
Cassava (<i>Manihot esculenta</i>)	556	Citayam
Sweetpotato (<i>Ipomoea batatas</i>)	1364	Pacet, Citayam, Cikeumeuh
Taro (<i>Colocasia esculenta</i>)	245	Cikeumeuh
Taro (<i>Xanthosoma</i> sp.)	126	Cikeumeuh
Bitter yam (<i>Dioscorea hispida</i>)	14	Cikeumeuh
Arrowroot (<i>Maranta arundinacea</i>)	34	Cikeumeuh
Edible Canna (<i>Canna edulis</i>)	63	Cikeumeuh
Lesser yam (<i>Dioscorea esculenta</i>)	17	Cikeumeuh
Water yam (<i>Dioscorea alata</i>)	20	Cikeumeuh
Elephantfoot-yam (<i>Amorphophallus</i>)	2	Cikeumeuh

In-vitro Conservation

In-vitro conservation is considered relatively safer approach to conserve clonal crop germplasm. Disregard the initial investment, this method can be more affordable and manageable. *In vitro* conservation is used for maintenance of plant organs or plantlets in a medium- term (some months up to some years) growth-limiting conditions. Cells-tissue are first grown on a gel and fed with suitable nutrients and hormones to give rise to entire plants, afterwards, being maintained under growth-limiting conditions on artificial culture media. The

cultures is also serve as sources of disease-free materials for germplasm transfers and distribution under regulated phytosanitary control, for multiplication and a source of explants for cryo-preservation.

FAO standard for in-vitro culture and slow growth storage include species-specific identification and determination of optimal storage conditions and regular monitoring system for checking the quality of the *in vitro* culture in slow-growth storage and possible contamination.

Prior to the in-vitro conservation, suitable media and conditions for growth of explants need to be developed which may involves surface disinfection procedures and germination medium. For some cases, basal medium may be determined based on culture of similar species and standard protocol can be used, but in many cases, explants media and growth conditions are critical. In this case, a custom-made protocols, media, and growth conditions are needed. Experimentation to achieve satisfactory slow growth is imperative. There are various modifications that usually made to culture media, including reduction levels of minerals, reduction of sucrose content, manipulation of the type and concentration of growth regulators, or inclusion of osmotically-active substances such as mannitol and paclo-butrazol.

In ICABIOGRAD, in-vitro conservation is applied to sweet potato, cassava, and taro. Procedures of series steps on disinfection and germination are carried out following published standard protocol and the Murashige and Skoog (MS) media is used as starting standar media with minor modification. Slow growth media is made by addition of osmotically-active substances such as mannitol and paclo-butrazol. Storage temperature set at 20 °C. In these media, culture can be maintained for 6 – 24 months.

Characterization and Evaluation

Characterization, an activity on the recording of characters or traits which are highly heritable or can be easily seen and are expressed in all environments, makes possible the easy and quick discrimination between accessions or phenotypes. Whereas, evaluation measure expression of traits that is usually influenced by the environment (Handbook of GB). Characterization and evaluation is an important prerequisite to effective use of the collection and allow detection of misidentifications during other genebank operations. A proper characterization will results in better insight in the composition of the collection and makes an important contribution towards rationalizing management procedures

FAO standard required that characterization should be based on standardized and calibrated measuring formats and follow internationally agreed descriptor lists. For orthodox seed, around 60 percent of accessions should be characterized during the first regeneration cycle. The trial should be carried out in at least three environmentally diverse locations and data collected over at least three year. For clonal crop, the data should be obtained for as many accessions as possible and molecular tools are important to confirm accession identity.

Characterization and evaluation in ICABIOGRAD is performed by referring to descriptor from IPGRI. Up to now, only small portion of the collection was characterized. he characterizations performed are morphological and agronomical characterization. The evaluation of major pest and diseases and some a-biotic stress were performed for several numbers of collections. The same condition is

for the nutritious value. Molecular characterization or DNA fingerprinting is started for limited collection of rice, soybean and sweet potato accession.

Documentation and Data Management

The inter-linked activities in Genebank management system produce various data. These accumulated data need to be documented and to be managed in a database system. Database system manages data of each accession of the collection and useful to help genebank management more efficiently and to support further research and development. Data management system is also an important aspect to support utilization of PGR effectively and efficiently and facilitate access and transfer information.

FAO standard required that passport data of the accessions be documented using FAO/Bioversity multi-crop passport descriptors MCPD and all data and information generated in the genebank relating to all aspects of conservation and use of the material should be recorded in a database.

The existing database for genetic resources in ICABIOGRAD is managed with application base on Microsoft Access The system is continually maintained and the database is routinely updated and validated to ensure the availability and accuracy of data & information and to facilitate faster and easier of the access. Some data /information were stored online.

Utilization and Distribution

In the scope of PGRFA, the concept of conservation is closely interlinked with utilization. The success of the conservation program is not merely revealed on the material have been maintained, but also on the utilization. The utilization of germplasm includes broad scope, including direct and indirect utilization. The primary utilization of genebank collection is for breeding purpose. However, the collection can also be used for re-introduction program and other research and studies. Utilization of the collection is depending on the availability of data and information of each accession.

According to FAO standard, distribution should be in compliance with national laws and relevant international treaties and conventions. For international transfer, samples should be accompanied by all relevant documents required by the donor and the recipient country. Time span between receipt of a request and the dispatch of the seeds should be kept to a minimum. Sample distribution should consider the availability of sufficient size of material in the stock. Associated information should accompany the material being distributed.

ICABIOGRAD genebank distribute collection based on request for research and development and education purpose. There is administrative mechanism on how to access genebank collections. Some information can be accessed online. In the genebank management system, there is condition whether the request is approved or rejected.

CONCLUSION

1. Some management practices in ICABIOGRAD gene bank are below standard in the coverage and the practices/activities.
2. ICABIOGRAD Gene Bank need more effort on maintenance of identity and genetic integrity by: 1). Completing confirmation step during acquisition, field plantation, and seed processing, 2). Providing the availability of

herbarium, specimen, and seed reference aside of the passport data, and 3). Additional molecular techniques to assess whether genomic stability has been maintained, especially for clonal accession.

3. ICABIOGRAD Gene Bank need more effort on maintenance of seed quality by better management during seed production, processing, and during the storing.
4. The need to maximize conservation by optimization the use of gene bank facilities, exploring new financial support, and safety duplication.

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