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Enhancing accessibility and conservation of plant tissue samples stored in silica gel, and developing a disaster plan for this collection at Royal Botanic Gardens, Kew

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Abstract

Many specimens in the Royal Botanic Gardens (RBG), Kew Plant Tissue Collection represent rare and endangered species from difficult to access regions of the world, including unique samples from a diversity of taxonomic groups. Whilst storing research materials in individual laboratories during use is accepted practice, it is unsuitable for safe long-term preservation. This paper describes the process for deposition of plant materials into the tissue collections, best practice in recuration to ensure long-term preservation and storage, and disaster planning.

Keywords: long-term storage, archival quality, biodiversity, best practice, CBD

Introduction

Botanists collect plant material e.g. for identification, genomic studies or bioprospecting of secondary metabolites. Depending on the purpose, different parts of the plant are sampled, e.g. flowers, leaves, roots, seeds, etc. For each of these parts and purposes, different protocols for field collecting exist, and are reviewed by Gemeinholzer et al. (2010). The advantages of storing plant material in silica gel for successful subsequent DNA-extractions are emphasized by Chase and Hills (1991), and this procedure has become standard in the field of Botany. Here we describe a standardized workflow for processing botanical material coming fresh from the field, to build up a plant tissue collection which can be used for both genomic and other biochemical downstream applications.

Total DNA extractions from plant tissue are still collections in the traditional sense, but require specific technologies that differ from herbarium or museum collections in many important ways. The development of best practice for what is often termed 'molecular' collections involves standardised methods for collection, long-term archival storage, retrieval and distribution. The preservation and long-term storage of material derived from biological specimens (e.g., DNA extracts) and associated data are essential to

ensure comparability and reproducibility in all areas of biological research (Corthals and DeSalle, 2005). In a centrally-managed repository, it is easier and more efficient to implement standardised procedures for modern conservation and long-term storage of silica gel dried plant tissues, and to manage access and security in a consistent and user-friendly manner, as well as adhering to the Convention of Biological Diversity (CBD). Adapting to best practice in the physical care, storage, and handling of archived material maximises life expectancy and the availability of high quality biological material for future research (ISBER, 2012). The amount of work, space, and weight needed in the field (and the herbarium), to collect and preserve suitable material for DNA studies is relatively small, given the potential benefits (Gaudeul and Rouhan, 2013).

Deposition of plant material in the tissue collections

At Royal Botanic Gardens (RBG), Kew, researchers are provided with collecting equipment and a standardised tissue sampling protocol (apps.kew.org/dnabank/SampleProtocol.pdf). As the samples collected in the field have only been preserved in a provisional way, additional handling and data management is necessary before they are deposited permanently in the research collection. In the case of plant tissue samples, permanent and safe physical space must be available and accessible. By permanent, it is understood that it will be available for storage of the collection for decades, if not centuries; by accessible, the samples can be inspected and used readily and without unnecessary delay and paperwork.

Once incorporated into a collection, voucher specimens may be examined by many researchers over time, so provision must be made for identification to be fed back to the collection and collection management system. Field identifications are not always accurate, and names can change as the understanding of particular groups develop. If the country of origin placed restrictions on the use of voucher material in the collection (or export) permit, such as stipulating that material may not be used for third-party DNA extraction, or not be sent on loan to another institution, then these restrictions need to be noted on the label of the respective specimen itself as well as in the management system of the collection housing the specimen (Savolainen et al., 2006, Gemeinholzer et al., 2010).

For final storage purposes, plant tissue dried in silica gel should be stored in archival quality resealable transparent bags bags (e.g. polyethylene zipper bags provided by Preservation Equipment Ltd.) with trace amounts of indicator silica gel (2–16 mesh, grade 42; RBG, Kew use 2-5 mm (2-5 mesh) orange-to-colourless indicating silica gel beads) in order to monitor the risk of rehydration, which can occur, for example, due to ageing containers. Ziplock bags have the advantage of being flexible, without risk of breaking (in contrast to, e.g., glass vials), inexpensive, and durable. In order to minimize exposure to air and humidity they should, in turn, be kept in tightly sealed plastic boxes (Prendini et al., 2002).

Re-curation

The re-curation process should start with plant tissue samples considered a priority e.g. current projects and type specimens. Samples are first transferred to un-buffered (archival quality) glassine envelopes with pointed forceps (or long flat forceps for large specimens in deep bags). The glassine envelope is placed in a zipped polyethylene bag. A small amount of indicating orange-to-colourless silica gel (2-16 mesh), and a label printed on acid free paper with all relevant data of the sample – as a minimum sample number, name, collector name and number (permitting auditing; see Figure 1) – should be added to the zipped polyethylene bag. The original collection bag (which might be, for example, a tea or coffee filter filled with the plant tissue sample) with the original information should be included in the outside bag as a final audit check point, but sealed separately from the sample in order to account for the risk of researchers having used non-archival materials (e.g. bags or pens) during their field work.

The recurated samples are now stored in hermetically closed containers at room temperature, ideally in a humidity-controlled environment for long-term archival storage. At RBG, Kew, we use transparent plastic containers in order to facilitate rapid checks of the humidity indicators in the silica gel without the need to open the lid of the containers. If the humidity can be maintained at a constant level rather than kept extremely low, this is adequate, but requires more frequent inspection of the collection and replacement of the indicator gel at more regular intervals (Figure 2).

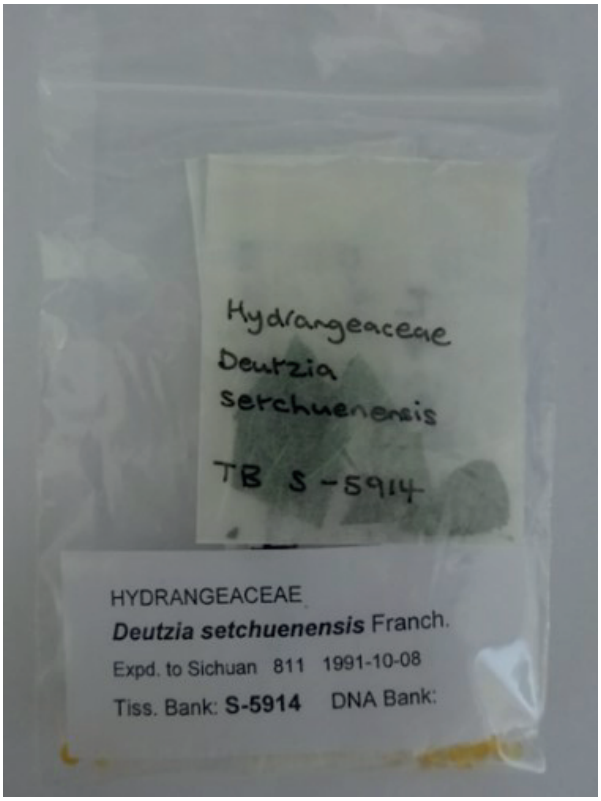

Figure 1 Recurated Sample. Image: Duque-Thüs, R., 2016.

Figure 2 Final Storage. Image: Duque-Thüs, R., 2016.

Archival quality material and storage

In the particular example of plant tissue samples stored in silica gel, there is a preference for 'preventative conservation'. This includes any action taken to prevent known damage occurring, for example by storing material in a suitable and secure environment or packing it in an appropriate way using archival quality material. 'Archival' or 'conservation' quality refers to materials that are physically durable and chemically stable. Several types of plastics and fabrics fall into this category. Such items are said to be 'inert', and do not release degradation products that can be harmful to collections. Using this type of material ensures the safety and stability of collections for long-term storage (Pasiuk, 2004).

Silica gel: health and safety

Fine crystalline silica gel allows greater surface coverage of leaf tissue, but only indurated products should be used to minimize the risk of silicosis. Crystalline silica powder or silica dust is colourless, has a higher hygroscopic capacity than silica gel, but is rarely used as it has the disadvantage of being irritating to the respiratory tract, may cause irritation of the digestive tract, and dust from the beads may irritate the skin and the eyes. Therefore, precautions for handling should be taken (Fischer Scientific, 2009). Crystalline silica dust can cause silicosis and must be used with face masks or under a laboratory hood or laminar flow cabinet (Gemeinholzer et al., 2010).

Required resources for the recuration of the tissue bank

- Pointed forceps for removing small pieces of specimen tissue from the original collection envelopes.
- Flat, long forceps for extracting large pieces of tissue from collection envelopes.
- Paintbrushes for cleaning fine silica dust from the plant tissue.
- Archival quality acid-free paper (ISO 9706) for labels.
- Unbuffered glassine envelopes (sizes S, M, and L) for storage of plant tissue.
- Zipped polyethylene bags (sizes S, M and L) for storage of glassine envelopes containing the plant tissue.
- Indicating silica gel (2-16 mesh) for monitoring of moisture levels in tissue samples.
- Transparent rectangular containers for the storage of Ziploc bags with glassine-enveloped plant tissue. For example, those containers used for professional food storage purposes from ADDIS of 4.6 litre, which are guaranteed acid-free.

Disaster plan

All disaster plans depend fundamentally on an underpinning of everyday collections management best practice and risk assessment. The first step is to identify the possible risks for the collection (Table 1).

Rescue priorities for the plant tissue collections in a disaster situation

- Type specimens.
- Unique samples e.g. extinct species or largely inaccessible (endangered species, permit problems, collecting from politically unstable countries, etc.).

Development of an emergency response team

In the event of a disaster, roles and responsibilities must be clear for everybody involved in the response. For this reason, a disaster response team must be established. It will consist of trained members of staff. Their email addresses and mobile phone numbers must be kept accessible. The team members and their roles will have to be discussed in more detail when the final storage location of the tissue sample collection is decided.

How to react after a disaster has happened?

Evacuation following a disaster must be as fast and controlled as possible. This will require appropriate modifications to existing infrastructure and equipment in the early stage of preparing the disaster plan, depending on the final location of the tissue bank.

Localisation of the samples evacuated is important. Maps with the current and suggested evacuation locations of the material have to be prepared, accessible, and shared with fire brigade officers, local fire marshals, and health and safety teams. It is essential to have the evacuated samples correctly stored in their recorded location to avoid the extra disaster that the loss of the samples would mean.

Recovery

Once a disaster has occurred, such as a broken pipe or a fire, a triage system is needed to decide which specimens are recoverable and which should be disposed of. This will be based on factors such as specimens' value and the amount of damage suffered by each specimen, for example, with the final decision on disposal to be approved by the trustees of the institution.

Table 1. Possible risks to plant tissue collections, and methods of mitigation.

Risk	Possible Consequences	Mitigation
Defective storage systems	Broken specimens. Broken containers.	Archival quality storage containers. Regular checks on specimen and container integrity.
Fire	Destruction of specimens by burning. Contamination with smoke and dust. Deterioration by water. Unknown chemical interactions between extinguishing agents and specimens or preservatives.	Following fire safety procedures. Storage of tissues in airtight and waterproof containers.
Flood	Deterioration by water.	Storage of tissues in airtight and waterproof containers. Establishment of an evacuation plan.
High air humidity	Mould growth. DNA degradation.	Monitoring of air and sample humidity. Replacing exhausted silica. Management of environmental conditions in storage area.
Heat	DNA degradation	Monitoring of temperature. Management of environmental conditions in storage area.
Pests	Destruction of specimens.	Establishment and implementation of an IPM protocol. Carrying out regular checks on specimens and containers.

Registration and evaluation of the damage

To evaluate the possible damage, it is necessary to follow an existing and established protocol (ISBER, 2012). In the case of the tissue bank there should be procedures such as:

1. Random selection and testing of a minimum of 10 samples by DNA extraction from a randomised list of samples.
2. Quality and quantity control of extracted DNA.

In May 2016, a flood occurred in the Jodrell laboratories, resulting in no losses or damage to the plant tissue collections due to best practice in storing the collection and adherence to the disaster plan previously developed (Kapinos, 2016).

Discussion

The advantages of the long-term storage of plant tissue collections are: 1) Improved access for researchers; 2) Long-time stable storage; 3) Health and safety risks of silicosis are minimised; 4) Compliance with Museum Accreditations Standards; 5) Taxonomically referenced collection available for long-term research; 6) Long-term availability of biodiversity data from the collection; 7) Possible future uses of dried whole plant tissue which can reference previous studies.

While facilitating access to samples is a key aim of the Plant Tissue Collection, RBG, Kew is committed to honouring the letter and spirit of the CBD and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and our agreements with partner countries; accordingly, some material is restricted. Kew has developed guidelines to ensure best practice for the acquisition and supply of genetic resources, based on the Principles on Access to Genetic Resources and Benefit Sharing, a document developed by 28 botanic gardens worldwide and endorsed by RBG, Kew in March 2001 (see Royal Botanic Gardens, Kew, 2004), and the Bonn Guidelines on Access to Genetic Resources and Benefit Sharing, developed under the CBD (Secretariat of the Convention on Biological Diversity, 2002). In line with the aims of the CBD, RBG, Kew's Material Supply Agreement (Royal Botanic Gardens, Kew, 2015) restricts the use of material to non-commercial use, including scientific research, education, and conservation. This encourages the recipient to share benefits fairly and equitably, and only allows for the transfer of material to a bona fide institution for non-commercial use. Likewise, all material obtained by fieldwork by RBG, Kew staff in overseas countries is bound by prior informed consent and legally mutually agreed terms.

It is important to point out that current Next Generation Sequencing techniques (High Throughput Sequencing (HTS)) can require larger quantities of material for DNA extractions; this could cause problems for botanic gardens and herbaria in the supply of plant material. For this reason, the Global Genome Biodiversity Network (GGBN, 2011; Seberg et al., 2016)) has started a public consultation on making the metadata from HTS libraries available through the data portal to help prevent HTS libraries becoming single use and to promote better use of these libraries (see GGBN, 2017).

Conclusion

Developing the long-term storage and disaster plan of the Plant Tissue Collection at RBG, Kew is an ongoing process. Additional samples stored throughout RBG, Kew are progressively being accessioned into the Tissue Collection, where they can be made available to the wider scientific community in a secure and consistent manner.

Unless there is a specific exemption, tissue collected for DNA extraction falls under CITES controls and transfer of plant tissue and DNA samples between countries are subject to CITES regulations.

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References

Chase, M.W., and Hills, H.H., 1991. Silica Gel: An Ideal Material for Field Preservation of Leaf Samples for DNA Studies.

Taxon, 40(2), pp.215-220.

Corthals, A., and DeSalle, R., 2005. An Application of Tissue and DNA Banking for Genomics and Conservation: The Ambrose Monell Cryo-Collection (AMCC). *Syst. Biol.*, 54(5), pp.823–831.

Fisher Scientific, 2009. Material Safety Data Sheet Silica Gel (Davisol*). [online] Available at:

<https://www.nwmissouri.edu/naturalsciences/sds/s/Silica%20Gel%20mesh.pdf>.

Gaudeul M. and Rouhan G., 2013. A plea for modern botanical collections to include DNA-friendly material. Trends in Plant Science, 18, pp.184–185.

Gemeinholzer, B., Rey, I., Weising, K., Grundmann, M., Muellner, A.N., Zetzsche, H., Droege, G., Seberg, O., Petersen, G., Rawson, D.M., and Weigt, L.A., 2010. Organizing specimen and tissue preservation in the field for subsequent molecular analyses. In: Eymann, J., Degreef, J., Häuser, C., Monje, J.C., Samyn, Y., and VandenSpiegel, D. (eds), 2010. ABC-Taxa, Volume 8 - Manual on Field Recording Techniques and Protocols for All Taxa Biodiversity Inventories. Ch.2, pp.129-157.

GGBN (Eds.), 2011. The GGBN Data Portal. [online] Available at: <http://data.ggbn.org>.

GGBN, 2017. HTS public consultation. [online] Available at: <http://wiki.ggbn.org/ggbn/HTS_Public_Consultation>.

ISBER, 2012. Best practices for repositories: Collection, storage, retrieval and distribution of biological materials for research (third edition). Biopreservation and Biobanking, 10(2), pp.81-161.

Kapinos, E., 2016. Personal communication.

Pasiuk, J., 2004. Safe Plastics and Fabrics for Exhibit and Storage" Conserve O Gram, 18/2, Washington, DC: National Park Service. [online] Available at: <https://www.nps.gov/museum/publications/conserveogram/18-02.pdf>.

Prendini, L., Hanner, R., and DeSalle, R., 2002. Obtaining, storing and archiving specimens and tissue samples for use in molecular studies. In: DeSalle, R., Giribet, G. and Wheeler, W.C. (Eds.), 2002. Methods and Tools in Biosciences and Medicine. Techniques in Molecular Evolution and Systematics. Basel: Birkhäuser Verlag AG. pp. 176–248.

Royal Botanic Gardens, Kew, 2004. Policy on access to resources and benefit-sharing. [online] Available at: <https://www.kew.org/sites/default/files/ABSPolicy.pdf>.

Royal Botanic Gardens, Kew, 2015. Non-commercial material supply agreement for DNA. [online] Available at: <http://apps.kew.org/dnabank/MTA.pdf>.

Savolainen, V., Powell, M.P., Davis, K., Reeves, G., and Corthals, A. (Eds.), 2006.

DNA and Tissue Banking for Biodiversity and Conservation: Theory, Practice and Uses. Kew Publishing, Royal Botanic Gardens Kew & IUCN: 168 pp.

Seberg, O., Droege, G., Barker, K., Coddington, J.A., Funk, A., Gostel, M., Petersen, G., and Smith, P.P., 2016. Global Genome Biodiversity Network: Saving a blueprint of the Tree of Life – A botanical perspective. Annals of Botany, 118 (3), pp.393-399.

Secretariat of the Convention on Biological Diversity, 2002. Bonn guidelines on access to genetic resources and fair and equitable sharing of the benefits arising out of their utilization. [online] Available at: <https://www.cbd.int/doc/publications/cbd-bonn-gdls-en.pdf>.