The collection and storage of plant material for DNA extraction: The Teabag Method

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ABSTRACT. Silica gel has become the most common instrument for preserving leaf material in the field for future DNA extraction. This has generally involved leaf material being placed in silica gel in zip-lock type bags. Although effective it often requires a large amount of silica gel and large number of plastic bags to be taken into the field, something which is problematic during long field trips to remote areas. It also has the disadvantage that if the silica gel becomes hydrated or the plant material damp, replacement of the silica gel is difficult and can result in contamination. An alternative method using empty teabags avoids the need to carry large amounts of silica gel and plastic bags into the field and reduces the difficulty of replacing hydrated silica gel during fieldwork and longer term institutional storage.

Keywords. Collection, DNA, plant, silica gel, storage, teabag

Introduction

The success of global initiatives such as the Angiosperm Phylogeny Group (Angiosperm Phylogeny Group 1998; Angiosperm Phylogeny Group II 2003; Angiosperm Phylogeny Group III 2009) and the Barcode of life (Hollingsworth et al. 2011) have relied very much on high levels of species sampling and the availability of suitable material for DNA extraction. Taxa from temperate zones, botanic gardens or that are cultivated are often well sampled but species found in the tropics, in particular in more remote areas, are poorly represented (Harris 1993). To address this problem, extraction of material from herbarium material suitable for DNA analysis has been investigated with varying degrees of success depending on age, preservation methods and storage conditions (e.g. Lister et al. 2008; Liston et al. 1990; Andreasen et al. 2009; Rogers & Bendich 1985; Sarkinen et al. in press). This practice, however, has raised concern from collection curators about the destructive nature of this sampling from often irreplaceable herbarium material. Extraction has also been problematic from herbarium specimens pressed in spirit or poisoned – a common practice in South

East Asia. The collection of plant material dried in silica gel, in addition to herbarium material, is now a common practice and helps avoid the difficulties of DNA extraction from herbarium material.

Chase & Hills (1991) suggested the collection of about 4–6 grams of fresh leaf material torn into pieces not exceeding 2 cm^2 and then placed into a zip-lock type sealable plastic bag with silica gel at a ratio of not less than 10 to 1. This is considered a highly effective method of drying material and minimising DNA degradation.

The storage of material in zip-lock bags appears effective but requires a large amount of silica gel and plastic bags to be taken into the field, something which can be problematic during long field trips to remote areas. It also has the disadvantage that if the silica gel becomes hydrated (a small hole in the bag will cause hydration in tropical environments) the replacement of the silica gel is difficult and its reuse can result in contamination. This paper summarizes a method which is becoming more widely used but currently unpublished. It should be useful to researchers wishing to refine their field practices.

Methods

An alternative method of drying and storing plant material for DNA extraction uses empty teabags such as those that are widely available in supermarkets and specialist tea shops. Instead of placing the material in silica gel in a sealable plastic bag, as suggested by Chase & Hills (1991), the material is placed inside the teabag with a collector label placed against the wall of the bag so that it is easily legible (Fig. 1A) and sealed (usually by folding the top over). The teabag is then placed in an airtight container and completely submerged in silica gel until completely dry (Fig. 1B). The container is shaken frequently over the first day to make sure dry silica gel is always in close contact with the plant material. Once the material is completely dry, the teabag can then be removed from the silica gel and placed in an airtight container which has a fresh layer of silica gel at its base (Fig. 1C) for longer term storage. This silica gel layer can be easily replaced if it becomes hydrated.

Fig. 1. A. Ripped leaf material placed in teabag with internal paper label. **B.** Teabag submerged in silica gel (regularly shaken). **C.** Longer term storage of teabags in sealable container with layer of silica gel at base. (Photos: P. Wilkie)

Results

In the field, plant material placed in teabags has been found to dry within the same time scale as material placed directly into silica gel in zip-lock bags. The amount of silica gel and zip-lock bags needed to be taken into the field has been reduced significantly and it has been much easier to replace hydrated silica gel in the field.

Material collected and stored in this manner over the past seven years at the Royal Botanic Garden Edinburgh appears equally effective as material stored using zip-lock bags at providing non-degraded DNA. Although not statistically rigorous, parallel QIAextractor (Qiagen) extraction of material from the same plant accession collected three years previously using both traditional and teabag collection and storage methods provided equivalent quantities and qualities of DNA, as assessed by gel electrophoresis and spectrophotometry. Polymerase chain reaction amplification of plastid loci *trn*L and *rbc*L was also confirmed. No contamination of material between collections has been recorded, indeed it is probable that this method reduces this risk, especially when having to replace silica gel, as the plant material remains contained within the teabag at all times.

The teabag method has simplified the management of collections during longer term institutional storage and has proven particularly useful in tropical environments which have to deal with very high ambient humidity and thus more regular changing of silica gel.

Discussion

This short paper suggests a modified method of collecting leaf material for subsequent DNA extraction. Much of the information gathered has been from our own experience, with a range of researchers using this method in the field in different continents over the past few years. Some variations to this method can be found on the web, such as the use of envelopes and coffee filters (http://evol.mcmaster.ca/~brian/evoldir/Answers/Leaf.Preservation.answers). The most obvious benefit to the field botanist is the reduced amount of silica gel and plastic bags needing to be transported to and from the field. In tropical climates the ability to easily change the silica gel has also been an important improvement, as it has been found that zip-lock bags are not 100% airtight (particularly when silica grains get into the mechanism), which can lead to rehydration of plant material. No obvious difference in efficacy of the material for DNA extraction has been noticed compared to the zip-lock bag method although we acknowledge that our trials did not provide statistical support for this observation. Indeed we hope that this paper may generate further investigations and publications on this topic.

Of equal importance to the long term preservation of plant material suitable for DNA extraction is the environmental conditions in which the desiccated material (be it in a teabag or zip-lock bag) is stored over the long term. As noted by Hollingsworth et al. (2011) there is a need for guidelines on the optimum storage conditions for

desiccated material, though it appears that the dryer, colder and more stable the environment, the less likely the DNA is to degrade.

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