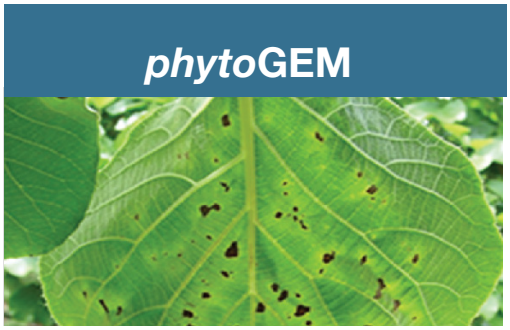
Exymes



A kiwifruit leaf infected with Pseudomonas syrigae pv. actinidiae.

Rapid automated extraction of high quality plant DNA using phytoGEM and the PDQeX2400

Dr Abishek Muralidhar

Introduction

Extracting DNA from plants can be challenging because of their tough cell walls and the inhibitory polysaccharides and tannins released during the extraction process. Currently available methods address these difficulties by using tissue grinding followed by silica beads, columns or organic solvents. These procedures are multistep making them labour intensive and time-consuming.

The phytoGEM extraction system from ZyGEM is a simple closed-tube method for extracting PCR-ready plant DNA. It uses a collection tool that crushes the material onto storages cards. Punches from the card are lysed using an aggressive thermophilic proteinase combined with a cocktail of cell wall degrading glycosyl hydrolases. The extraction is performed automatically in the PDQeX2400 device which both lyses the cells and purifies the DNA through a proprietary polymer matrix.

To demonstrate the extraction efficiency from the phytoGEM plant DNA extraction system, six plants were selected: Arabidopsis thaliana, Oryza sativa (Rice), Zea mays (Maize), Triticum aestivum (Wheat), Solanum lycopersicum (Tomato) and Citrus limon (Lemon). Both qPCRs and end-point PCRs were used to evaluate the integrity and quality of the extracted DNA.

Methods

Sample preparation

Healthy leaves from the six plants were crushed onto the storage cards using the *phyto*GEM sampling tool (Fig. 1).

DNA extraction

4 mm circular punches were removed from the storage cards using a Harris punch. Two punches were placed into the PDQeX extraction tubes. The following reaction mixture was prepared and dispensed into each tube:

- 2 µl *phyto*GEM;
- 2 µl *prep*GEM;
- 20 µl 5X Buffer;
- 76 µl H_2O

Tubes were sealed by inserting the tapered cap and gently flicked to remove air bubbles and to ensure that the punch discs were completely immersed in the liquid. These were then processed using the PDQeX "Plant" extraction program. The program took a total of 15 minutes.

Quantification and assessment of the extracted DNA

2 µl of extract was used for qPCR amplification in a 10 µl reaction containing the following: 5 µl Perfecta™ SYBR®Green Fastmix™ (Quanta Biosciences), 0.5 µl each of forward and reverse primers and 2 µl H_2O.

Endpoint PCRs were performed to amplify products of different molecular weights from both nuclear and chloroplast genes using 5 µl PCR mix (Quanta Biosciences), 0.5 µl each of forward and reverse primers and 2 µl H_2O. Amplicons were visualized using agarose gel electrophoresis.

Results

All *phyto*GEM extractions gave good quality DNA that produced clean qPCR plots (Figure 2). Quantitative Comparison of *phyto*GEM and QIAGEN spin columns showed that *phyto*GEM gave higher DNA yield per mm^2 for most leaf types. (Table 1). The QIAGEN kit, however out performed phytoGEM with lemon leaf samples with respect to yield.

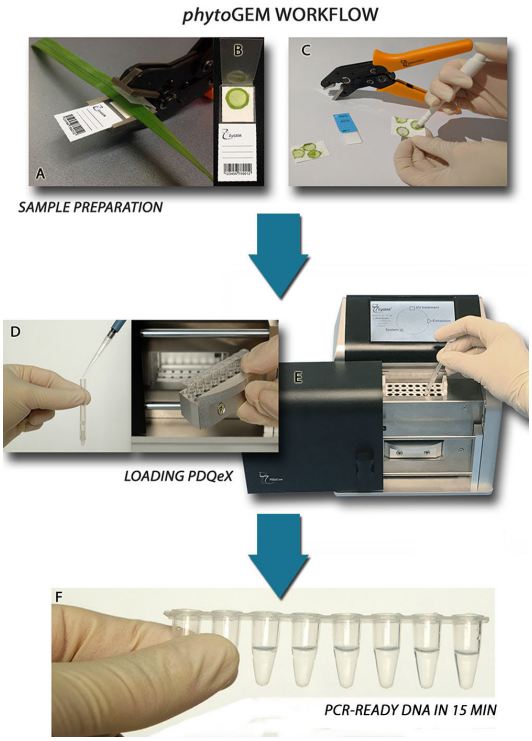


Figure 1: The steps for plant DNA extraction using the ***phyto***GEM system. Leaf samples are crushed using the ***phyto***GEM crusher tool (A) onto storage cards (B). Circular punches are taken (C) and transferred into PDQeX tubes (D). Tubes with punches and reagents are inserted into the PDQeX (E) and run through an extraction program. High quality, clear plant DNA (F) is ready for downstream applications.

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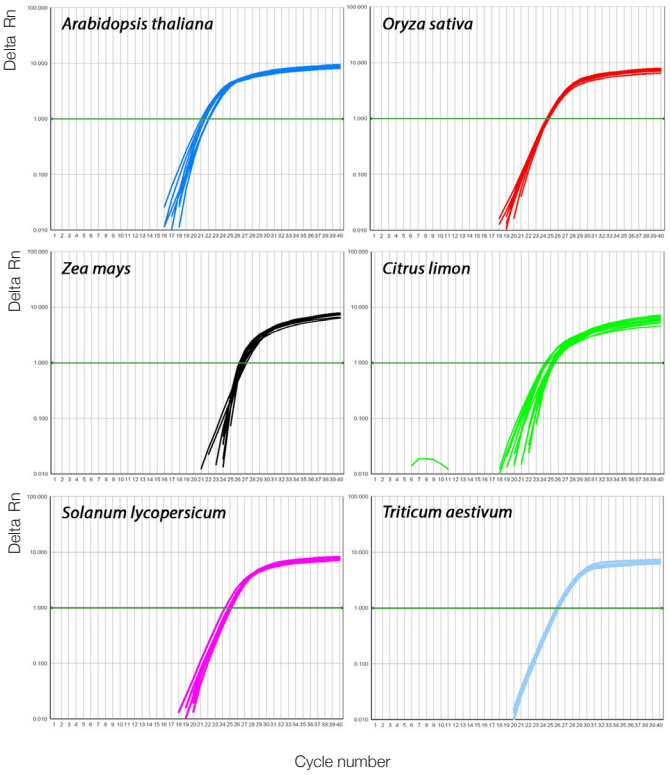

Figure 2. qPCR plots of alcohol dehydrogenase (*adh*) gene fragments amplified from *phyto*GEM extractions from 6 plants.

Figure 3 shows the average DNA yield for different numbers of punches used in the PDQeX extractions. Yield is proportional to the number of punches. The data demonstrate the flexibility of the method in that it allows the user to decide whether to maximise yield or conserve the sample.

Species	Method	Area (mm^2)	Total (ng)	Conc (ng / µl)	Recovery (ng/mm^2)
Arabidopsis	QIAGEN	1024	1515	15.15	1.47
(*A. thaliana*)	*phyto*GEM	50.24	94	0.94	4.34
Rice	QIAGEN	958	1791	17.91	1.87
(*O. sativa*)	*phyto*GEM	50.24	199	1.99	5.95
Maize	QIAGEN	633.94	806	8.06	1.27
(*Z. mays*)	*phyto*GEM	50.24	120	1.2	2.38
Tomato	QIAGEN	436.32	1398	13.98	3.20
(*S. lycopersicum*)	*phyto*GEM	50.24	360	3.6	7.17
Wheat	QIAGEN	1000	2816	28.16	2.8
(*T. aestivum*)	*phyto*GEM	50.24	110	1.1	2.18
Lemon	QIAGEN	679	5019	50.19	7.39
(*C. limon*)	*phyto*GEM	50.24	123	1.23	2.45

Table 1. Comparison of DNA yields between *phyto*GEM and the Plant Mini Kit from QIAGEN. Extractions were made from 5 different plants. The *phyto*GEM extractions recovered substantially more DNA per mm^2 from *A. thaliana, O. sativa, S. lycopersicum* and *Z. mays*.

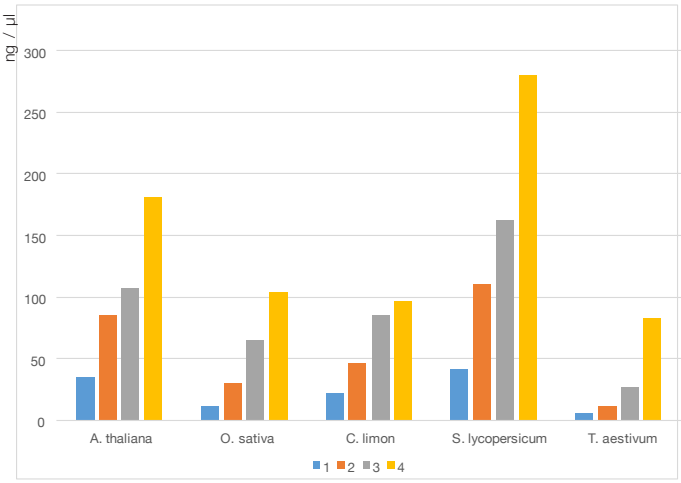

Figure 3. Concentration of DNA in extracts produced using the PDQeX from different plants using 1 – 4 punches. Data are compiled from 5 extractions from each plant species.

*Phyto*GEM-extracted DNA performs equally well with end-point PCR of larger DNA fragments. Amplicons from nuclear (*adh, act2*) and chloroplast genes (*rbcL, matK, psbA*) of sizes ranging from 200-2300 bp were successfully generated from the six species (Figure 4).

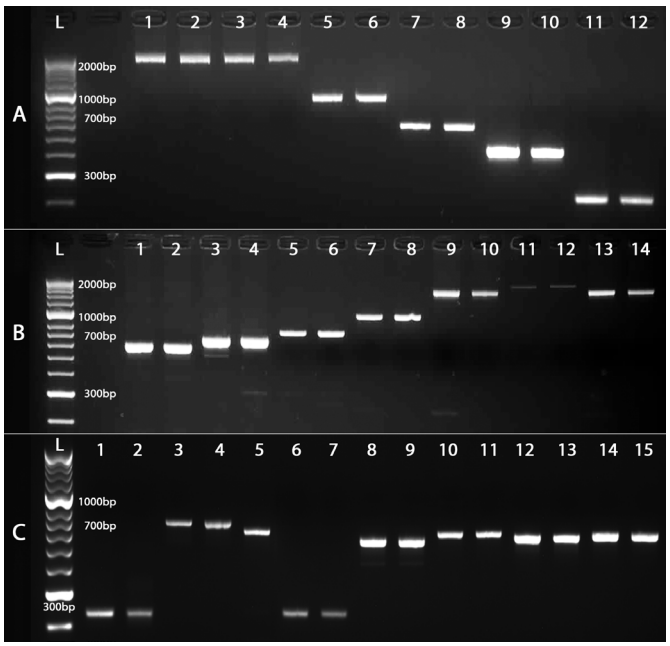

Figure 4. Agarose gel images showing amplicons generated from plant extractions. Fragments were generated using primer combinations targeting sequences in 5 genes: *adh* (alcohol dehydrogenase), *act2* (actin 2), *mat*k (maturase K), *rbc*L (RuBisCO large subunit) and *psb*A (photosystem II) ranging from ~200–2300 bp.

(A) Arabidopis: 1-4: act2, 5-6: matK, 7-8: rbcL, 9-10 psbA, 11-12: adh
(B) Rice: 1-2: rbcL, 3-4: psb, 5-14: various fragments of adh.
(C) 1-2: lemon adh, 3-4: lemon rbcL, 5: lemon psb, 6-7: maize adh, 8-9: maize psb, 10-11: maize rbcL, 12-13: tomato psb, 14-15: tomato rbcL.

Discussion

This study demonstrates the automated *phyto*GEM extraction system and its ability to rapidly produce PCR-ready DNA from both monocots and dicots. The method is fast (~15 minutes) and hands-off making it ideal for high-throughput genetic screening of plants.

References

[1] Doyle & Doyle, 1990; Biotechniques, 12: 13–15.
[2] Lickfeldt et. al, 2002; Hort.Science, 37: 822–825
[3] Tamari et. al, 2013; J Biomol Tech., 24: 113–118

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