QuickExtract – Rapid and efficient extraction of PCR-ready genomic DNA from plant and seed samples

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Introduction

The screening of large populations and the associated challenges in automation, chemistry and cost are major scientific and business drivers in plant breeding. The QuickExtract™ Plant DNA Extraction Solution can be used to rapidly and efficiently extract PCR-ready genomic DNA from most plant leaf samples using a simple, one-tube protocol that takes only 8 minutes.

This study shows the successful use of QuickExtract. The influence of grinding on the quality of genotyping results was investigated for multiple plant species. Leaf and seed material were used for testing.

PCR based genotyping methods such as KASP® or BHQplus® Probes were tested. In addition, it is shown that the QuickExtract lysate can be stored and re-used even days later.

Materials and methods

A. Determination of the impact of grinding

Whole seeds from wheat, tomato, hulled sunflower and pepper were either ground or incubated whole with QuickExtract. 100 µL of QuickExtract was added to tomato and pepper seeds, 200 µL was added to wheat seeds and 300 µL was added to sunflower seeds. Samples were processed according to the QuickExtract workflow in Fig 1, and diluted 4 and 16 fold prior to PCR amplification with KASP on the IntelliQube®.

B. Evaluate BHQplus and KASP chemistries for relevant crop types

QuickExtract Solution was added to 24 samples of seed and leaf material of multiple crop types according to Fig 1 and Table 1. Samples were incubated at 65 °C for 8 mins, diluted 2-8 fold (Fig 2) and PCR amplified on the IntelliQube with KASP and BHQ chemistries (see Fig 3).

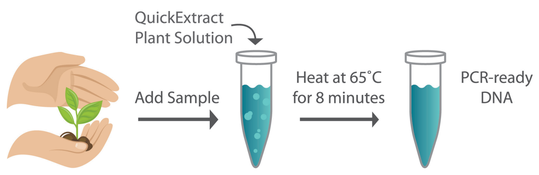

Figure 1: Overview about the QuickExtract workflow.
After cell lysis, inhibitory cell components are degraded, whereas the released DNA can be inserted directly into the PCR after further heat incubation.

Crop	Tissue Type	Processing	Grinding required	Mass (mg)	QuickExtract Buffer added (µl)
Corn	Seed	Chipped	Optional	~20 mg	200
				~100 mg	400
	Leaf	Punches	Optional	1-2 punches	100
				5 punches	200
				20 punches	400
Wheat	Seed	Whole	Required	~35 mg	200
	Leaf	Cut	Required	~5-10 mg	100
				~20 mg	200
				~45 mg	400
Rapeseed	Seed	Whole	Optional (Grinding slighly better)	~5 mg	100
	Leaf	Punches	Grinding not tested	~5-10 mg	100
Soy	Seed	Half	Grinding not tested	~80 mg	200
Tomato	Seed	Whole	Optional	~3 mg	100
	Leaf	Punches	Optional	5 punches	200
				15 punches	400
Pepper	Seed	Whole	No Grinding	~8 mg	100
	Leaf	Punches	No Grinding	5 punches	200
				15 punches	400
Cotton	Seed	Whole	Required	~100 mg	200
		Hulled	Required	~50 mg	200
	Leaf	Punches	Not tested	2 punches	200
Sunflower	Seed	Whole	Required	~100 mg	200
		Hulled	Optional	~50 mg	200-300
	Leaf	Punches	Not tested	2 punches	200

Table 1: *Sample and pre-treatment of validated crops, seeds or leaves, grinding requirement conditions, starting weight, and volume of QuickExtract required.*

C. Stability of extracted DNA for at least 4 weeks at 4 ˚C

Corn and tomato leaf samples were extracted with QuickExtract. The DNA was PCR amplified using 2 different KASP assays for each crop type. The sample plate was stored at 4 °C and the experiment was repeated 1 month later (see Fig 4).

Results

A. Determination of the impact of grinding

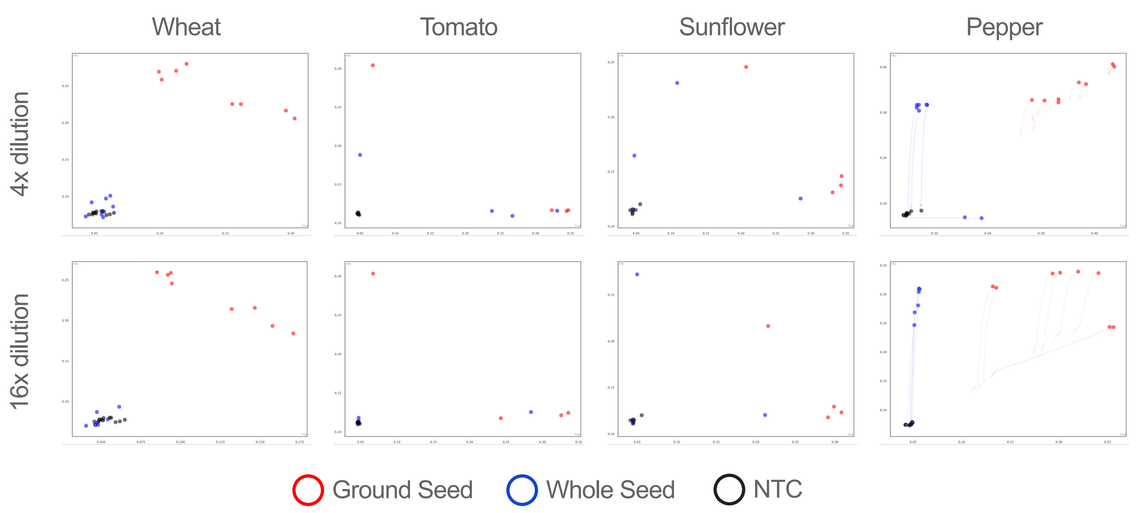

Figure 2

B. Evaluate BHQplus and KASP chemistries for relevant crop types

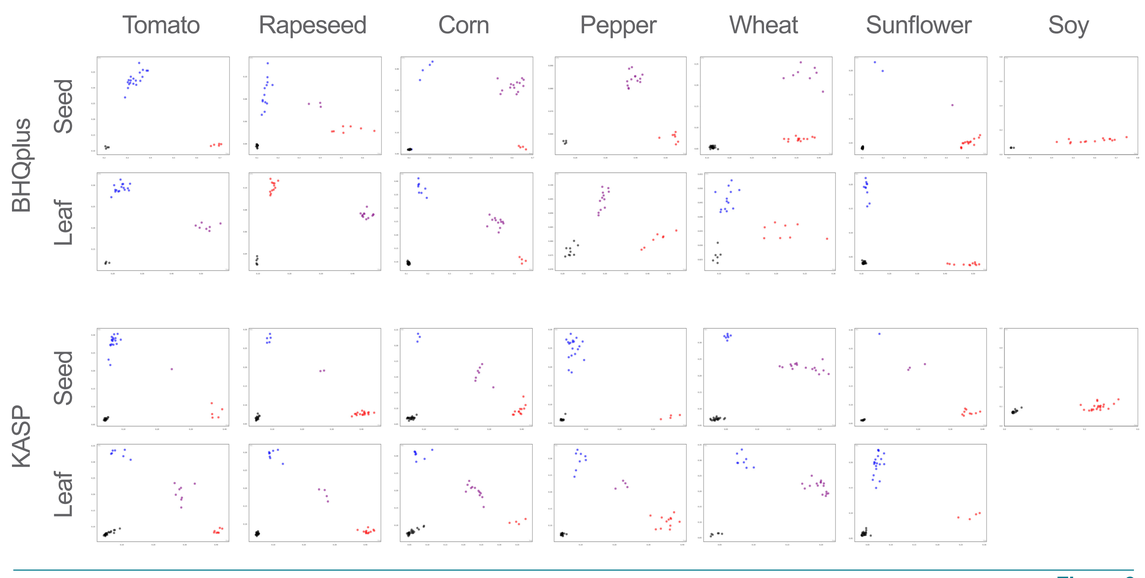

Figure 3

C. Stability of extracted DNA for at least 4 weeks at 4 ˚C

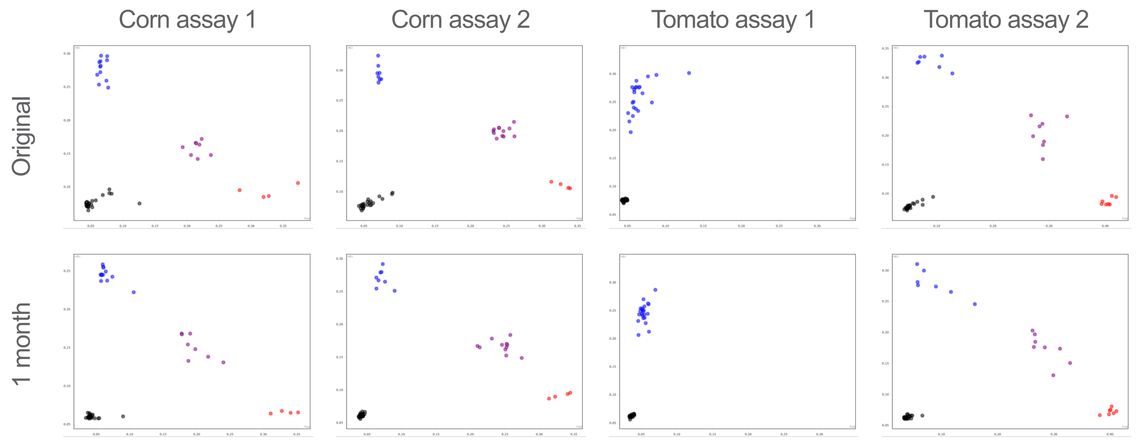

Figure 4

Conclusion

The results show that grinding needs to be determined for each sample used. QuickExtract allows genotyping of tomato and sunflower seeds without grinding. Grinding was required for amplification with wheat seeds, whereas grinding was inhibitory to PCR for pepper seeds. Similar results were obtained with leaf material from these crops (data not shown). Efficient cleaning of the seeds due to coating with chemicals need to be considered.

QuickExtract can be used to extract DNA from leaf and seed material from multiple types of plants for endpoint PCR applications (KASP and BHQplus probes). The results show whether to grind the sample and sample mass to QuickExtract volume must be empirically determined for each crop and sample type. Volume of the processed sample to use for amplification must also be optimised.

Good genotyping results were obtained with the QuickExtracted samples even after storage for 4 weeks at 4 ˚C.

Summary

- PCR-quality DNA may be extracted from leaf or seed material from multiple plant species.
- The processed sample is stable if stored at 4 ˚C for 1 month or for several years when stored at -20 ˚C.
- Prior to large scale processing of multiple samples the following should be considered:
  - Grinding the leaf or seed material prior to processing
  - Optimising the ratio of sample mass to QuickExtract Solution volume
  - Diluting the processed sample before downstream processing

Figure 2: The effect of grinding on seed material.
Wheat, tomato, sunflower and pepper seeds were either ground (red circles) or extracted whole (blue circles) with QuickExtract. Samples were diluted 4 fold (top) or 16 fold (bottom) prior to PCR amplification with KASP on the IntelliQube. Water was used as negative controls (NTC).
Figure 3: Evaluate BHQplus and KASP chemistries for relevant crop types *Cluster plots for 7 crops (leaf or seed samples), using KASP and BHQplus Probes The DNA in the QuickExtract Plant DNA Extraction Solution may be further purified using either sbeadex® or the MasterPure™ DNA purification kit (data not known).*
Figure 4: Stability of DNA stored at 4 ˚C. *DNA extracted from corn and tomato with QuickExtract was PCR amplified (original) with two KASP assays and then stored at 4 °C for 1 month. The samples were then again PCR amplified against the same two assays (1 month).*

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