Steinbrenner Laborsysteme GmbH

magtivio

MagSi-DNA Plant CLS

Application Note *| DNA extraction from vegetable seeds, field crops and oil seeds for high-throughput genotyping*

Introduction

Plant breeding generates seeds with traits that allow increases in crop productivity, resistance to diseases and drought. Quality inspection of seeds by production companies often includes analysis with PCR-based genotyping using specific genetic marker sets ranging from four to thirty individual markers, depending on the crop species and variety. Seed quality criteria may include a maximum inbred percentage or other criteria requiring large numbers of seeds per batch to be analyzed. Although there is variation in yield and quality within and between seed species, it is crucial to obtain DNA of high quality that can be used in such analyses.

MagSi-DNA Plant CLS is intended for fast and cost-effective DNA extractions from all plant samples and includes reagents optimized for processing cotyl, leafs and seeds. This application note provides an extensive quantity and quality analysis of DNA extracted from seeds of nine different plant species, including vegetable seeds, field crop seeds and oil seeds, showing the suitability for use in genomic applications (e.g. genotyping by PCR or DNA sequencing). Seeds of cucumber (*Cucumis sativus*), tomato (*Lycopersicon lycopersicum*), sweet pepper (*Capsicum annuum*), corn (*Zea mays*), sugar beet (*Beta vulgaris*), wheat (*Triticum aestivum*), rape (*Brassica rapa*), sunflower (*Helianthus annuus*), and soy (*Glycine max*) were used.

Materials and methods

All seeds used for this study were obtained from a local wholesale market. Sunflower seeds were cut in two parts, and one half of the seed was transferred to a microtube for DNA extraction. From all other species, one whole seed was transferred to a microtube per DNA extraction. Before mechanical disruption (8 mm stainless steel beads, 5 x 1 min at 1500 rpm) in the 2010 Geno/Grinder® (SPEX® SamplePrep), corn seeds were incubated in lysis buffer for 2-3 hours. Soy seeds were soaked overnight in water, after which the water was removed. No pretreatment was applied for the other seeds before homogenization.

After homogenization, 500 µL Lysis Buffer VG (tomato, cucumber, pepper, sugar beet, rape, soy) or Lysis Buffer PL (corn, wheat, sunflower) was added and samples were incubated at 65 °C for 30 minutes in a ThermoMixer C (Eppendorf). Disrupted and lysed samples were centrifuged for 15 minutes (6000 x g) to pellet cell debris. Afterwards 400 µL sample lysate was transferred and used as input for automated DNA purification on the PurePrep 96 System with a final elution volume of 150 µL.

DNA concentrations and purity of the eluates were measured by UV-VIS with the NanoDrop™ One according to manufacturer's instructions (Thermo Scientific™). The presence or absence of inhibitors was evaluated by Real-Time PCR on the AriaMx Real-Time PCR system (Agilent) with primers targeting the tRNA-leucine gene. From the samples, 2 µL undiluted and 1:10 diluted DNA was used in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).

Results

Yield

DNA concentrations from all seeds tested are presented in Figure 1. DNA concentrations were highly dependent on the plant species and seed size, and ranged from 8 to 224 ng/µL. Highest DNA concentrations were obtained from oil seeds and lower concentrations from the small vegetable seeds.

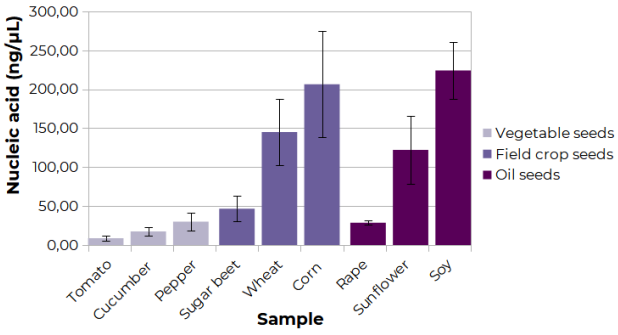

Figure 1. *DNA concentrations extracted from vegetable, field crop and oil seeds, measured by UV-VIS with the NanoDrop™ One. The data are presented as mean (n=3, ±1 SD).*

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Purity

In Figure 2, purity ratios measured by UV-VIS of the extracted DNA samples are presented. All A260/A280 purity ratios are ≥1.6, indicating high quality DNA without contaminations.

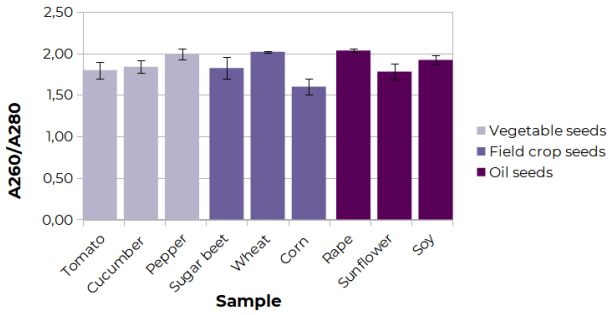

Figure 2. *DNA purity ratios for tomato, cucumber, pepper, corn, sugar beet, wheat, rape, sunflower and soybean seeds obtained by UV-VIS with the NanoDrop™ One. High purity ratios are measured for all seeds. Data are presented as mean (n=3, ±1 SD).*

PCR

PCR results are presented in Figure 3. Ct values were reported for all samples without dilution. Dilution of DNA samples resulted in the expected increase of the Ct value (appr. 3.3 per 10-fold dilution), indicating the absence of PCR inhibition.

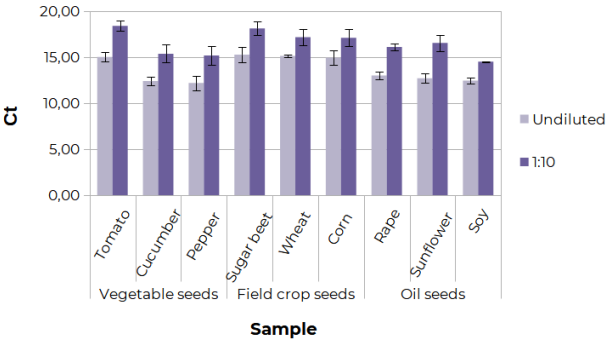

Figure 3. *PCR results from extracted vegetable, field crop and oil seed DNA with primers targeting the tRNA-leucine gene. The data are presented as mean (n=3, ±1 SD).*

Discussion and conclusion

The data presented demonstrates that DNA can be successfully extracted from a variety of vegetable, field crop and oil seeds. The variations in obtained DNA concentrations, within and between seed types, are related to natural differences such as size and weight. However, all seeds generate high purity ratios and are suitable for PCR analysis, and the final volume of purified sample allows for a high number of individual assays to be performed. When requiring higher DNA concentrations for NGS-based genotyping, elution volumes can be adjusted or extraction can be done with multiple seeds as input.

It can be concluded that MagSi-DNA Plant CLS provides a suitable extraction method for DNA extraction from vegetable, field crop and oil seeds for genotyping assays by seed production companies and other agriculture laboratories. The extraction protocol can be carried out with minimal equipment requirements, and is easily automated using a magnetic particle processor and/or liquid handling workstation. The MagSi-DNA Plant CLS kit includes different lysis buffers optimized for seeds or leaf material as is also available with a ready-to-use rQ CLS Extraction plate already containing the magnetic beads and binding buffer.

Literature

- *Product Manual MagSi-DNA Plant CLS, magtivio B.V.*
- *NanoDrop One UG, 269-309102, ThermoFisher Scientific*
- *Dwivedi et al, 2020. First the seed: Genomic advances in seed science for improved crop productivity and food security. Crop Science. 2021; 61:1501-1526.*
- *Verma et al, 2022. Application of Recombinant DNA technology in Agriculture: A Review. International Journal of Advanced Research in Biological Sciences. 9(3): 138-146.*
- *Lauba et al., 2010. Development of primer and probe sets for the detection of plant species in honey. Food chemistry. 2010; 118: 979-986.*

Ordering information

Art. No.	Description	Amount
MDKT00260096	MagSi-DNA Plant CLS	96 preps
MDKT00260960	MagSi-DNA Plant CLS	10 x 96 preps
MDKT00260196PF	rQ MagSi-DNA Plant CLS *	96 preps
MD0200142596PF	rQ Plant CLS Binding Plate **	25 pcs

* All kit components except lysis buffers provided pre-filled in 96 DeepWell plates for PurePrep 96 / KingFisher™ Flex 96

** Pre-filled binding plate contains beads and binding buffer suited for PurePrep 96 / KingFisher™ Flex 96

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