Semi-automated DNA isolation from cereal crops using the Te-MagS™ module

Sanja Baric, Stefanie Unterholzner and Josef Dalla Via
Research Centre for Agriculture and Forestry Laimburg, I-39040 Auer/Ora, Italy

Members of the Research Centre for Agriculture and Forestry Laimburg, led by Sanja Baric (front row, second from right), together with the Director of the Research Centre, Josef Dalla Via (front row, far right)

The Research Centre for Agriculture and Forestry Laimburg works closely with the farming industry in South Tyrol in a variety of agricultural research projects, from fruit and wine production to plant protection, vegetable cultivation, agricultural chemistry and fish farming. The researchers have recently applied a semi-automated procedure for extracting DNA from large numbers of cereal crop samples, using the Te-MagS™ module, to increase throughput and reduce the labor bottleneck.

The Molecular Biology Laboratory at the Research Centre for Agriculture and Forestry Laimburg in South Tyrol, northern Italy, is currently involved in a research project that focuses on the genetic characterization of local cereal landraces. In addition to botanical and agronomic information, the project is using genetic data to establish and maintain a germplasm collection. The study aims to analyze microsatellite markers from large numbers of samples and, in order to achieve this, it was necessary to develop a semi-automated DNA extraction method that would yield high quality DNA from various grain species.

Automating plant DNA isolation using magnetic bead technology

Isolation of DNA from plant tissue represents a crucial step for the quality and outcome of subsequent downstream applications, such as PCR, sequencing or genotyping. However, this step can also be a considerable bottleneck in many extensive plant genotyping studies. Plant cells are surrounded by a rigid cellulose-containing cell wall that must be mechanically disrupted to release nucleic acids into solution. Furthermore, plant cells are rich in polysaccharides and secondary metabolites that can be co-purified with DNA and, subsequently, might inhibit enzymatic reactions. Traditional cetyl-trimethylammonium bromide (CTAB)-based protocols for DNA isolation from plant material involve several centrifugation steps, make use of harmful organic solvents, and generally have a low potential for automation.

To overcome these issues, we established an automated DNA extraction procedure using a Te-MagS module, configured to hold 48 microcentrifuge tubes, integrated with a Freedom EVO® 100 liquid handling workstation with four channels for disposable tips and lower disposable tip eject option. DNA binds reversibly to paramagnetic silica-coated particles in solution, and buffers used for extraction are aspirated by pipetting while the paramagnetic particles are captured by magnets. No further centrifugation steps or vacuum filtration are required and, after preparation of the plant lysate, the DNA isolation process runs without any additional manual intervention.

DNA extraction procedure for cereal crops

Our protocol uses the Wizard® Magnetic 96 DNA Plant System (Promega, Madison, USA) and comprises two major steps: (i) manual preparation of the plant lysate and (ii) automated DNA isolation (Figure 1). Fresh leaf tissue obtained from young cereal seedlings is homogenized directly in Lysis Buffer A supplied with the DNA extraction kit. After centrifugation of the homogenate, the supernatant is manually transferred to 1.5 ml microcentrifuge tubes – ready to be placed in the Te-MagS unit on the Freedom EVO workstation (Figure 2). The manual transfer of the lysate is considered an important step to avoid carryover of plant debris, which could potentially clog the pipetting tips during automated liquid handling. Once the tubes containing the lysate have been placed into the Freedom EVO, the extraction procedure is fully automated and no further manual handling is required. The tubes with the final DNA eluates from all 48 samples can be removed after 90 minutes.

Results

The semi-automated DNA purification protocol using the Te-MagS yielded high quality DNA from different wheat species and hybrids as well as from oat and rye (Figure 3). DNA quantity generally ranged from 100 to 300 ng per mg of fresh plant tissue. Accurate amplification of diverse microsatellite loci indicated that DNA isolates were free of PCR-inhibiting substances. By the application of the semi-automated procedure it was possible to at least double the throughput for DNA isolation in comparison to the conventional approach. Moreover, automation could save about 60 % of manual labor time, which might be particularly beneficial for laboratories with limited human resources.

Summary

The semi-automated method for DNA extraction established in the Research Centre for Agriculture and Forestry in Laimburg, was set up to fulfill medium throughput requirements. However, if required, the processing speed and throughput of magnetic bead purification can be increased by using any Freedom EVO workstation with an eight-channel liquid handling arm integrated with a Te-MagS module configured to hold 96-well microtiter plates. Automating the Wizard® Magnetic 96 DNA Plant System on the workstation could allow sufficient amounts of high quality genomic DNA to be obtained from several cereal crops for analysis in extensive population studies, assisted breeding programs, disease diagnosis or GMO detection.

The present work is part of the GENE-SAVE project, which was funded by the European Union under its Interreg IIIA program between Italy and Austria, and the Governments of South Tyrol (Italy) and Tyrol (Austria). The authors thank Guido Cimoli, Application Specialist from Tecan, for his support during the establishment of the automated DNA extraction procedure.

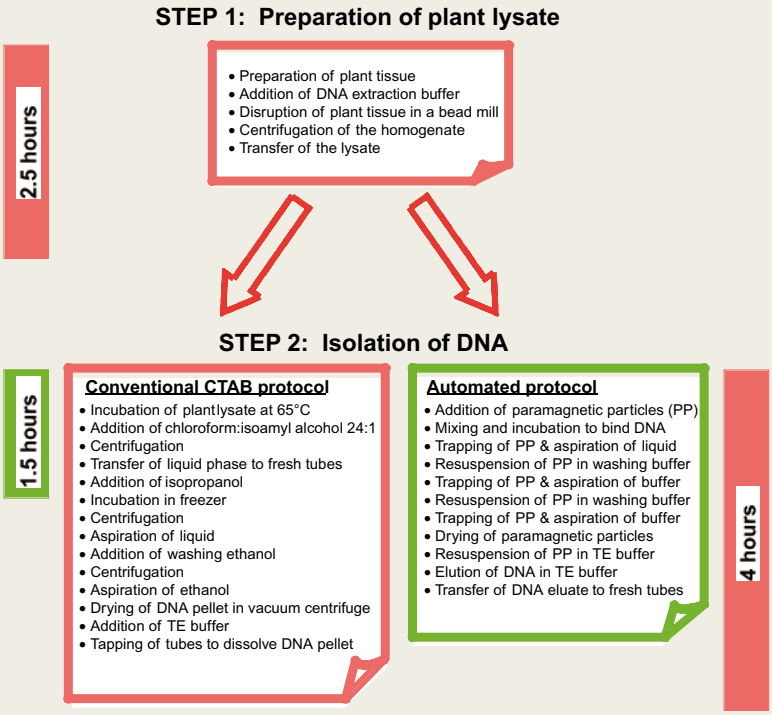

Figure 1: Comparison of the automated (left) and the conventional CTAB-based protocol (right) for plant DNA extraction (Step 2). The automated protocol uses the Wizard® Magnetic 96 DNA Plant System (Promega) on a Tecan Freedom EVO 100 workstation with four pipetting channels and the Te-MagS magnetic separation unit. The manual procedure for the preparation of plant lysate from 48 samples (Step 1) remains identical for both extraction types. Fully automated steps are coded in green, while those requiring manual intervention are coded in red.

Figure 2: Collaborator of the Molecular Biology Laboratory preparing the Tecan Freedom EVO 100 for automated DNA extraction.

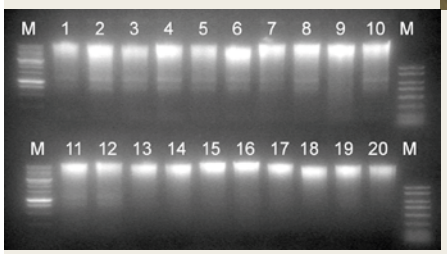

Figure 3: Genomic DNA obtained from different cereal species or hybrids. Nucleic acid was isolated from approximately 30 to 40 mg of fresh leaf material from young seedlings, which was ground directly in 300 µl Lysis Buffer A (Wizard® Magnetic 96 DNA Plant System, Promega) using a bead mill (Mixer Mill 300; Retsch, Haan, Germany). After centrifugation, 125 µl of the supernatant was transferred to 1.5 ml microcentrifuge tubes and placed on the Freedom EVO. DNA was captured by the addition of MagneSil™ Paramagnetic Particles (Promega). Following two washing steps, DNA was eluted in 75 µl of TE buffer, pH 7.5, and transferred to fresh microcentrifuge tubes. Five µl of DNA eluate was run on a 1 % ethidium bromide stained agarose gel in TAE buffer M size marker; 1, 2 Triticale; 3 Aegilops squarrosa; 4 Triticum boeoticum; 5 T. monococcum; 6 T. dicoccoides; 7 T. dicoccum; 8 T. turgidum; 9 T. aestivo-compactum; 10 T. sphaerococcum; 11, 13, 14 T. aestivum; 12 T. spelta; 15, 16, 17 Avena sativa; 18, 19, 20 Secale cereale.