Low Input Genomic DNA Extraction Protocol for Sorghum Leaves, suitable for Next Generation Sequencing

Coumba Fall[1], Louis K. Prom[2], Mame P. Sarr[3], Adamou Haougui[4], and Clint W. Magill[1]

[1]Department of Plant Plathology and Microbiology, Texas A&M University, College Station, Texas

[2]U.S. Department of Agriculture, College Station, Texas

[3]Centre National de Recherches Agronomiques de Bambey, Senegal

[4]Institut National de la Recherche Agronomique du Niger, Niamey, Niger

Abstract: Sorghum is characterized by its high genetic diversity and potential of adaptation to biotic and abiotic stresses. The use of marker-assisted selection would help to faster sorghum breeding , hence enable an optimized use of the crop's potential. As a first step of most of molecular biology-based technology, an efficient and high yielding DNA extraction method should be used. Here, we tested a low input CTAB-based method that can be used in developing countries' laboratories, with downstream use for next generation sequencing.

Key-Words: Sorghum. DNA-Extraction, Low-Input, Next Generation Sequencing, Marker-Assisted Selection

Introduction:

Sorghum is the fifth most important cereal crop grown in the world and is used as a source of food, feed and biofuel. Its high genetic diversity and potential resistance to abiotic and biotic stresses, make it a promising value crop in the context of climate change. Molecular biology tools are an efficient way to faster breeding procedures. For example, Marker-Assisted Selection would help to faster the sorghum breeding procedures . As an initial step, genomic DNA extraction should be done properly to enable whole genome sequencing of the sorghum lines, cultivars or landraces of interest. However, high polyphenol compounds present in sorghum hamper, the quality and yield of the extracted DNA. Here, we tested a low input, CTAB-based genomic DNA extraction protocol reproducible in developing countries' laboratories, that could be used for downstream application as Next Generation Sequencing.

Material and Methods:

Sorghum seeds from Senegal and Niger were grown in the PLPM insectary room facility at Texas A&M University. The genomic DNA was extracted following a modified protocol from Doyle and Doyle (1987) and Kale et al. (2020). The DNA was then visualized on a 1% TAE agarose gel stained with ethidium bromide. The yield and quality was also assessed using a QuickDrop spectrophotometer (Molecular Devices).

Results and Discussion:

The genomic DNA extracted yielded between 163-295 µg/ml with fragments of 6-10 kb (fig.1). The average A260/A280 ratio was 1.878 and for the A260/A230 2.05 (Table1). The extracts seem therefore suitable for whole genome sequencing and next generation analysis, according to the requirements of the Genomics and Bioinformatic services of Texas A&M Agrilife Research at College Station.

Conclusion:

The CTAB-based method tested in this study does not require the use of liquid nitrogen that can be a limitation in developing countries. In addition, most of the reagents can be found without high-cost inputs.

Acknowledgement:

This study is made possible through funding by the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet through grants from American People provided to the United States Agency for International Development (USAID) under cooperative agreement number AID-OAA-A-13-00047. The contents are the sole responsibility of the authors and do not necessarily reflect the views of USAID or the US Government.

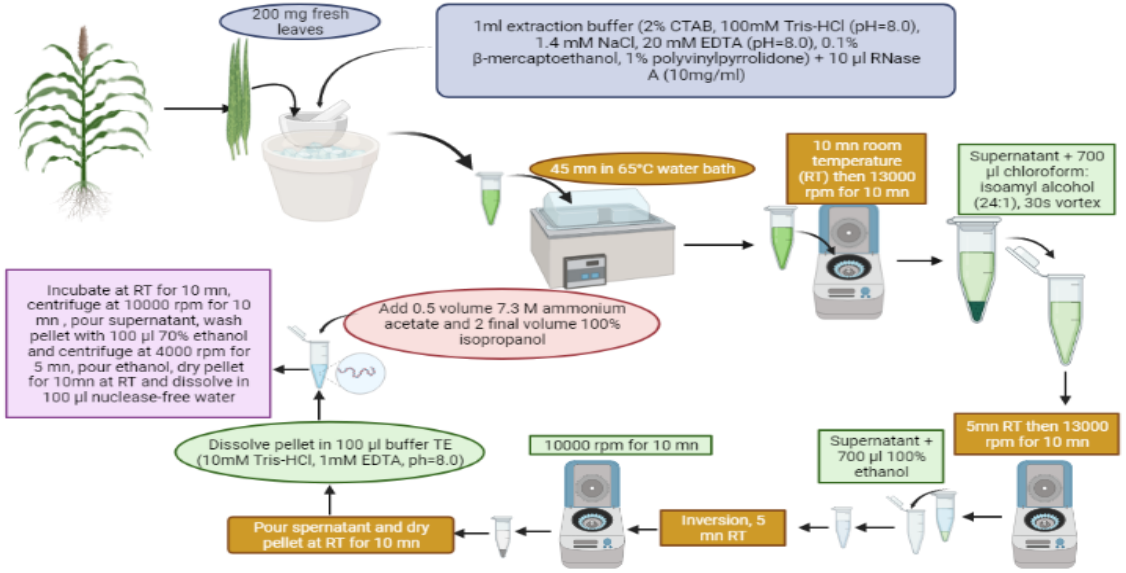

Table1: Average genomic yield and absorbance ratios

Yield (µg/ml	A260/A230	A260/A280
224.5	2.05	1.878

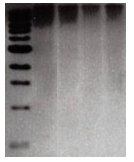

Fig.1: visualization of the extracts on 1% TAE agarose gel

Created in BioRender.com

Literature cited

Kale et al. 2020 https://doi.org/10.22271/chemi.2020.v8.i1p.8409

Doyle and Doyle 1987. Phytochemical Bulletin, Vol. 19 (1): 11-15, 1987