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DNA isolation from mature endosperm

(Based on DNA isolation protocol by Mauricio Lopes)

The conditions for DNA isolation from mature endosperm should be optimized to avoid precipitation of starch with genomic DNA. To overcome this problem, a sarcosyl buffer is used and samples are not heated during extraction. To improve intact DNA yields, kernels (30 DAP or older) can be stored at – 80 C or lyophilized, which also facilitates the grinding and flour weighting.

Procedure

Dissect the endosperm removing the pericarp. Embryonic tissues can also be used;

Lyophilize the tissue – OPTIONAL- for 24 hours (6 hours @ -20 C and 18 hours @ 0 C);

Move it to a pre-chilled mortar and grind it making a fine flour;

Weigh 50-200 mg of flour in a eppendorf tube;

Add 600 ul of extraction buffer (see below);

Vortex, mixing up the flour and buffer well;

Let it stand @ RT for 10 min.;

Add 600 ul of Phenol-Chlorophorm, vortex it vigorously;

Transfer to a PHASE LOCK tube, if available;

Spin @ 14000 rpm for 10 min.;

Transfer supernatant to a new tube, add 5 ul of RNAse;

Incubate @ 37 C for one hour;

Add 450 ul of cold isopropanol;

Mix and incubate @ -80 C for 30 min.;

Spin @ 14000 rpm for 10 min.;

Pour off supernatant and wash the pellet with 500 ul of 70% Ethanol;

Dry the pellet @ RT;

Add 50 ul of water, ressupend the pellet overnight @ 4 C;

Spin for 15 sec.

Run a gel with Lambda DNA standards to check quality and determine concentration.

Buffer and reagents

Extraction Buffer	100 mM TRIS pH 8.5 100 mM NaCl 200 mM EDTA pH 8.0 1% Sarcosyl
RNAse	10 mg/ml (Boil to inactivate any DNAse activity)
Isopropanol	Kept @ -20 C