NPPC protocol on RNA extraction from plant sample using CTAB method (modified Doyle and Doyle, 1987; Aladizgeh et al 20023)

Materials Needed:

- Sample
- Homogenizer
- FastPrep
- Beating beads
- Weighing balance
- 2ml /1.5 ml microcentrifuge tubes
- Pipettes and sterilised tips ((10µl, 200µl, 1000µl)
- Centrifuge
- Vortex mixer
- Incubator or water bath

Reagent Solutions:

- Lysis buffer / CTAB extraction buffer (2% (v/v) CTAB, 100 mM Tris-HCl, 25 mM EDTA, 2 M NaCl)
- 2.5%m/v PVP-40 (Polyvinylpyrrolidone
- 2% β-mercaptoethanol)
- Chloroform: isoamyl alcohol (24:1 v/v)
- 2.5 M lithium chloride (LiCl) or Isopropanol
- 70% ethanol
- Nuclease-free water
- Sterile distilled (molecular grade) water or TE buffer

Procedure:

1. Label all tubes and plastic pouch before starting extraction: two sets of 2 ml tubes and one set of 1.5 mL tube. Turn on the water bath with the temperature set at 65°C.
2. Grind 100 mg (0.1 g) plant tissues (e.g. leaf, shoot, root) in 1000 µl of prewarmed (at 65 °C) lysis buffer.
3. Transfer crushed sample to 2 ml PCR or eppendorf tube and add 10 µl β-mercaptoethanol and vortexed for 1 min.
4. Incubated at 65 °C in a water bath for 10 minutes (shake the tubes by inverting the tubes gently after 5 minutes).
5. Centrifuge at 13000 rpm for 4 min at room temperature (~ 20 °C – 22 °C).
6. Collect supernatant 900 µl into 2 ml PCR tube.
7. Add an equal volume (900 µl) of chloroform: isoamyl alcohol (24:1) and vortexed for 1 minutes.
8. Centrifuge at 13000 rpm for 10 minutes at 20 °C.
9. Transfer upper aqueous phase (600 µl) to 1.5 ml PCR tube gently, without disrupting the solid and organic phases.
10. Add an equal volume (600 µl) of cold Isopropanol to each tube. (Isopropanol should be stored at −20°C).
    Or transfer 600 µl into tube which half the amount (300 µl) of lithium chloride (7.5M) was added, and stored at −20 °C for 15–20 min.

11. Precipitate at −20°C for 1 hr if to be continued on the same day or at 4°C overnight if to be continued on the next day.
12. Centrifuge samples at 13000 rpm for 30 min at 4 °C.
13. Discard the supernatant and resuspend the pellet with 500 µl ice-cold ethanol (80%v/v).
14. Vortex slightly/mix by hand for few seconds by inverting.
15. Centrifuge pellets at 13000 rpm for 5 min at 4 °C.
16. Again, decant the tube and let the pellet air-dry for approximately 30–40 min.
17. Resuspend the pellet in 50 µl of RNase-free water or nuclease free water.
18. Store RNA at −20°C till further use.

Reference

Aladizgeh. F.M., Jabbari. L., Khayam., N. R, Aalami. A., Atwell.B.J., Haynes. P.A.2023. A universal protocol for high-quality DNA and RNA isolation from diverse plant species. PLoS ONE 18(12): e0295852. https://doi.org/10.1371/journal.pone.0295852