Cyanide quantification in plant samples

In most plant tissues, cyanide is stored in form of cyanogenic glucosides. To measure the cyanide content in a given sample, you have to break down these glucosides.

Cyanide is highly volatile. Thus, do obtain exact data you ALWAYS have to work under cool conditions to lower activity of enzymes involved in decomposition of cyanogenic glucosides.

We use gas-tight Thunberg vessels to break down cyanogenic glucosides and not to lose any cyanide.

1. Weight you plant (leaf) samples. Avoid wilting of leaf material.
2. Homogenize (grind) leaf material with buffer (Na_2HPO_4 solution; 0.067 mol/l)
3. Centrifugation of samples (13,000 rpm, 4˚C)
4. Transfer supernatant in new tube and put on ice

Prepare the Thunberg-Vessels

1. Add 0.45 ml NaH_2PO_4 solution (0.067 mol/l) to the main chamber of the vessel
2. Add 0.1 ml NaH_2PO_4 solution (0.067 mol/l)
3. Pipet 0.6 ml 0.2 mol/l NaOH to the side bulb
4. Add 0.05 ml sample and close lid of the Thunberg-Vessel immediately
5. Incubate at 30 °C for 20 min
6. Stop reaction by inverting the closed Thunberg-Vessel several times. Make sure liquids of side bulb and main container are mixed thoroughly.

Prepare reagent tubes for dye reaction

1. Add 0.1 ml 0.1 mol/l HCL
2. Add 4.8 ml ddH_2O
3. Add 0.1 ml sample
4. Vortex
5. Add 1 micro spoon reagent 1A
6. Vortex
7. Add 1 micro spoon reagent 2A
8. Vortex (thoroughly!!)
9. Add 3 drops of reagent 3A
10. Vortex and wait for 5 min
11. Pipet 1 ml in a cuvette and measure at 585nm against a blank (= a sample which was prepared identically but without sample. Add Na_2HPO_4 solution (0.067 mol/l) instead.

Reference

Ballhorn D.J., Lieberei R. & Ganzhorn J.U. (2005) Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore-plant interaction: The importance of quantitative data. J Chem Ecol 31:1445-1473.