6. PLANT NUTRIENT ANALYSIS

V.Selvamani, and Neenu. S

Division of Crop Production

Central Plantation Crops Research Institute, Kasaragod – 671 124, Kerala.

Preparation of Samples

To collect leaves, the plantation should be divided into homogenous areas, taking into account the plant's ages, nutritional status and plant health, as well as soil variability. In each homogeneous area, which should not exceed 10 ha, leaflets should be collected from a minimum of 20 plants. The exception to the rule of the most-recently-matured leaf is the analysis of Ca, Cu, B, and S, which are relatively immobile in the plant. Therefore, an analysis of the mature leaves in this case may not reveal the Ca, B, Cu, or S deficiency in the younger leaves. When a nutrient deficiency of this nature is suspected, young (not fully expanded) leaf tissue is needed for analysis.

According to the analytical procedures for specific purposes either the fresh material or the dry material is taken. Moreover, since dried material can be stored for long periods, for most of the analytical studies the dried material is preferred. Drying of the samples may be done in an oven at 70-80°C. Over heating should be avoided. Best material may be obtained by for drying 18-24 hours. For moisture determination, however, drying should be continued at 105°C until a constant weight is obtained. In the case of tissues of other organs of the palm the specific period of heating should be standardized.

The tissue samples are then ground well in a mechanical mill with stainless steel blades. The ground tissue is then sieved through a 60 mesh stainless steel sieve. The ground samples are then stored in clean bottles or polythene vials with proper labelling. In the case of the samples that are stored for long period, a further drying over calcium chloride in desiccators just before weighing the required quantity for analysis, should be done.

Method of Analysis

1.Total Nitrogen in Plant Samples

Nitrogen plays important role in the synthesis of protein responsible for metabolic activities in plants. Nitrogen content of plant parts varies between 0.2 to 0.6 % of dried

materials and green leaves contain higher N then stem, flowers and seeds. One common plant analysis is that of nitrogen (N) by Kjeldahl method. However, wet ashing with H_2SO_4 and H_2O_2 is also used for eliminating the use of selenium in the former method.

Principle

The plant samples are digested with sulphur salicylic acid mixture. Organic and nitrate nitrogen is converted to ammonium sulphate and the ammonia gas is distilled into boric acid and titrated with standard sulphuric acid. The nitrate content present in the sample form nitrocompounds by the reaction of salicyclic acid in acid medium.

Apparatus

Block-digester, Kjeldahl Distillation unit, conical flask, Pipettes, measuring cylinders etc.

Reagents

- *Sulfuric Acid (H_2SO_4)-salicylic acid*

Dissolve 1g of salicylic acid in 30 ml of concentrated H_2SO_4.

- *Digestion mixture*

Mix 25g of K_2SO_4 with 5g of $CuSO_4.5H_2O$ and 0.5 g of metallic selenium powder by grinding in a mortar.

- *Sodium Hydroxide Solution (NaOH), 40%*
- *Mixed indicator*

0.1g of bromocresol green + 0.07g of methyl red in 100ml of 95% ethanol

- *Boric Acid Solution (H_3BO_3), 2%*

Dissolve 20g of H_3BO_3 in distill water and dilute the contents to 900 mL. add 20 mL of bromocresol green + methyl red mixed indicator solution. Then add 0.1N NaOH drop wise till solution becomes reddish purple. Make up the volume to 1 litre.

Procedure

A. Digestion

1. Mix and spread finely ground (Cyclone mill) plant sample in a thin layer on a sheet of paper until it looks uniform.
2. Weigh 0.5 or 1g of dry plant material, and transfer quantitatively into a 100-ml digestion tube.
3. Add a few pumice boiling granules, and add about 3 g catalyst mixture using

4. Add 10 ml concentrated sulfuric acid containing salicylic acid using a dispenser, and stir until mixed well.
5. Allow to stand overnight
6. Place tubes in a block-digester set at 100°C for 20 minutes and digest at low flame, and remove the tubes to wash down any material adhering to the neck of the tube with the same concentrated sulfuric acid. Thoroughly agitate the tube contents, and then place the tubes back on the block-digester set at 380°C for 2 hours after clearing.
7. After digestion is complete, remove tubes, cool, and bring to 100 ml volume with distilled water.

B. Distillation

1. Prior to distillation, shake the digestion tube to thoroughly mix its contents. And pipette 10 ml aliquot into a 100 ml distillation flask.
2. Add 10 to 15ml of 40% NaOH to make the contents alkaline of distillation assembly is washed with small amount of distill water 2 to 3 times.
3. Before adding NaOH, boric acid mixed indicator solution should be kept ready at the receiving end of condenser outlet so that outlet is dipped in boric acid.
4. Carry out distillation by passing steam into distillation flask and the colour of boric acid mixed indicator solution changes from reddish purple to green and continue distillation for some more time to trap all the NH_3 released from distillation of sample.
5. After distillation, bluish green coloured ammonia trapped boric acid is titrated against 0.01N H_2SO_4 till colour changes to purple releasing boric acid with the formation of $(NH_4)_2SO_4$.
6. Run a blank without the plant material to check for contamination and to ensure precision.

$$\% \text{ N in plant or soil sample} = \frac{(S-B) \times N \text{ of } H_2SO_4 \times \text{Volume of digest} \times 0.014 \times 100}{\text{Weight of plant or soil sample} \times \text{Aliquot taken for distillation}}$$

where,

S = Volume of standard H_2SO_4 used in sample titration

B = Volume of standard H_2SO_4 used in blank titration

2.Total Phosphorus in Plant Sample

Principle

The concentration of P in the plant samples is usually about one tenth of the N content. The concentration varies with plant parts and crop growth stage. The medium level od P in most crops is less than 0.3% on dry weight basis. Plant P is converted into orthophosphates during digestion. These orthophosphates react with molybdate and vanadate and give yellow coloured unreduced vando molybdo phosphoric heteropoly complex in nitric acid medium. The yellow colour is attributed to the substitution of oxyvanadium and oxymolybednum radicals for the oxygen of phosphate. The intensity of this yellow colour is directly proportional to the concentration of phosphates in the plant samples which can be read in spectrophotometer. Yellow colour developed in 30 minutes and is stable for 2 to 8 weeks

Apparatus

Spectrophotometer or colorimeter, 410 nm wavelength, Block-digester, beakers, volumetric flasks, pipettes, and funnels.

Reagents

- *Perchloric Acid ($HClO_4$), 60%*
- *Nitric acid (HNO_3)*
- *Diacid mixture*

HNO_3: $HClO_4$ (9:4 ratio) mix 900 mL of HNO_3 with 400 mL of $HClO_4$

- *Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid*

Dissolve 22.5 g ammonium heptamolybdate [$(NH_4)_6Mo7O_{24}.4H_2O$] in 400 mL distill water (a). Dissolve 1.25 g ammonium metavanadate (NH_4VO_3) in 300 ml hot distill water (b). Add (b) to (a) in a 1L volumetric flask, and let the mixture cool to room temperature. Slowly add 250 ml concentrated nitric acid (HNO_3) to the mixture, cool the solution to room temperature, and dilute to 1L volume with distilled water.

- *Phosphorus standard solution*

Prepare 100 ppm of P standard stock solution by dissolving 0.2195g of KH_2PO_4 (AR grade) distilled water and make upto 500ml.

- *Working P standards*

Prepare 0.5,1, 2, 3, 5, 7, 7, 10, 12, 15, 18 and 20 ppm P working standard solution by pipetting out 0.25, 0.5, 1.0, 1.5, 2.5, 3.5, 5.0, 6.0, 7.5, 9.0 and 10 mL of stock P solution respectively into 50 mL volumetric flasks separately. Add to each 10ml of

vanadate molybdate reagent and make up the volume to 50 mL with distilled water and shake. Allow to stand for 30 minutes and yellow colour will be developed.

Procedure for plant sample

1. Transfer 0.5 to 1.0 gm of plant sample into a 100 mL conical flask or dry digestion tubes and wet the sample with 10ml of conc. HNO_3. Let it stand for at least 2 hours or overnight. Pre digestion is required to avoid bumping or violent reaction on addition of diacid mixture to plant sample.
2. Then gently heat on a hot plate until the volume of the contents is reduced to about 4ml or even less and the material turns white sand.
3. Remove the flask from hot plate and allow it to cool. And add distilled water and make the volume upto 100 mL.

$$\% \text{ P in plant or soil sample} = \frac{\text{ppm from graph} \times \text{Volume of digest} \times \text{Volume made after colour development} \times 100}{\text{Weight of plant or soil sample} \times \text{Aliquot taken for} \times 10^6}$$

3.Total Potassium in Plants

Principle

The plant digest containing alkali and alkaline earth metals fed to flame photometer, it vapourises to gaseous state. Atoms of some specific elements (Na or K) take energy from flame and get excited to the higher state. The excited state of the atom does not remain stable for longer time as it is unstable and reverts to its original state (ground level). While returning to the original level, the atoms loose there energy in the form of radiation. The characteristic colour and wavelength of radiation indicates the type of element (red or pink coloured radiation is due to K-776nm). The intensity of emitted radiation indicates the concentration of the particular element in test samples. The intensity of this fluorescent electromagnetic radiation can be measured by photosensitive detector or photocell of the flame photometer.

Reagent

- *Perchloric Acid ($HClO_4$), 60%*
- *Nitric acid (HNO_3)*
- *Diacid mixture*

HNO_3: $HClO_4$ (9:4 ratio) mix 900 mL of HNO_3 with 400 mL of $HClO_4$.

- Working standards

Prepare 1000 ppm K by dissolving 1.9069 g KCl in 1 litre distilled water. Dilute a known concentration from 1000 ppm to get 100 ppm K. From this, prepare working standard solutions containing 1, 2, 3, 4, 5, 6, 7, 8 and 10 ppm K with suitable dilution using distilled water

Procedure

1. Transfer 0.5 to 1.0 g of plant sample into a 100 mL conical flask or dry digestion tubes and wet the sample with 10 mL of conc. HNO_3. Let it stand for at least 2 hours or overnight.
2. Pre digestion is required to avoid bumping or violent reaction on addition of diacid mixture to plant sample.
3. Then gently heat on a hot plate until the volume of the contents is reduced to about 4ml or even less and the material turns white sand. Remove the flask from hot plate and allow it to cool. And add distilled water and make the volume upto 100 mL.

Measurement

1. Transfer a known quantity of sample digest into a 50 mL volumetric flask make up the volume with distilled water.
2. Feed the diluted solution of plant digest and record the flame photometer reading.

Calculations

$$\% \text{ K in plant sample} = \frac{\text{Sample reading (ppm) X Volume of digest X Dilution factor X } 100}{\text{Weight of plant sample}}$$

4.Estimation of Ca, Mg, S and micronutrients

The triacid extract prepared above can be used for the estimation of total Ca and Mg of the tissue samples. The procedures are same as that described for soil analysis. For the estimation of sulphur, diacid (HNO_3 - $HClO_4$) in the ratio 10:4 can be used for digestion. For trace elements double distilled water is to be used for dilution etc. and is estimated by using atomic absorption spectrophotometer.