

## A COMPARISON OF DIMETHYL SULPHOXIDE (DMSO) AND ACETONE EXTRACTS FOR THE DETERMINATION OF CHLOROPHYLL IN *HEVEA* LEAF TISSUE

Many plant physiological, ecological and horticultural studies require comparative analysis of leaf chlorophyll density. Water soluble solvents such as methanol, ethanol, acetone, pyridine and acetone plus ethyl acetate are in use for the extraction of chlorophylls (Strain and Svec, 1966). The conventional chlorophyll extraction method (Arnon, 1949) involves grinding the plant tissues in 80 per cent acetone with subsequent centrifugation to remove solid plant materials. This method is slow and tedious. Moreover, extracts are unstable and require immediate spectroscopic analysis. Shoaf and Lium (1976) and Hiscox and Israelstam (1979) have shown that dimethyl sulphoxide (DMSO) is superior to acetone in the extraction of chlorophylls in a wide range of algal as well as angiosperm and gymnosperm materials. They have shown that spectroscopic analysis need not necessarily be immediate since DMSO extracts are stable.

Studies on screening of *Hevea* clones for characters such as photosynthetic efficiency invariably involve estimation of chlorophylls and quite often a large number of samples are to be analysed. In laboratories with limited manpower and facilities a rapid extraction procedure in which the extractant is fairly stable, so that spectrophotometric analysis could be carried out over an extended period, is necessary. The success of DMSO method has been established in other plant materials and hence it was attempted to check its fitness in *Hevea* foliage. The study was aimed at standardisation of the DMSO extraction

method for *Hevea* foliage and its comparison with acetone extraction method.

Mature leaves from eighteen trees of *Hevea brasiliensis* (clone RRIM 600), at two and a half years growth, were collected. Fresh leaf discs taken from each sample were transferred to a beaker containing 7 ml of DMSO (E. Merck Limited). The chlorophyll was extracted in the fluid without maceration by keeping on a water bath at 65-70°C for varying times viz., 15, 30 and 60 min. The extract was made up to 10 ml with DMSO and the OD was read at 645 and 663 nm in a Shimadzu UV 160 A spectrophotometer against DMSO blank. Chlorophyll, extracted in 80 per cent acetone (Arnon, 1949), served for comparison.

The samples from DMSO extracts (taken from 30 min incubation period) as well as the acetone extracts were transferred to vials, sealed and stored between 0 - 4°C. The OD values at 645 and 663 nm were read after 24, 48, 72 and 96 h for determination of chlorophyll contents.

The mean values for chlorophyll content in DMSO extracts and acetone extracts are given in Table 1. There was no significant difference between acetone extraction and DMSO extraction for 15 min as evidenced by a non-significant paired 't' test value denoting that extraction for 15 min in DMSO compares well with acetone extraction. This, however is a deviation from the observations reported for some angiosperm and gymnosperm materials by