Pak. J. Bot., 13(1): 103-106, 1981.

A RAPID METHOD FOR CHLOROPHYLL DETERMINATION

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Abstract

A rapid method for colorimetric determination of chlorophyll a,b and total chlorophyll has been suggested by using a single wavelength 645 nm.

Introduction

The chlorophyll as an index for determination of photosynthetic ability has been reported by many workers (Hinghnin, 1950; Starnes & Haddley, 1965). Relation between higher photosynthetic activity and high yield has been reported in various crop plants (Wallace & Munger, 1966; Ishar & Wallace, 1967). The chlorophylls constitute about 10% of the dry weight of the chloroplast (Zelitch, 1971). Chlorophyll b differs from chlorophyll a in, that the methyl group in ring B is replaced by formyl group (CHO). This slight difference changes the absorption spectrum in organic solvents so that the maximum in the red which occurs at 660 nm for chlorophyll a is now at 643 nm for chlorophyll b with a lower absorption coefficient (French & Young, 1956). In green plants chlorophyll a is present in several different forms but all these revert to ordinary chlorophyll a on extraction with organic solvents (French, 1961). *In vivo,* chlorophyll a has an absorption maximum in red at 663 nm and also absorbs a longer wavelength, 650 nm (Holt, 1965).

Various workers have suggested different formulae, for colorimetric determination of various chlorophyll pigments, e.g. Arnon (1949) and Zscheile & Comar (1941) for determination of Chlorophyll a,b and total chlorophyll; Duxbury & Yentsch (1956) for determination of chlorophyll a Plummer (1971) for total chlorophyll. Of these the formulae suggested by Arnon (1949) are more commonly used and require three wavelengths i.e., 663, 652, and 645 nm for the determination of chlorophyll a,b and total. In the present work, the determination of chlorophylls has been worked out using a single wave-length (645 nm).

Table 1. Values of Chlorophyll a, b and total chlorophyll as computed by different formulae (Values are means of 100 samples)

Name of Chlorophyll pigments	Amount (g/g fresh weight sample) By Proposed Formulae	By Arnon's Formulae	By Zscheile & Comar's formulae	By Duxbury & Yentsch's formulae	By Plummer's formulae	t–Value
Chlorophyll a	0.00216	0.00212	0.00215	0.00216	–	0.00061
Chlorophyll b	0.00112	0.00112	0.00112	–	–	0.0055
Total Chlorophyll	0.00378	0.00384	0.00375	–	0.00374	0.00009

Materials and Methods

Hundred samples from various parts of different plant species at different stages of growth were analysed for chlorophyll determination. Samples one gram each were weighed and after grinding the green material with pestle and mortar the chlorophylls in each sample were extracted with a mixture of acetone and ethyl alcohol (4:1) by repeatedly homogenizing in a blender. Supernatant solution containing chlorophylls was decanted and made upto a known volume with extracting solution. For using Zscheile & Comar's coefficients, this extract was transferred to ethyl ether. Absorbance of these solutions were recorded at 645, 652, 663, 665 and 630 nm wavelengths and concentration of cholrophyll a,b and total was determined by formulae of above mentioned workers. Correlation was worked out between absorbance at 645 nm and 663 nm and also between 645 nm and 652 nm. Using these correlations, simplified formulae are proposed following algebric substitution in the formulae of Arnon (1949). Arnon's formula for total chlorophyll determination gives slightly higher values than chlorophyll a+b (Yoshida *et al.*, 1976). Keeping the above fact in view the ratio of absorbance between 652 and 645 nm has been reduced from 1.69 to 1.50 at the time of its substitution in Arnon's formula to get the more accurate results for total chlorophyll. The values of chlorophylls obtained by the proposed formulae were compared with the values obtained by other formulae of Arnon (1949), Zscheile & Comar (1941), Duxbury & Yentsch (1956) and Plummer (1971).

Results & Discussion

It was observed that absorbance at 663 nm was 2.50 times that of 645 nm and absorbance at 652 nm was 1.69 times that of 645 nm. Using this information the formulae of Arnon (1949) are modified as under:

Chlorophyll a g/g fresh weight sample = OD at 645 X 0.02969
Chlorophyll b g/g fresh weight sample = OD at 645 X 0.01096
Total Chlorophyll g/g fresh weight sample = OD at 645 X 0.04347

This modification is based on the fact that every compound has got a specific absorption spectrum and absorption of any particular wavelength of this spectrum will be directly proportional to absorption at other wavelengths. Wavelength 645 nm is common in absorption spectrum of both chlorophyll a and b hence can be used for estimation of both. As evident from Table 1, the difference in values obtained by proposed formulae as well as by other formulae is statistically insignificant therefore, formulae suggested here

can be conveniently used for the determination of chlorophyll a,b and total chlorophyll in higher plants.

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