Original Research Paper

Volume - 13 | Issue - 02 | February - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Botany

SPECTROPHOTOMETRIC ANALYSIS OF CHLOROPHYLL AND CAROTENOID PIGMENTS IN NON- LEGUMINOUS CROPS IN EXISTING CLIMATIC SCENARIO

Dr ShivaliKharoliwal	Department of Botany University of Kota, Kota, (Rajasthan) India.
Dr.Ankita Choudhary*	Department of Botany University of Kota, Kota, (Rajasthan) India.*Corresponding Author
Dr. Poonam Sharma	Department of Botany University of Kota, Kota, (Rajasthan) India.
Dr.Mridula Khandelwal	Department of Botany University of Kota, Kota, (Rajasthan) India.

ABSTRACT Chlorophyll a and chlorophyll b and carotenoids are the main photosynthetic pigments present in plants and are good index of photosynthetic activity. Extraction of chlorophyll by acetone extraction method was carried out in non-leguminous plants by spectrophotometer. The aim of this study is to relate the amount of concentrations of chlorophyll and carotenoids between plants.

KEYWORDS : carotenoids, chlorophylls, spectrophotometric analysis, non- leguminous crops, extraction, Solvent

Introduction

All plants require steady state of nutrients to accomplish their physiological functions. A depletion in overall leaf chlorophyll content reduces the amount of solar radiation that can be absorbed which in turn reduces photosynthesis. The measurement of chlorophyll content can help in yielding important information regarding biotic stress factors operating in nature. By employing measurements of chlorophyll content we can gain valuable insights into plant yield and in turn can reduce fertilizer usage.

Methodology

Selection of Plants:

The non-legumes fodder crops selected for the experiments were vizBrassica hirta and RaphanussativusLinn. All the plants were cultivated in the field in Kharif season. For experimentation, the crops were harvested at pre-flowering stage and were brought to laboratory from the cultivation site.

Sample Collection:

For the experimentation the leaf samples were collected from the field in field in fresh and clean polythene bags and were brought to the laboratory for spectrophotometric analysis of pigments. While bringing the leaf samples to the laboratory, precautions were taken so as to avoid the mechanical or other damage. All the samples were washed under tap water to remove dust particles ,unwanted particles from the surface of leaves and were then analysed for the determination of Chlorophyll-a, Chlorophyll-b and total Chlorophyll .

Analytical Procedure:

The Quantitative estimation of chlorophyll-a, chlorophyll-b and total chlorophyll was carried out by the method of Arnon (1949). 1g fresh leaf material was taken and homogenized with 80% acetone and centrifuged at 5000 rpm for 5 min. Supernatant was adjusted to 100 ml in the volumetric flask. The absorbance (O.D.) of this extracted solution was measured at 430, 645 and 663λ. From these readings concentrations of chlorophyllsand carotenoids pigment were determined by using following formula/equation:

The absorbance (O.D.) of this extracted solution was measured at 430,645 and 663λ. From these readings concentrations of chlorophylls pigment were determined by using following formula:-

Solvent Formula / Equation
80% Acetone

$$\text{Chlorophyll -a mg/g tissue} = \frac{12.7\,(\text{O.D } 663\lambda.) - 2.69(\text{ O.D } 645\lambda.) \times V}{a \times 1000 \times W}$$

$$\text{Chlorophyll -b mg/g tissue} = \frac{22.9(\text{O.D } 645\lambda.) - 4.68\ (\text{O.D } 663\lambda.) \times V}{a \times 1000 \times W}$$

$$\text{Total chlorophyll mg/g tissue} = \frac{20.2\ (\text{O.D } 645\lambda.) + 8.02\ (\text{O.D } 663\lambda.)}{a \times 1000 \times W}$$

Where,
a = 1 (light pathlength)
V = Final volume of chlorophyll extract in 80% acetone=50ml
W = Fresh weight of tissue extracted in grams.

Table-1
The Spectrophotometric determination of absorbance of Chlorophyll at different wavelengthsin non-leguminous crops:-

Crop name	Mean Optical density at 645nm	Mean Optical density at 663nm	MeanOptical density at 430nm
Brassica campestris	0.912	1.192	1.462
Raphanussativus	0.619	1.432	0.00

Table-2
The Spectrophotometric determination of absorbance for Chlorophyll at different wavelengths of non leguminous crop plants:-

Crop name	Chl-a	Chl-b	Total chlorophyll	Ratio
Brassica campestris	1.268	1.5306	0.886	1.43
Raphanussativus	1.6521	0.7473	0.294	2.21

A=Absorbance, Ch-a=Chlorophyll-a, Ch-b=Chlorophyll-b, Totalchl.=Total Chlorophyll.

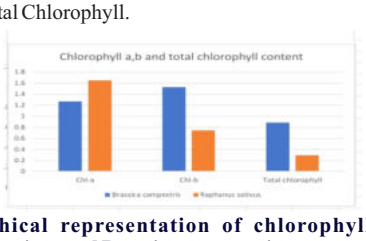

Fig 1Graphical representation of chlorophyll pigments inRaphanussativus and Brassica campestris

Result and Discussion:

Physiological state of plant is largely governed by the pigments present

in the leaf. Chlorophyll-b pigments acts by transferring the light it absorbs to chlorophyll-a(Bojovic B & Stojanov,2005).The content of foliar pigment varies depending on species(Ferus P &Kosovar M ,2001;Kambleet.al,2015;)). Variation in leaf pigments (chlorophyll and Carotenoid) and theirrelationship are effected by both internal factors (Kourilet.al, 1999Porra JR, 2002) and environmental conditions (Bondada BR &Syvertsen JP,2003;Shibghatallah et.al,2013Sardoconet.al,2014). The study has revealed that the Chlorophyll-a ranges from 1.268 to 1.65 mg/g and chlorophyll b ranges from 1.53 to 0.747mg/g(Table 1,fig1)and the total chlorophyll chl (a+b) ranges from 0.29 to 0.88 mg/g(Table 2)in Brassica campestris and Raphanus sativus respectively which are in concordance with earlier studies.

Conclusion

The quantitative analysis of photosynthetic pigment showed that chlorophyll a was high in Raphanuss sativus followed by Brassica campestris . Further, the chlorophyll content can be used as indicators of plant health stress and nutritional deficiencies and to study the effect of changing climatic conditions on chlorophyll content in plants .

Acknowledgements

We are thankful to the Department of Botany, University of Kota, Kota, and Rajasthan.

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