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Short communication

Growing conditions influence non-destructive estimation of chlorophyll in leaves of *Valeriana jatamansi*

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ABSTRACT

Leaf chlorophyll (Chl) content is an indicator of physiological conditions and nutritional status of a plant. However, the conventional methods for estimating the Chl content in leaves are analytical based destructive and time consuming, and these methods do not allow to study on the same sample over time. *Valeriana jatamansi*, a perennial medicinal herb, is now endangered and at the edge of becoming extinct in India. It is therefore a pressing need to conserve and maintain this species in their natural habitat. One experiment was conducted to ascertain whether the CCM-200, a hand-held Chl meter, could be effectively used to estimate Chl content of *V. Jatamansi* in a non-destructive manner. The regression equations between CCM-200 reading and actual chlorophyll values have been developed for three accessions of *V. jatamansi* grown under two different conditions. In the present data-sets, second-degree polynomial regression model has been proven to be the best model with higher R^2 ($R^2 = 0.852–0.964$, $P \leq 0.01$) and lower ACI values (94.47–171.78) for all the cases (except plastic film greenhouse grown accession-3). For accession-5 and accession-7, the model predicted values of total Chl were very close to conventionally measured values with an RMSE < 3.50 μg cm^{-2}. However, the calibrated models were circumstances-specific.

1. Introduction

Chlorophyll, the green pigment of plants, traps the light energy for the production of carbohydrate through the process of photosynthesis. However, the amount of solar radiation absorbed by a leaf depends upon the total photosynthetic pigment content (Riccardi et al., 2014). Chlorophyll also regulates many physiological functions of the plants. Leaf chlorophyll content is a key indicator of the physiological status (Steele et al., 2008), stress (Datt, 1999) and nutritional status (Filella et al., 1995; Wu et al., 2008) of a plant. It has also been reported that photosynthetic activity and primary production of plants are reduced at low concentration of chlorophyll in leaves (Filella et al., 1995; Richardson et al., 2002). Hence, the status of the chlorophyll content in leaves evaluates the overall productivity of a plant.

The chlorophyll concentration in leaves is generally determined through extraction of pigment in an organic solvent such as acetone (Arnon, 1949), methanol (Cenkey et al., 2010), dimethylsulfoxide (Netto et al., 2005) etc., followed by the spectrophotometric reading of the absorbance by the chlorophyll solution. Though laboratory based methods are accurate, they require destructive sampling, expensive equipment and chemicals, and are relatively time-consuming and laborious. Moreover, these methods do not allow to study the same leaf over time. On the other hand, it has been proven that the techniques for non-destructive estimation of chlorophyll based on absorbance and reflectance of light by the leaves are time-saving and simple for a number of species such as maize (*Zea mays* L.) and soybeans (*Glycine max* L. Merrill.) (Gitelson et al., 2005), birch (*Betula pendula* Roth.), wheat (*Triticum aestivum* L.) and potato (*Solanum tuberosum* L.) (Uddling et al., 2007), stevia (*Stevia rebaudiana* Bertoni) (Pal et al., 2013) and sugar maple (*Acer saccharum* Morsh.) (Van den Berg and Perkins, 2004).

Valeriana jatamansi, a perennial medicinal herb of the Valerianaceae family, is widely found in the temperate zone of the western Himalaya at an altitude of 1300–3300 m.a.s.l (Rather et al., 2012). In India the species is now endangered and at the edge of becoming extinct (Nayar and Sastry, 1998) due to over-exploitation from its natural habitat to meet the burgeoning demand. It is therefore a pressing need to conserve and maintain this species in their natural habitat. The non-destructive technique for estimation of chlorophyll content in the leaves will help to understand the physiological and nutritional status of this plant under natural habitat.

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However, to the best of our knowledge, no investigation has been carried out so far for estimation of chlorophyll in a non-destructive manner on *V. jatamansi* under different growing conditions.

The chlorophyll content meter CCM-200 (Opti Sciences, USA), among several chlorophyll content meters available in the market, is widely used to determine the chlorophyll content in leaves of different species in a non-destructive manner. This instrument provides a chlorophyll content index (CCI) value that indicates relative chlorophyll content instead of actual chlorophyll content. Thus, for direct estimation of chlorophyll content with the help of chlorophyll meter, it is necessary to formulate a suitable mathematical relationship between chlorophyll meter output and actual chlorophyll data. Hand-held chlorophyll meters measure the transmittance by leaves at two different wavelengths of light: 660 nm (red) and 940 nm (near-infrared). The red light is strongly absorbed by chlorophyll, whereas the near-infrared light is used as a reference wavelength to adjust the differences due to leaf structure (Markwell et al., 1995).

The mathematical relationships between CCI value and total chlorophyll content vary with species (Sandoval-Villa et al., 2002; Yamamoto et al., 2002; Uddling et al., 2007; Silla et al., 2010), growing conditions (Bullock and Anderson, 1998; Jifon et al., 2005), and specific leaf weight (Thompson et al., 1996). Thus, an independent mathematical model should be developed for each species for a particular zone. The objectives of this study were thus to evaluate (1) the feasibility of a non-destructive field method for estimation of chlorophyll content in different accessions of *V. jatamansi* leaves with the help of CCM-200; and (2) whether the relationship differs among the accessions within growing conditions, between plastic film greenhouse and open-field-grown plants.

2. Materials and methods

2.1. Plant material and location

Three accessions of *V. jatamansi* having contrasting leaf characteristics were used as a plant material in this study. All the three accessions of *V. jatamansi* were grown in both plastic film greenhouse and open field conditions. The experiment was conducted at the experimental farm of Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology (32°06′ 05″ N; 76°34′10″E; altitude 1395 a.m.s.l), Palampur, India, during 2014. In this study, 70 leaf samples appearing very pale to dark green in colour were collected from each accession and growing conditions. Out of 70 leaf samples, 50 were used to establish the mathematical relation or model between CCI and actual chlorophyll content. The remaining (20) samples were used to validate the models. The features of the leaves for all three accessions under both the conditions are presented in Table 1.

2.2. CCM reading and chlorophyll extraction

From each sample, five readings were recorded from different locations of leaves (except mid-vein) with a CCM-200 hand-held chlorophyll meter which calculates the non-dimensional chlorophyll content index (CCI). The readings were averaged to make a single representative CCI value for each leaf sample. Immediately after CCI readings, 30 leaf discs (0.158 cm^2 each) were cut with a standard leaf punch. Discs were removed from the mid lamina area of each leaf where CCI was measured. chlorophyll was extracted from all (30) leaf discs with 80% acetone. After recording the absorbance at 645 nm and 663 nm with a spectrophotometer (UV-2600 UV–vis Spectrophotometer, Shimadzu), the total chlorophyll was quantified with the classic equations of Arnon (1949).

$$\text{Chlorophyll}\,a = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times (\text{solution}/\text{leaftissue}) \times 1000$$

$$\text{Chlorophyll}\,b = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times (\text{solution}/\text{leaftissue}) \times 1000$$

$$\text{Totalchlorophyll} = \text{Chlorophyll}\,a + \text{Chlorophyll}\,b.$$

The chlorophyll content was expressed in μg cm^{-2} green leaf tissue, whereas the unit of solution and leaf tissue were in mL and cm^{-2}, respectively. A_{645} and A_{663} are the absorbance values at 645 and 663 nm, respectively.

2.3. Data analysis

To develop the best regression model for estimating the chlorophyll in a non-destructive manner, the data set of 70 samples of each accession was randomly separated into two parts. The first part having 50 samples was used to develop the different regression models (e.g. linear, power, exponential and polynomial) relating to CCI and actual chlorophyll content. The goodness-of-fit of the models was tested with the help of the root mean square error of the developed models (RMSE). The Akaike's information criteria (AIC) value, which is the indicator of the actual success of the any mathematical model, was also calculated for each accession and condition. The remaining samples (20) were used to calculate the root mean square error of validation (RMSR) for testing the efficiency of the developed model. Standard formulae were used for calculating RMSE and AIC

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{n}(Y_i - X_i)^2}$$

where, Y_i and X_i represent the model predicted and observed values, respectively.

$$\text{AIC} = n[\ln(SS/n)] + 2K$$

where, n is the number of observations, SS the sum of the square of the vertical distances of the points from the curve and K is the number of parameters in the regression equations. In this study,

Table 1
The characteristics of the leaves for all three accessions under both the conditions.

Accession	Growing conditions	Samples (N)	Average leaf length (cm)	Average leaf width (cm)	Moisture content (%) in leaf	Specific leaf weight (mg cm^{-2})
Accession- 3	Plastic film greenhouse	50	6.39	5.90	92.15	25.95
	Open field	50	3.88	3.24	88.90	33.38
Accession- 5	Plastic film greenhouse	50	7.19	5.95	90.63	24.40
	Open field	50	4.63	3.49	86.83	26.98
Accession- 7	Plastic film greenhouse	50	6.72	5.15	90.36	30.37
	Open field	50	4.80	3.42	86.94	30.81