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Research article

Estimation of the chlorophyll concentration in seven *Citrus* species of Kokrajhar district, BTAD, Assam, India

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Abstract: Green plants synthesize a wide range of primary and secondary metabolites in different quantity. Chlorophylls belong to the primary metabolites that give the color of the leaves and fruits, especially in the unripe stage. The spectral properties of chlorophylls are essential in harvesting light energy and in the transduction of absorbed light energy for photosynthesis. Like other plants, the variation of leaf colour, as well as photosynthetic activity in Citrus species, is dependent on chlorophyll concentration. Chlorophyll content determines the photosynthetic capacity of the plant per unit area of leaf, stress and nutritional deficiencies. The study of chlorophyll content in seven *Citrus* species growing in different localities of Kokrajhar district of Assam has shown some variability among them as an individual character.
Keywords: Citrus leaf - Pigment - Photosynthesis - Metabolite - Productivity.

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INTRODUCTION

Chlorophylls are the most important green pigments in plants for the photosynthetic process (Bhatia & Parashar 1997). Higher plants contain Chl *a*, Chl *b*, accessory pigments and several additional forms of chlorophyll. The Chl *a* and Chl *b* are the best known among five main types of chlorophyll and are most commonly found in all autotrophic organisms except pigment containing bacteria. Chl *a* has an empirical formula of $C_{55}H_{72}O_5N_4Mg$ and the empirical formula of Chl *b* is $C_{55}H_{70}O_6N_4Mg$. Chl *a* usually appears blue-green and Chl *b* is yellow-green (Devlin & Witham 1997). Both Chl *a* and Chl *b* pigments are associated with light harvesting processes (Ferus & Arkosiova 2001), which are solely responsible for photosynthesis in higher plants.

Chlorophyll concentration in leaves is an indicator of plant health (Porra 2002). The chlorophyll a:b ratio also indicates the developmental state of photosynthetic apparatus in plants. It has a determinative role in growth and development of higher plants. The chlorophyll content also indicates the photosynthetic capacity per unit area of the leaf (Kozlowski *et al.* 1991) that determines the rate of photosynthesis in the plant (Dickman & Kozlowski 1968). Determination of chlorophyll content as an indirect method of estimating the productivity also provides a good understanding of the photosynthetic regime of plants (Bojovic & Stojanovic 2005).The chlorophyll content increases with leaf development and then decreases with the senescence phenomenon (Pereyra *et al*. 2014). The rate of photosynthesis is also higher in flowering and fruiting branches of sub-tropical fruit species in comparison to non-fruiting branches (Avery 1977). However, the pigment is a factor that might also be responsible for the colour variation of leaf in different *Citrus* species. The present study was undertaken for estimation of chlorophyll content in the mature leaf of *Citrus* plant growing in Kokrajhar district, Assam.

MATERIALS AND METHODS

Seven *Citrus* species namely *C. limon* Burm f, *C. medica* Linn., *C. aurantifolia* Swin., *C. reticulata* Blanco., *C. nobilis* Lour., *C. jambhiri* Lush., and *C. grandis* (L.) Osbeck. as reported by Gogoi & Basumatary (2017) were selected for the present study. The matured leaves were collected from the selected *Citrus* spp. growing in different areas of Kokrajhar during the month of December 2017. One gram leaf from each species is measured

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and cut into fine pieces and then grinded with mortar and pestle. Thereafter, 20 ml of 80% acetone and 0.5 g of ($MgCo_3$) powder was added and further grinded gently following the method of Kamble *et al*. (2015). The mixture was then incubated at 4ºC for 3 hours. The mixture was centrifuged at 2500 rpm for 5 min and the supernatant was transferred to a 100 ml volumetric flask and the volume was made up to 100ml with the addition of 80% acetone and the solution was used for chlorophyll estimation (Fig. 1). The absorbance of the

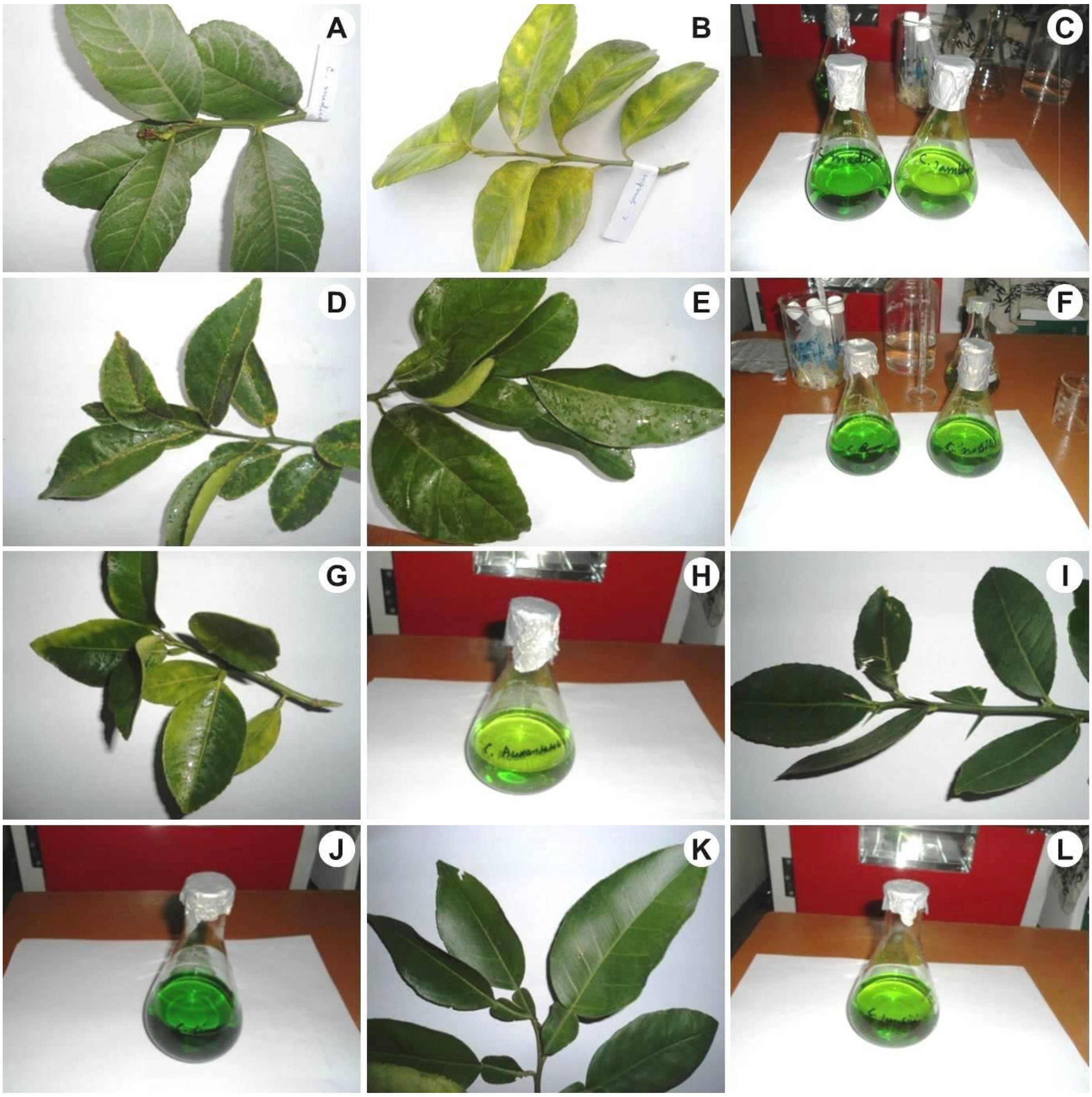

Figure 1. Leaves and their chlorophyll extraction in acetone solution: **A,** *Citrus medica*; **B,** *Citrus jhambiri*; **C,** Chlorophyll extract of *C. medica* & *C. jhambiri*; **D,** *Citrus nobilis*; **E,** *Citrus reticulata*; **F,** Chlorophyll extract of *C. nobilis* & *C. reticulata*; **G,** *Citrus aurantafolia*; **H,** Chlorophyll extract of *C. aurantafolia*; **I,** *Citrus limon*; **J,** Chlolorophyll extract of *C. limon*; **K,** *Citrus grandis*; **L,** Chlorophyll extract of *C. grandis*.

solutions was measured at 645 nm and 663 nm in LABTRONIC Spectrophotometer LT-39 taking the 80% acetone solution as blank (Sadasivam & Manickam1996). The reading was taken in a triplicate sample and average was considered for calculation of chlorophyll content. The chlorophyll *a*, *b* and *a* + *b* (total chlorophyll) contents were calculated out by applying the following (Arnon 1949) formulae:-

$$\text{mg chlorophyll } a/\text{g tissue} = \frac{12.7(\text{A}663) - 2.69(\text{A}645) \text{ x V}}{1000 \text{ x W}}$$

$$\text{mg chlorophyll } b/\text{g tissue} = \frac{22.9(\text{A}645) - 4.68(\text{A}663) \text{ x V}}{1000 \text{ x W}}$$

$$\text{mg total chlorophyll/g tissue} = \frac{20.2(A645) + 8.02(A663) \times V}{1000 \times W}$$

Where, A = absorbance at specific wavelength
V = final volume of chlorophyll extract in 80% acetone
W = fresh weight of tissue extracted

RESULTS AND DISCUSSION

Table 1. Chlorophyll concentration in seven *Citrus* spp. found in Kokrajhar district of Assam.

S.No.	Name of Species	O.D. Readings		Chl *a* (mg g^{-1})	Chl *b* (mg g^{-1})	Total Chl (mg g^{-1})
		645 nm	663 nm			
1	*Citrus limon* Burm f.	0.554±0.137	1.277±0.002	1.47	0.61	2.08
2	*Citrus medica* Linn.	0.404±0.003	0.904±0.002	1.03	0.50	1.53
3	*Citrus aurantifolia* Swin.	0.231±0.014	0.326±0.032	0.35	0.38	0.73
4	*Citrus reticulata* Blanco.	0.397±0.001	0.765±0.002	0.87	0.55	1.42
5	*Citrus nobilis* Lour.	0.354±0.001	0.699±0.002	0.79	0.48	1.27
6	*Citrus jhambiri* Lush.	0.269±0.004	0.456±0.004	0.51	0.41	0.92
7	*Citrus grandis* (L.) Osbeck.	0.339±0.010	0.583±0.010	0.65	0.49	1.14

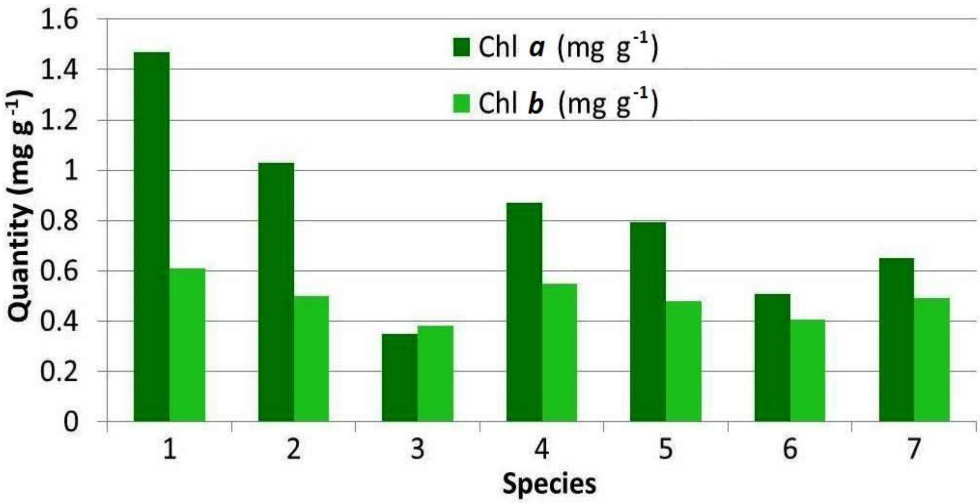

Figure 2. The variation of chlorophyll *a* and *b* content among seven *Citrus* species.

The study has revealed that the Chl *a* ranges from 0.35 to 1.47 mg g^{-1} and Chl *b* ranges from 0.38 to 0.61 mg g^{-1} and the total chlorophyll Chl (*a*+*b*) ranges from 0.73 to 2.08 mg g^{-1} in seven *Citrus* spp. From the result it is also seen that *C. limon* Burm f. has the highest concentration of Chl *a* and Chl *b* whereas *C. aurantifolia* (Christm.) Swin. was found to have least quantity of Chl *a* and Chl *b* among the seven species (Table 1 & Fig. 2). Among the seven citrus species the highest total chlorophyll content was found in *C. limon* (2.08 mg g^{-1}) followed by *C. medica* (1.53 mg g^{-1}), *C. reticulata* (1.42 mg g^{-1}), *C. nobilis* (1.27 mg g^{-1}), *C. grandis* (1.14 mg g^{-1}), *C. jambhiri* (0.92 mg g^{-1}) and *C. aurantifolia* (0.73 mg g^{-1}) respectively (Table 1 & Fig. 3). Similarly, Chl *a* was also found to be highest in *C. limon* (1.47 mg g^{-1}) followed by *C. medica* (1.03 mg g^{-1}), *C. reticulata* (0.87 mg g^{-1}), *C. nobilis* (0.79 mg g^{-1}), *C. grandis* (0.65 mg g^{-1}), *C. jambhiri* (0.51 mg g^{-1}) and *C. aurantifolia* (0.35 mg g^{-1}) respectively. The second highest in quantity of Chl *b* was found in *C. reticulata* (0.55 mg g^{-1}) followed by *C. medica* (0.50 mg g^{-1}), *C. grandis* (0.49 mg g^{-1}), *C. nobilis* (0.48 mg g^{-1}), *C. jambhiri* (0.41 mg g^{-1}), and *C. aurantifolia* (0.38 mg g^{-1}) respectively. The highest variation of chlorophyll *a* and *b* were observed in *C. limon*. The quantitative variation of chlorophyll content in *Citrus* spp. may be due to the health condition of the plants, habitat condition, leaf surface area and nutrients of the soil. Earlier Bojovic & Stojanovic (2005) reported that chlorophyll and carotenoid content in wheat cultivars depends on the presence of mineral elements in the substrate and plant physiological and environmental factors. Moreover, the application of plant growth regulators in higher concentrations has also positive effects on leaf chlorophyll content (Sardoei 2014).

According to Pandey & Sinha (1998) Chl *a* and Chl *b* occur together in the higher plants in the ratio of 2:1. The typical Chl *a/b* for shade plants is about 1.6–2.2 and daily maximum sunlight exposed plants have the typical Chl *a*/*b* content approximately 2.6–3.4 (Anderson 1986). In *Citrus* species the Chl *a*/*b* was observed in between 0.73–2.08 which is much lower than Anderson's view. The reason of dissimilarities of Chl *a*/*b* with other plants may be due to the presence of inactive light harvesting complexes in *Citrus* resulting effect in growth and development. Several literature and pieces of evidence suggest that the Chl *a*:*b* ratio plays an important role to higher plants to adapt to new light regions to make optimal use of ambient light intensities and quantities (Arnon 1949). The ratio of Chl *a* and *b* is stable in fully green leaves at nearly 3, but it can vary depending on the physiological status of the plant (Kouril *et al*. 1999). An average Chl *a*:*b* ratio of 0.81:0.49 was observed in *Citrus* plants. Bondada & Syvertsen (2003) reported that the nitrogen-deficient leaves of *Citrus reticulata* Blanco contain less chlorophyll per unit area, but a greater chlorophyll *a*:*b* ratio than N-fertilized leaves. The Chl *a*/*b* in maximum sunlight exposure and shade plants reflect the adaptation of the chloroplast to prevailing intensity through regulation of the amount of photosystem I (PS I) relative to photosystem II (PS II)

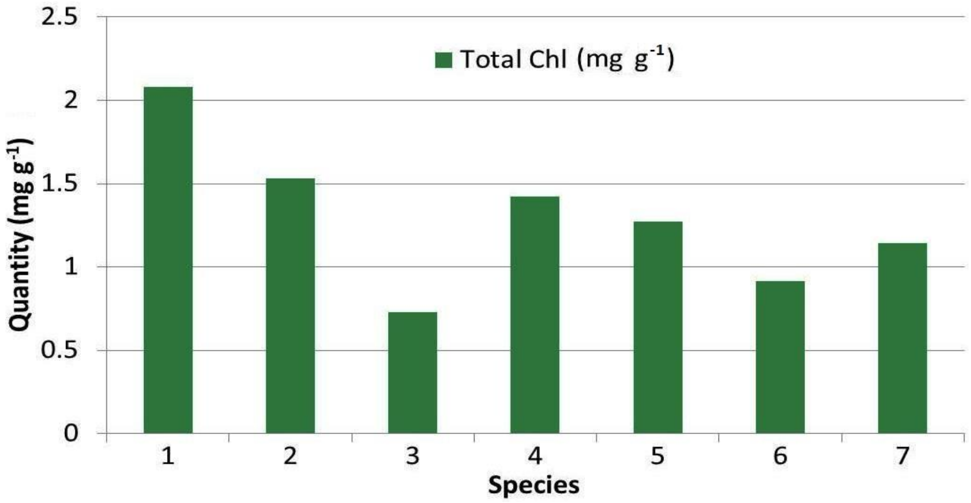

Figure 3. Total chlorophyll content in seven different *Citrus* species.

and the size and composition of the light harvesting complexes (LHCs) of each photosystem. Moreover, the leaf colors of a plant can be used to identify stress level due to its adaptation to environmental change (Shibghatallah 2013). Therefore, from the observation it can be said that the *C. limon* and *C. medica* were well adapted under local environmental condition. Kamble *et al*. (2015) opined that the leaf chlorophyll concentration plays a vital role in maintenance of photosynthetic mechanism as well as plant metabolism. Apart from these the seasonal variation and maturity of the leaf also affect the concentration of chlorophyll content in leaf and chlorophyll *a*:*b* ratio remains substantially lower in plants growing in high CO_2 condition (Cave *et al*. 1981). Nevertheless, in the present investigation, a variability of chlorophyll content in the *Citrus* species observed but it has a scope to consider as tools for identification of *Citrus* species and varieties. From the (Table 1 & Fig. 2 & 3), an inference can be made that the Chl *a*, Chl *b* and total chlorophyll content in mg/g leaf tissue of different *Citrus* species are an individual character of each *Citrus* species studied. Remarkable variation of green colour of the leaf was observed as the same is dependent on the variability of chlorophyll content in the species. The alteration of Chlorophyll content may due to the factors like water, soil, temperature stress etc. which indirectly affect the leaf area, morphology, thickness and chloroplast distribution.

CONCLUSION

The study provides a reliable data on chlorophyll content of seven citrus species growing in different localities of Kokrajhar district. The quantitative analysis of photosynthetic pigment showed that the photosynthetic potential and primary productivity is highest in *Citrus limon* followed by *C. medica* among the seven *Citrus* spp. Further, the chlorophyll content can be used as indicators of plant health stress and nutritional deficiencies. Our findings may be helpful in the further studies to monitor the effect of changing micro-climate on chlorophyll content in citrus plants in terms of temperature, water, carbon dioxide concentration and soil condition of Kokrajhar district of Assam.

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