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Changing pattern of chlorophyll content and carotenoid in different flushes of five litchi varieties

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Abstract
Litchi is one of the fascinating fruit crops due to its high demand and export potentiality. Enhancing its productivity is one of the great achievements. Increasing photosynthetic activity through exploiting photosynthetic components are major target. The carotenoid and chlorophyll content are considered to be one of the major indicative of the physiology and phenology of plants. Therefore the present work has been shaped to analysed photosynthetic activity at chlorophyll level at initial stage of flush emergence. Five varieties like Purbi, Bedana, Shahi, China and Dehrarose have been taken as study materials for quantification of chlorophyll content in leaves of three different flush. Chlorophyll is directly related with photosynthetic efficiency of plants and it makes an indirect estimation of possible nutrient status in crop plants. Data were analysed and result revealed variation in chlorophyll content and carotenoids among the varieties and also in different flushes. Bedana showed highest in chlorophyll'a' (1.01 mg g^{-1} fr wt), b (0.59 mg g^{-1} fr wt) and total chlorophyll (1.8 mg g^{-1} fr wt) as compared to rest of the variety studied whereby China showed lowest. Second, highest chlorophyll 'a'content was noticed in Dehrarose, Chlorophyll 'b' was highest in Bedana. In early variety like Shahi the chlorophyll content showed maximum in the first flush than from the second and third flush. However, in the mid variety like purbi and Bedana chlorophyll content showed maximum in third and first flush as compared to all three flush as well as in late variety like China and Dehrarose chlorophyll content showed maximum in second flush as compared to first and third flush. Carotenoid content was also measured maximum in Bedana (0.12 mg g^{-1} fr wt) followed by Shahi (0.11 mg g^{-1} fr wt), Dehrarose (0.087 mg g^{-1} fr wt), Purbi (0.079 mg g^{-1} fr wt) and China (0.056mg g^{-1} fr wt).

Keywords: chlorophyll, carotenoid, litchi varieties

Introduction
The litchi (*Litchi chinensis* Sonn.) is one of the important subtropical evergreen fruit trees and native of South China grown for its excellent fruit quality, characteristics pleasant flavour and attractive colour. Litchi belongs to Sapindaceae or soapberry family which contains 125genera and 1000 species [1]. India is the second largest litchi producing country next only to China and has a good export potential. Litchi being specific in climatic requirement, it has been confined to a few states and amongst Bihar state recorded with 74 percent of litchi production. Litchi farming has become the source of livelihood for millions of people as it provides both on-farm and off-farm employment [2]. Thus, litchi cultivation and enhancing its production and productivity is of great concerned especially in the state like Bihar.

Litchi produces leaf flushes twice or thrice commonly in a year in a fruiting tree after harvest till panicle emergence viz. early (after harvest), mid (August to October) and late (after November) [3]. Determination of flushing time and management of flush cycle, has been reported as an important trait for productivity of litchi [4]. Sometimes, poor fruit set in litchi has also been generalized by a failure of pollination and fertilization. The reasons may be many but trees fail to flower some season or fail to fruit set and carry a reasonable crop depending on the environmental factors [5]. The lack of flowering has been discussed in several reports due to unfavourable weather or timing of shoot during the cooler months of the year [6]. Olesen *et al.*,(2002) [7] observed that the importance of flushing management in terms of bearing shoot for flowering and fruiting in litchi and Chang (2008) [8] suggested that poor fruit retention would result from insufficient flushes on the bearing shoot, whereas poor flowering could occur when the bearing shoot produce immature flush and leaves.

However, the duration and interval of successive flushes in litchi seems to be strongly dependent on the vigour of the tree [9], irrigation, radiation and temperature [10]. The photosynthetic rate also plays a key role for the energy availability in the plant, which is again control directly or indirectly by chlorophyll contents and its stability [11]. Chlorophyll contents and its contribution towards photosynthetic activities have been reported in other fruits like apple [12].

The Carotenoid occurs in photosynthetic tissue along with chlorophyll to protect them from photo oxidative damage. Carotenoids to protect their stem and leaves from the energy of sun. However, lesser information is available on flushing pattern and panicle emergence in litchi plants under sub-tropical conditions. Keeping this in view, the present work was undertaken to investigate changing patterns of chlorophyll contents and carotenoids in respective flush of litchi trees. The approaches developed in this study could be used to investigate the influence of chlorophyll and carotenoids in different flush concerning responses to flowering and fruit set in litchi.

Materials and Methods

Plant materials and experimental design

The experiments were carried out at Bihar Agricultural University, Sabour during 2017-18. seventeen years old uniform sized trees of litchi cultivar Purbi, Bedana, China, Shahi and Dehrarose space at eight metre. The experiment was laid out in Randomised block design with four replications. Regular monitoring was done to study the time of flush emergence and flowering. Twenty five flush was tagged in each treatment at all stage of flush emergence and observation on flushing pattern, panicle emergence and flowering pattern were recorded.

Morphological traits measurement

Changing pattern in plant morphological traits was also studied. For the morphological study, parameters such as flush length (cm), flush diameter (cm), number of leaves, number of leaflet per leaves were measured.

Chlorophyll estimation

Chlorophyll contents a, b and total chlorophyll was estimated using acetone method with little modification as given by Arnon (1949) [13]. Leaf samples were collected at initial stage of flush emergence. Fully expanded leaf was used as materials for extraction and estimation of chlorophyll. 0.2 gram of freshly collected leaf material (devoid of mid- rib) were homogenized in 8 ml 80% acetone using mortar and pestle. The homogenate was then centrifuge at 4°c for 15 min at 15000rpm. The supernatant collected carefully read the absorbance at 663 and 645 nm. Chlorophyll ‘a’ and ‘b’ and total chlorophyll are determined by using the formula given below:

Chlorophyll ‘a’ = $[(12.7*A_{663}) – (2.69*A_{645})]*V/1000*W$
Chlorophyll ‘b’ = $[(22.9*A_{645}) – (4.68*A_{663})]*V/1000*W$
Total Chlorophyll = $[(8.02*A_{663}) + (20.2*A_{645})*V/1000*W$

Carotenoids estimation

Estimation of carotenoids was performed by the method of Hendry and Price (1993) [14] with little modification. Leaf sample of 0.2 g was homogenized in 80% acetone. As mentioned in the chlorophyll estimation process, carotenoids were extracted and after centrifugation supernatant was used for spectrophotometric reading. An absorbance was recorded at three different wavelengths such as 663nm, 645 nm and 480 nm. Carotenoids content was calculated using the formula:

[A480 + (0.114*A663) – (0.638 – A645)]*V/1000*W

Here, A = Absorption, V = Total volume, W = weight of sample (gram)

Concentration of chlorophyll and carotenoids are expressed in mg g^{-1} fr wt.

Results and Discussion

Generally, three flushes are produced in litchi between fruit harvest to panicle emergence [15]. Time of emergence of different flushes has a profound influence on the floriferousness of the shoot. It was clearly shown by the previous report that the flush maturity earliest (before the winter period) produce floral shoots, while flushes maturing quite late produce vegetative shoots [16]. Flush emergences time and duration of flushing and its influence towards the fruit is variety specific. Therefore, to investigate the role of flushing in the litchi varieties and patterns in chlorophyll contents and also in carotenoids, experiment has been designed.

Morphological patterns of flush in five litchi varieties

The mid season flush mainly appearing in the month of Sep-Oct is considered to be of more significance in litchi [16]. The date of flush emergence ranged from 28/06/17 to 06/02/18 in five varieties. Dehrarose was earliest to initiate first flush emergence followed by Shahi. Variation in the date of emergence of flush, panicle and developmental stages have direct contribution in fruit setting as they are controlled by progression in seasonal temperature [17]. Also, the litchi varieties can be distinguished depending upon the colour of new flush and season of flushing [2].

Table 1: Morphological traits of flush: number of flush, date of flush emergence, flush length (cm), flush diameter (cm), number of leaves per flush, number of leaflet per leaves) in five litchi varieties.

Varieties	Flush					No. of leaves/ flush	No. of leaflet /leaves
		Date of emergence	**Colour**	**Length (cm)**	**Diameter (cm)**		
Shahi	1st	12-07-17	Light Green	36.4	3.1	4-6	5-7
	2nd	22-09-17					
	3rd	02-02-18					
Purbi	1st	22-07-17	Light Reddish brown	39.6	3.4	4-6	5-7
	2nd	04-10-17					
	3rd	07-12-17					
Bedana	1st	12-08-17	Dark Reddish brown	38.7	2.2	3-5	4-6
	2nd	29-10-17					
	3rd	20-01-18					
China	1st	19-08-17	Pinkish green	32.8	3.0	3-5	5-7
	2nd	16-10-17					
	3rd	29-01-18					
Dehrarose	1st	28-06-17	Reddish green	37.2	3.8	5-7	4-6
	2nd	18-09-17					
	3rd	06-02-18					

In reference to this report, the result showed that variation in leaf colors amongst the five varieties. Shahi showed light green, Purbi- light reddish brown, Bedana- Dark reddish brown, China- pinkish green and Dehrarose- reddish green respectively (Table 1). Moreover, differences in flush development has been discussed in the same table, where litchi Purbi is the longest of flush with 39.6 cm followed by Bedana litchi with 38.7 cm. Number of leaves per flush was also noted for the all five varieties, in which late variety litchi Dehrarose has been noticed with maximum number of leaves i.e.5-7 number and widest flush diameter of 3.8 cm but variety china bears only 3-5 number of leaves in a flush. The wide diversity of leaf flushing pattern reflect the fact of possible variability in flowering, fruiting and survival strategies evolved in litchi tree species[18]. Singh and Kushwaha (2006)[18], also reported the importance and contributions of leaf flushing towards litchi trees adaption under a strong seasonal subtropical climate.

Table 2: Chlorophyll contents (a, b, total) and carotenoids in three different flush of five litchi varieties. Concentration of chlorophyll and carotenoids are expressed in mg g^{-1} fr wt. The values are mean of four eplications.

Plant variety / Flush		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
Shahi	Flush1	0.729	0.525	1.538	0.107
	Flush2	0.629	0.269	0.952	0.052
	Flush3	0.524	0.239	0.445	0.028
Purvi	Flush1	0.360	0.346	0.706	0.033
	Flush2	0.671	0.331	1.001	0.057
	Flush3	0.749	0.361	1.169	0.079
Bedana	Flush1	1.014	0.591	1.719	0.116
	Flush2	0.752	0.531	1.052	0.077
	Flush3	0.399	0.326	0.860	0.022
China	Flush1	0.491	0.278	0.769	0.044
	Flush2	0.517	0.373	0.889	0.056
	Flush3	0.352	0.201	0.433	0.046
Dehrarose	Flush1	0.714	0.385	1.098	0.087
	Flush2	0.937	0.279	1.346	0.068
	Flush3	0.482	0.247	0.728	0.042

Changing patterns of Chlorophyll contents (a, b, total) in five litchi varieties

Variation in the contents of chlorophyll was noticed amongst the varieties studied and also in flushes. Data depicted in Table 2 revealed highest chlorophyll contents in Bedana, chlorophyll a (1.01 mg g^{-1} fr wt), b (0.59 mg g^{-1} fr wt) and total chlorophyll (1.8 mg g^{-1} fr wt) followed by Dehrarose as compared to rest of the varieties studied. Litchi variety, China showed lowest in chlorophyll contents with chlorophyll a (0.350 mg g^{-1}), chlorophyll b (0.201 mg g^{-1}), and total chlorophyll (0.433 mg g^{-1}). This decreased in chlorophyll concentration in china variety may be due to its bushy appearance that shaded on the preceding leaves. Lowest concentration in china is in evidence of the lower photosynthetic rate in china litchi variety as reported by Hieke *et al*.[19]. This alteration in chlorophyll might be for protection and enhancement of photosynthetic activity in the plant. The results also suggest that variation of chlorophyll content is seemingly to be more variety specific and flushing pattern. In early variety litchi, Shahi showed maximum chlorophyll contents in the first flush than from the second and third flush. However, in the mid variety, litchi Purbi and Bedana chlorophyll content was maximum in third flush and in late variety like China and Dehrarose chlorophyll contents were noticed maximum in second flush as compared to first and third flush (Table 2). These changes in chlorophyll concentration reflected changes in the colour of leaves which were red green, pinkish, light green etc. Increase in total chlorophyll concentration at 8.5 fold during development from young red leaves to mature dark green leaves in litchi was reported earlier by Heike *et al*. [19, 20]. Alteration in chlorophyll ratio to use the light efficiently has been discuss as plant leaf adaptation when exposed to high light intensity in fruits *Chamaecyparis obtusa* forests[21] *Satsuma mandarin* [22].

Changing patterns of carotenoids in five litchi varieties

Carotenoids are generally studied for its light harvesting ability and photoprotection activity. Under adverse stress conditions their roles become more pronounce[23]. Carotenoids contents in plants also provide complementary information on plant canopy physiological status[24-25]. Data presented in table 2 revealed a trend of carotenoids content with that of chlorophyll content in all the studied five litchi varieties. Maximum carotenoids were measured in mid variety Bedana (0.12 mg g^{-1} fr wt) followed by an early variety Shahi (0.11 mg g^{-1} fr wt), and then late variety Dehrarose (0.087 mg g^{-1} fr wt), Purbi (0.079 mg g^{-1} fr wt) and China (0.056mg g^{-1} fr wt) measured the lowest carotenoids concentration amongst five varieties of litchi taken in the present study (Table 2). As far as flushing has concerned, in early litchi Shahi variety, the carotenoid content showed maximum in the first flush than from the second and third flush. However, in the Purbii and Bedana, carotenoid content showed maximum in third and first flush respectively. China and Dehrarose litchi variety showed maximum carotenoid content in second and first flush respectively. The result also revealed an increasing concentration of carotenoids with increasing chlorophyll content. This may be due to the shielding activity of carotenoids towards chlorophyll oxidation under high light intensity [26].

Conflict of interest

None

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