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PHYSIO-BIOCHEMICAL STATUS OF SHOOTS RELATED TO LITCHI FLOWERING

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ABSTRACT

Litchi produces leaf flushes, flowers and fruit on terminals of new growth. The reproductive phase is associated with the ability to alternate between the production of vegetative and reproductive buds. The stress factors like water stress and cold winter are the triggering factors to induce dormancy which promotes flowering. The flushes maturing earliest (before the winter period) produce floral shoots, while flushes maturing quite late (flushing just prior to floral induction) produce vegetative shoots. Significant reduction in the winter flushing (November and December) was recorded in paclobutrazol and KNO_3 treated trees as compared to the control. 'China' litchi has lowered photosynthetic rate (*A*), transpiration rate (*E)*, internal CO_2 concentration (*Ci*), stomatal conductance (*gs*) and Leaf temperature (*Tl*) than 'Shahi' litchi. Floral shoots had more *A*, *E* but less *Ci*, *gs* over non-floral shoots. Leaves of floral and non-floral branches had reflected significant variation in bio-chemical status. *Chlorophyll a, b* and *total chlorophyll* contents were higher during flowering stage over floral bud differentiation stage irrespective of treatments. '*China*' litchi had lesser content of total carbohydrate (total CHO) and reducing sugar (RS) content than '*Shahi*' litchi. The total CHO and RS content increased from FBD stage to flowering stage but in contrast the proline and phenol content reduced during the same period. Although low temperature appears a pre-requisite for floral induction in lychee but there is strong relationship between flowering and flush emergence just before FBD. Harvesting of fruits with few leaves, post harvest cincturing (Girdling), use of paclobutrazol (PBZ) or KNO_3 during September-October may be used effectively in the sub-tropics to inhibit winter flushing and induce synchronous flowering.

KEY WORDS: Flushing, Floral, Maturity, Photosynthetic rate, Physiology, Shoots

INTRODUCTION

In India, erratic flowering, intensive vegetative growth and irregular bearing are typical of most litchi orchards specially variety of 'China' litchi. If the bud length exceeded a certain extent (2-3 mm), they could further develop only as vegetative shoots. Litchi produces leaf flushes, flowers and fruit on terminals of new growth. The reproductive phase is associated with the ability to alternate between the production of vegetative and reproductive buds. The stress factors like water stress and cold winter are the triggering factors to induce dormancy which promotes flowering. The vegetative flushing just prior to floral induction results in poor or no initiation and often vegetative shoots [10]. The new flushes emerge 3-4 time after harvest *viz.* early (after harvest), mid (August to October) and late (after November) season; the early and mid-season flushing influenced the yield, whereas the late season flushing do not have any contribution towards yield[11]. Shukla and Bajpai[14] observed that vegetative flushing in the last week of November hardly produced any panicle in March apparently due to immaturity of these shoots to differentiate flower buds in the month of December and January. The photosynthetic rate of the leaves is an important measure for the energy which is available to the plant in all growth stages such as leaf flushing, flowering and fruit development. It influences the assimilate transport in the plant and consequently the yield formation[6]. Photosynthetic rate and stomatal conductance are higher in non- flowering branches as compared to the flowering ones, in both cultivars of mango (Totapari and Langra) [13]. Gibberellin-inhibiting tree-growth regulators such as paclobutrazol (PBZ) have been shown to reduce shoot elongation, leaf expansion, and stem diameter growth of many tree species[17], by improving water use efficiency and consequently increasing drought tolerance and the quality of produced fruits by a change in the distribution pattern of photosynthetic products, thereby, diverting them towards reproductive growth and the formation of flower buds, fruit set and fruit growth. The paclobutrazol (PBZ) induces flowering in China because after 2 months of treatment, paclobutrazol treatment records higher amount of total non-structural carbohydrates (TNC), reducing sugars (RS) in the branches and leaves whereas the total nitrogen content (TN) decreases, thus resulting in a higher ratio of TNC/TN in the treated trees than in the control[1]. KNO_3 can enhance flowering especially in tropical regions where cold temperature for floral induction may not be sufficient.

However, information on the relationship of leaf gas exchange parameters, shoot biochemical statuses with growth, flowering and fruit development in litchi are scarce. Hence, in the present investigation, we have made an attempt to study the variations of leaf gas exchange parameters and shoot physiology in flowering and non-flowering shoots of regular (*cv*. Shahi) and irregular (*cv*. China) bearing litchi cultivars. The present study has also been designed to investigate the effect of type of shoots, flushing time, PBZ and potassium nitrate on other physiological and biochemical characteristics of litchi cultivars. The results reported here are useful for documenting the sink/source relationship during flushing/ or flowering and for developing strategies on canopy management in terms of enhancing fruit production in litchi.

MATERIAL & METHODS

Twelve year old uniform sized trees of litchi cv. China and Shahi spaced at 8 x 8 m were selected for this experiment. The experiment was laid out in Randomized Block Design with three replications, and single tree was treated as a unit/treatment. Studied factors were 4 levels of PBZ at 0, 1.0, 2.0, 3.0 and 4.0 g a.i. per meter canopy diameter as soil drench underneath tree canopy during October, 2014 and two levels of potassium nitrate (100 and 200 mg/L) which were sprayed in the morning hours with a rocker sprayer (after adding Tween-20 as a surfactant) at fifteen-day intervals from 15th September to 15 October. To monitor the time of flush emergence and flowering, 10 shoots in each direction were tagged *(i.e*. 40 shoots per tree) and percentage of flowering was calculated by dividing no. of flowered shoot over numbers of shoots tagged after harvesting. All the observations were recorded on 40 flowering and non-flowering shoots in each cultivar. These floral and non floral shoots were tagged during flowering/fruiting (March-April) and same shoots were undertaken for measurements of other parameters during vegetative phase (July-August).

A portable gas exchange system (CIRAS-2; PP Systems, Made in USA) was used with a PLC 6(U) universal leaf auto cuvette in closed-circuit mode for measurement of photosynthetic rate (*A*), transpiration rate (E*)*, internal CO_2 concentration (*Ci*), stomatal conductance (*gs*) and Leaf temperature (*Tl*). Photosynthetic pigments like c*hlorophyll a*, *b*, *total chlorophyll* were extracted by the non-maceration method using DMSO[8]. The total carbohydrate (CHO) contents of leaves were estimated by the anthrone method [15]. Reducing sugar (RS) contents were estimated in leaves following the method outlined by Nelson and Somogyi in Thimmaiah[15]. A rapid colorimetric method [2] was employed to estimate proline contents. The total phenolic content of was measured using Folin- Ciocalteu's method with some modifications[4].

Differences between treatments were determined by Analysis of Variance (ANOVA) using SAS General Linear Model procedure[12]. Treatment means were compared using least significant differences (LSD) at the 5% level of significance.

RESULTS & DISCUSSION

Gaseous Exchange Parameters

Net photosynthetic rates (*A*) were significantly lower in flowering branches as compared to the non-flowering ones in both the cultivars. China litchi has lower photosynthetic rate (*A*), transpiration rate (*E)*, internal CO_2 concentration (*Ci*), stomatal conductance (*gs*) and Leaf temperature (*Tl*) than Shahi litchi. Results of present study (table 1) indicated that during vegetative phase, 'China' litchi itself and floral shoots of both the cultivars, had higher net photosynthetic rate (*A*) than floral phase.

TABLE 1: Gas exchange parameters in flowering and non-flowering shoots of litchi cultivars Shahi and China

Cultivars	Shoots	Photosynthetic rate (*A*) (m mol CO_2) $m^{-2} s^{-1}$)		Transpiration rate (*E*) (m mol $H_2O \cdot m^{-2}s^{-1}$)		Internal CO_2 Concentration (*Ci*) (m mol CO_2 mol^{-1} air)		Stomatal Conductance (*gs*) (m mol(H_2O) $m^{-2}s^{-1}$)		Leaf temperature (*Tl*) (^{0}C)	
		*VP	**FP	*VP	**FP	*VP	**FP	*VP	**FP	*VP	**FP
China	Floral	12.90	2.24	7.90	0.94	338.00	310.00	48.96	34.66	32.90	35.00
	Non Floral	9.70	2.36	8.90	1.35	357.66	199.00	51.90	27.13	33.36	33.95
	CD (p= 0.05)	NS	0.08	0.84	0.09	4.54	7.30	2.11	3.67	1.20	1.79
Shahi	Floral	8.36	2.85	6.83	1.35	325.00	235.00	41.33	29.06	35.80	35.15
	Non Floral	7.60	2.29	5.60	1.04	319.66	238.00	42.83	29.23	34.56	34.75
	CD (p= 0.05)	0.11	0.09	0.79	0.08	6.67	2.60	0.56	0.06	0.78	0.61

**VP: Vegetative phase; **FP: Floral Phase*

Irrespective of type of shoots, *A* and transpiration rate (*E*) drastically reduced during flowering phase, and China litchi affected the most. Shivashankara and Mathai[13], also found photosynthetic rate and stomatal conductance higher in non-flowering branches as compared to the flowering ones in regular as well as irregular cultivars of mango. During vegetative phase (means during flushing), both type of shoots (floral and non floral), recorded higher *A*, *E*, internal CO_2 concentration (*Ci*) and stomatal conductance (*gs*) than at flowering and fruiting, may be attributed to plants starts recovery of exhaustion due to heavy crop load in preceding season. The reduction in photosynthetic rate could be mainly due to reduction in carboxylation efficiency owing to the presence of inhibitors in the leaves of flowering branches[13]. During flowering, all those attributes show reducing trend due to flowers and fruits were acting as sink and high intense sunlight during flowering and fruiting might be inhibitory to photosynthetic pigments. Leaf temperature (*Tl*) was recorded high during floral phase (April-May) (table 1) due to existence of summer season in northern India with high light intensity. Although, *Ci* is very less during flowering in both the cultivars, but utilizing CO_2 for *A* reduced due to photo-oxidation of chlophyll as well as high leaf temperature during FP.

Leaf Biochemical Status

Leaves of floral and non-floral branches had reflected significant variation in bio-chemical status (table 2). '*China*' litchi had lesser content of total carbohydrate (total CHO) and reducing sugar (RS) content than '*Shahi*' litchi. The total CHO and RS content increased from FBD stage to flowering stage but in contrast the proline and phenol content reduced during the same period in both cultivars; however floral shoots had less content than non-floral shoots. C: N is considered as an important factor in the regulation of flowering in fruit crops. A high ratio has been postulated promotory to flowering whereas opposite beneficial for vegetative growth[3]. Floral shoots of 'China' litchi had reduced reducing sugars (RS), total carbohydrates (Total CHO) and proline content during FBD over vegetative growth phases. There was not much variation in proline contents in both the cultivars but it was reduced from FBD to flowering stage as in case of leaf total phenol content. The increase in C: N ratio is ascribed as the consequence of increased carbohydrate availability [9], which is necessary for the induction of flowering. Thus, maintenance of high C: N ratio may be one of important characteristic associated with the paclobutrazol for the floral bud induction in tropical fruits like mango. Thunyarpar[16] also found that available carbohydrate analysed as total non structural carbohydrate or starch were found to be accumulated before flower initiation and leaf flushing in litchi and longan.

TABLE 2: Leaf bio-chemical status in flowering and non-flowering shoots of litchi cultivars Shahi and China

Variety	Shoots	Total CHO (%		Reducing Sugar (%)		Proline (μg g^{-1})		Total Phenol (mg per 100 GAE^)	
		During FBD#	Flowering	During FBD	Flowering	During FBD	Flowering	During FBD	Flowering
China	Floral	4.12	4.38	3.00	3.09	39.53	33.27	36.97	35.17
	Non Floral	4.19	4.41	3.04	3.27	39.93	32.94	38.98	35.11
	CD (p= 0.05)	NS	0.052	0.34	0.21	0.42	0.06	0.38	0.66
Shahi	Floral	4.24	4.40	3.05	3.24	40.57	34.48	38.16	35.11
	Non Floral	4.21	4.40	3.06	3.23	39.91	34.61	38.19	36.03
	CD (p= 0.05)	0.071	0.061	0.31	0.23	0.41	0.09	0.41	0.57

#FBD: Floral bud differentiation, ^GAE: Gallic acid equivalent

Effect of paclobutrazol and potassium nitrate on flushing and chlorophyll status of leaves

To regulate flowering in alternate bearer 'China' litchi, paclobutrazol (PBZ) was applied through trunk soil line pore (TSLP) method and potassium nitrate sprayed on tree canopy and then leaf *chlorophyll a, b* and *total chlorophyll* contents, time of emergence of flushes, subsequently flowering percentage were estimated. The leaf *chlorophyll a, b* and *total chlorophyll* contents were assessed during fruit bud differentiation and at flowering stage.

Paclobutrazol and potassium nitrate treatment induced profuse flowering in litchi cv. China. Significant reduction in the winter flushing (November and December) was recorded in treated trees as compared to the control as shown in Table 3. It was clearly shown that the flushes maturing earliest (before the winter period) produce floral shoots, while flushes maturing quite late (December) produce vegetative shoots. The vegetative flushing just prior to floral induction results in poor or no initiation and often vegetative shoots (table 3). Application of paclobutrazol and KNO_3 during September-October brings no flushing or mild flushes during FBD stage (table 3), led to flowering in most of the branches. The lychee leaf flushes are strong sinks for assimilates from the rest of the plant. These assimilates can come from current assimilation or stored reserves [7]. The flowering in KNO_3 may be due to breaking of dormancy of shoots.3.0 to 4.0 g PBZ application brought no flushing during November and December which led to flowering. Higher dose of cultar (5 ml/m^2 plant spread) proved better than the lower dose (3 ml/m^2 plant spread) in controlling vegetative flush and increasing flowering and yield[5]. 100% flowering was assured by spray of KNO_3 with normal flushing in August and mild or no flushing during November-December. The mid-season flush (appearing in August-October) is of more significance in litchi cvs. Bedana, Bombai and Deshi, whereas the early season flush (appearing in July) is the desirable vegetative flush in the rest of the cultivars with respect to yield [11].

TABLE 3: Flushing pattern and flowering affected by application of paclobutrazol (PBZ) and potassium nitrate (KNO_3) in litchi cv. China

Treatment	Period of flush emergence			Per cent of floral branches (%)
	August	November	December	
1.0 g PBZ	No flushing	Mild flushing	Mild flushing	33
2.0 g PBZ	Mild flushing	Mild flushing	Mild Flushing	33
3.0 g PBZ	No flushing	No flushing	No flushing	33
4.0 g PBZ	Mild flushing	No flushing	Mild flushing	66
1 % KNO_3	Mild Flushing	No flushing	No flushing	66
2 % KNO_3	Normal flushing	Mild flushing	Mild flushing	100
Un-treated	Mild flushing	No flushing	Normal flushing	Nil

Results in fig. 1 and table 3 indicated that application of PBZ has a significant effect on leaf biochemical status, inhibition of winter flushing and flowering in litchi cv. China at the 5 % probability level. C*hlorophyll a*, *b* and *total chlorophyll* contents were higher during flowering stage over floral bud differentiation stage irrespective of treatments (fig. 1). It was found that maximum *chlorophyll b* content was recorded with 3.0 g PBZ per m canopy diameter followed by tree received 1 % KNO_3 and 4.0 g PBZ per m canopy diameter. 2.0 g PBZ and no application of PBZ or KNO_3 reduced *chlorophyll b* content. Control trees (un-treated with PBZ and KNO_3) had higher *chlorophyll a* content than treated trees. The *total chlorophyll* was found to be at par with most of the treatments. Leaf arrangement in China litchi is bushy in appearance which imparts shade on preceding leaves, and shaded leaves had a lower photosynthetic rate, so the plant performance and particularly flower induction were adversely affected in China litchi [7]. Some researcher[6,7], also found an 8.5-fold increase in total chlorophyll concentration during development from young red leaves to mature dark green leaves in litchi.

Our results indicated that the production of an adequate number of flushes by a bearing shoot, with a sufficient area of mature leaves and carbohydrate reserves, is one of the critical factors determining high fruit retention in litchi. Although low temperature appears a pre-requisite for floral induction in litchi but there is strong relationship between flowering and timing of flush emergence and its inhibition during winter. The slight fluctuation in temperature and rainfall pattern [during winter] disturb phasic changes, and harvesting of fruits with few leaves, post harvest cincturing (Girdling), use of paclobutrazol (PBZ) or KNO_3 can effectively employed in the sub-tropics to induce synchronous flowering. Further experiments on whole tree basis will be required to identify the potential status of remote carbohydrate resources, and the relative role of carbohydrates reserves and leaf photosynthesis compensation caused by application of paclobutrazol/or KNO_3 or practicing girdling on fruit set and fruit growth in litchi.

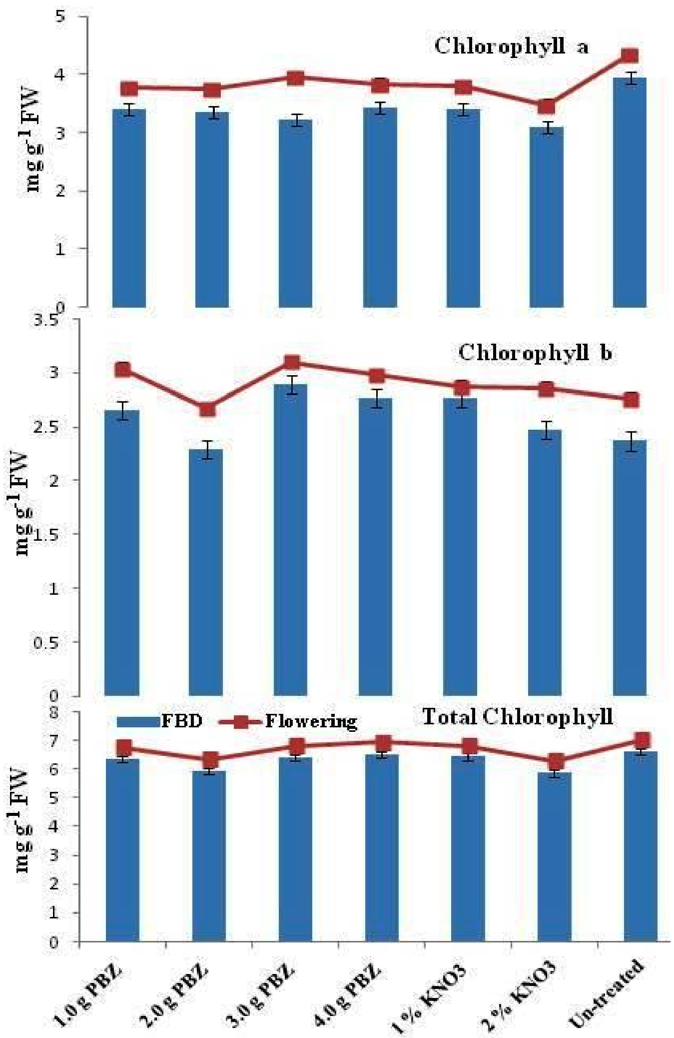

FIGURE 1: Leaf chlorophyll a, b and total chlorophyll contents affected by paclobutrazol and KNO_3 in litchi cv. China (*line diagram depict ±mean during flowering and bar for flowering; vertical bar denotes standard error of means*)

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