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Influence of bearing regulated chemicals and girdling on leaf chlorophyll, Sugars, and leaf nutrient status in litchi cv. China

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ABSTRACT

A field experiment was conducted to study the effect of bio-regulators and girdling on the leaf chlorophyll, sugar content, and leaf nutrient status of litchi cv. China. The group of bio-regulators viz. KNO_3 (2.0 %),Prohexadione-Ca (pro-Ca) (0.5 gL^{-1}), Salicylic acid (SA) (2000 ppm), KH_2PO_4 (0.5 %) and Spermidine (Spd) (0.01mM) were applied as foliar spray and Paclobutrazol (PBZ) (2.5 g a.i. per m canopy diameter) solution was poured around the shallow circular trench (about 10 cm deep), i.e. beneath trunk soil line. Besides foliar sprays of various bio-regulators, the girdling (removal of bark as 3 mm width in 75 % branches) was also practiced during the end of September month. During FBD and flowering the maximum content of chlorophyll a, b and total chlorophyll were reported in SA treated tree. SA treated trees also showed an increment in total sugar (23.38 %) over control (12.67%). Reducing sugar was found to be maximum with PBZ treated tree @ 2.5 g (11.3 %) over Pro-Ca at flowering and FBD stage (8.73%) and (6.8%), respectively. PBZ was recorded with the highest amount of nitrogen (1.94%) during the FBD stage, over Spermidine (0.01mM). During flowering, girdling led to the highest nitrogen content. The leaf phosphorous and potassium content recorded maximum with a tree sprayed with spermidine (0.01mM). The maximum calcium content was recorded with PBZ. The highest magnesium content was recorded with SA during the FBD and flowering stage (0.54 % and 0.44% respectively). However, during flowering girdling caused the highest boron content (55.00 ppm). A significant difference was also observed for N, P, K, Ca, Mg and B due to different bioregulators and girdling. However, leaf K and Ca were non-significant but the application of bioregulators showed a significant effect on N, P, Mg and B contents.

Key words : *Bio-regulators, FBD, Girdling, KNO_3, Leaf nutrient, Litchi, PBZ, Prohexadione-Ca.*

Introduction

The litchi (*Litchi chinensis* Sonn.) is one of the important subtropical evergreen fruit trees. India is the second-largest litchi producing country next to China and has a good export potential. However, it has been confined to a few states due to its specific climate requirement. India is the second-largest producer of litchi after China with an annual growth of 4.30% in area and 7.20 % in production in the country. Bihar as such accounts for 32 % of area and 42% production of litchi in the country.

The basic and strategic research programs in litchi aim at developing technologies for enhancing productivity, quality, and value addition for increased net returns. Alternate bearing is one of the major problems in litchi. To prevent alternate fruit set and stabilize yield, the flush growth should be

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restricted to 0.6 – 2.0 cm in October-November and that leaves should be removed from flush growth > 10 cm which is a tedious process for commercial production (Singh *et al.,* 2016). Increasing photosynthetic activity through exploiting photosynthetic components is a major target. The chlorophyll is considered the major indicator of the physiology and phenology of plants. Photosynthetic efficiency of plants is directly related to chlorophyll content and it makes an indirect estimation of nutrient status in plants.

The uses of various bio-regulators were reported by several workers as an alternative approach to minimize cracking, fruit drop, and above all ensuring regular flowering. Plant growth regulators are known to enhance the source-sink relationship and lead to the translocation of photo assimilates. It improves physiological efficiency including photosynthetic ability and it leads to effective partitioning of accumulates from source to sink (Solaimalai *et al.,* 2001). Potassium plays a major role in different processes like photosynthesis, respiration, ion absorption and transport, protein synthesis and enzyme activation. KH_2PO_4 is a very cost-effective fertilizer and it is consumed very quickly. Due to a shortage of potassium, many metabolic processes are affected like photosynthesis and translocation. Paclobutrazol (PP333) belongs to the triazole group of fungicides is a plant growth regulator. It is an antagonist of gibberellin and has been found that leaf chlorophyll content can be increased with the foliar spray of paclobutrazol (4 to 8 L ha^{-1}) in 'Fuji' apple (Kim *et al.,* 1990). Recently another GA biosynthesis inhibitor with low toxicity and limited persistence has been used for controlling the vegetative growth in apple (Byers *et al.,* 2004).

No universal nutrition program seems to be available for lychee and poor nutrition is likely to be one of the major factors contributing to fluctuating yields. Much research is required before we use nutrition management to manipulate the growth and fruiting cycle of lychee and maintain tree health, tree size, and fruit quality (Menzel and Simpson, 1987). The NPK level in autumn and fruiting shoots of litchi is related to the growing season. The N, P, K content in the leaves of fruiting shoots is in the order of N > K > P for each growth stage. The maximum value of the N: P: K ratio is found at the blossoming to harvest stage. The great amount of NPK stored in leaves consumed from flower differentiation to fruit developing stage, especially at the blossoming stage. The relative P and K content decrease about 12.8 to 41.5% and 0.9 to 30.3% respectively (Fan *et al.,* 2005).

The girdling in litchi proved beneficial for flower induction. Its most immediate effect is to stop the basipetal movement of assimilates through the phloem, which results in an accumulation of carbohydrates above the girdle (Di Vaio *et al.,* 2001). SA chemically known as 2-hydroxy benzoic acid is synthesized by plants. It is one of the crucial regulators of photosynthesis because it affects the chloroplast and leaf structure, stomatal closure, chlorophyll, and carotenoid content (Fariduddin *et al.,* 2003). Enhancement of the level of chlorophyll and carotenoid pigments, photosynthetic rate, and modifying the activity of some of the important enzymes are other roles assigned to salicylic acid. It induces specific changes in leaf anatomy and chloroplast structure. Exogenous Polyamines, including putrescine (Put), spermidine (Spd), and spermine (Spm) have been employed to regulate floral and fruit development and ripening in a lot of plants. It leads to a high growth rate and active cell division during the initial stage of fruit development (Galaston, 1983). Considering the above point in view, an experiment was conducted to evaluate the influence of this bio-regulator and girdling on chlorophyll, leaf sugars attribute, and micronutrient status of the shoot tips in litchi cv. China

Materials and Methods

Study site and experimental materials

The field experiment was conducted during the 2018-2019 and 2019-2020 seasons at the experimental Farm of ICAR-National Research Centre on Litchi, Musahari, Muzaffarpur, Bihar. The experimental site was situated at 26^0 5' 87'' N *latitude*; $85^0$26' 64'' E *longitude* at an elevation of 210 m above the mean sea level having a sub-tropical climate. The experimental soil was alluvial with sandy loam texture and is calcareous having pH 7.5 - 8.0. Twelve-year-old uniform-sized trees of litchi cv. China spaced at 8 x 8 m were selected for this experiment. The chosen design was a Randomized Block Design (RBD). The experimental trees (3 trees of each treatment = 24 trees) were maintained under uniform cultural practices.

Treatments

The details of treatment were, foliar spray of KNO_3

(2.0 %), Prohexadione-calcium (0.5 g a.i. /L) (pro-Ca); Paclobutrazol (PBZ) (2.5 g a. i. per canopy diameter) (PBZ); girdling with 3 mm intensity practiced in 75 % branches; foliar spray of salicylic acid (SA) @ 2000 ppm; KH_2PO_4 @ 0.5 % and spermidine (Spd) @ 0.01Mm. All the treatments were imposed during the last week of September (after 100 days of harvesting). The entire experiment was replicated three times with a single tree assumed as one unit (replication). Thus, the total number of trees was 24 (8 trees of particular cultivar for each treatment). The experimental trees were tagged in such a manner as to avoid border effects and each tree was surrounded by four trees. The proper weighing of different chemicals was done and the stock solution of each chemical was prepared by dissolving them in water. The final volume was made to 5 litre by adding water i.e. enough for spraying one tree. All the chemicals except PBZ were sprayed in the evening hour with a rocker sprayer (after adding Active-20 as a surfactant) by the end- September. For the application of PBZ, a shallow circular trench (about 10-cm-deep) was dug at a radius of 1 m from the base of each tree. PBZ in the water-soluble form ''Cultar'' was obtained from ICI (Indonesian Operations, Jakarta). The Burondkar and Gunjate (1993) method of soil drenching was followed, in which 10 small holes (10–15 cm depth) were made in the soil around the collar region (2.5 feet away from the tree trunk). A homogeneous solution of PBZ was prepared by dissolving it in 5 liters of tap water and 500 ml of this solution was drenched in each hole. Control trees were drenched with tap water. For girdling, a stripe of bark was removed in a circular fashion around the selected primary branch with the help of a pruning saw. The phloem portion was removed carefully without damaging the xylem. All the experimental trees were given uniform cultural practices as recommended by ICAR-NRCL, Muzaffarpur, Bihar.

Estimation of Chlorophylls, reducing and total sugars

The chlorophyll contents (chlorophyll a, b and total chlorophyll) of the leaves were analysed at the flower bud differentiation stage and flowering following the method as suggested by Barnes *et al* . (1992). Fully matured open leaf was chosen as the experimental sample for chlorophyll estimation. Accurately weighed 0.5 g of clean leaf sample was immersed in 10 ml of dimethyl sulphoxide (DMSO) AR grade. The samples were incubated at 80 ^{0}C for four hours in hot air oven. After incubation, it was taken out and 1 ml of the solution was diluted to 5 ml with DMSO and the sample then read on a spectrophotometer at 638, 645 and 663 4 nm using pure DMSO as blank. Chlorophyll a, b and total chlorophyll were calculated according to the following formula:

Chlorophyll 'a' = [(12.7*A663) – (2.69*A645)]*V/1000*W

Chlorophyll 'b' = [(22.9*A645) – (4.68*A663)]*V/1000*W

Total Chlorophyll = [(8.02*A663) + (20.2*A645)*V/1000*W

Reducing sugar (RS) contents were estimated in leaves following the method outlined by Nelson and Somogyi in Thimmaiah (2004). Exactly 100 mg of each sample was macerated and extracted twice with 5 ml 80% (v/v) hot ethanol (40 ºC). The supernatant was collected and evaporated to dryness in a water bath at 80 ºC. Thereafter, 10 ml of distilled water was added to dissolve the sugars. From this, a 0.2 ml aliquot was pipetted into a test tube and the volume increased to 2.0 ml by adding autoclaved double distilled water. One ml of alkaline copper tartrate solution was added and the sample was kept in a water bath at 100 ºC for 10 min. After cooling to 25 ºC, 1.0 ml of arseno-molybolic acid reagent was added to each tube and the volume made up to 10 ml with double-distilled water. After 10 min, the absorbance (blue colour) was read using a spectrophotometer at 620 nm. The total amount of RS present in each sample was then calculated from a standard curve.

The total sugar (TS) contents of the leaves were estimated by the anthrone method (Thimmaiah, 2004). Exactly 100 mg of young leaf tissue from each tree was hydrolyzed with 5.0 ml 2.5 M HCl in a boiling water bath for 3 h, then neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 2,000 g for 15 min at ambient temperature. The supernatant was collected and a 1.0 ml sample was used for analysis. Four ml of anthrone reagent was added to this aliquot and heated to 90 ºC in a water bath for 10 min. The sample was cooled rapidly and the green to dark green colour that formed was measured at 630 nm in a spectrophotometer, as above.

Estimation of leaf nutrients

The leaf samples were collected from all directions

at the flower bud differentiation stage and during flowering. The leaf nitrogen was determined by Kjeldahl method and phosphorus by vanado-molybdate colour reaction method. Potassium content was estimated by a microprocessor based flame photometer using specific filter (K filter) and LPG flame. Calcium and magnesium were determined by atomic absorption spectrophotometer using nitrous oxide-acetylene and air-acetylene flame (oxidizing), respectively. For estimation of B (boron), half a gram of the powdered leaf sample was kept in a silica crucible and ashed in a muffle furnace at 550 °C for 5 hours. The ashed sample was acidified with 1:1 HCl and kept in a water bath for 15 minutes and made with up to 25 ml with quartz double distilled water. From the extract, boron content was determined colorimetrically using Azomethine-H reagent, and the absorbance was read at 420 nm.

The mean was computed for the data on various attributes, whereas a two-factor analysis of variance (ANOVA) using a randomized block design (RBD) was conducted with SAS® 9.2 statistical software. The least significant differences (LSDs) between means at $P_{0.05}$ and the standard error (SE) of means were computed. Parameters means values were used to perform correlation analyses to test whether the variables are correlated in the population.

Result and Discussion

Variation in the content of chlorophyll was noticed among different bearing regulated chemicals and girdling. Data depicted in the Table 1 revealed that chlorophyll a (*chl a*) (8.36 mg g^{-1} FW), b (4.89 mg g^{-1} FW) total chlorophyll (*total chl*) (13.25 mg g^{-1} FW) was found highest in tree sprayed with salicylic acid (SA) @ 2000 ppm during FBD and flowering (C*hl b*: 10.39 mg g^{-1}FW), while least *chl a*, *b* and *total chl* was recorded in control trees (3.51, 2.92, 7.22 mg g^{-1}FW, respectively). During flowering and FBD stage chlorophyll b (*chl b*) was insignificant amongst means of various crop regulating chemicals and girdling. However, the *chl b and total chl* content also improved a lot due to Prohexadione-Ca @ 0.5 g (4.72, 11.75mg g^{-1} FW, respectively) over other treatment. At FBD stage, least *chl b* was recorded with spermidine (*Spd*) @ 0.01mM (3.48 mg g^{-1} FW) but at flowering stage the same treatment recorded highest content of *chl b* (6.52 mg g^{-1}FW) followed by KH_2PO_4 (4.60 mg g^{-1} FW). Our results were in congruence with findings of Shi *et al*. (2006) who noticed that exogenous application of SA significantly increased net photosynthetic rate which might be due to the improvement in the functioning of photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or by chlorophyll biosynthesis. According to Hayat *et al.* (2010), SA also enhanced photosynthesis by prohibition of chlorophyll oxidase enzyme activity in tomato. Singh *et al.* (2017) also found higher chlorophyll a, b and total in litchi during flowering stage over FBD stage irrespective of any treatments.

The leaf total sugar (*TS*) was maximum in SA treated trees (23.38 %) followed by *Spd* treated tree (21.14 %), while least in untreated tree (12.67 %) (Table 1). The *TS* improved a lot (23.38 %, 22.19 %, respectively) due to SA, during FBD and flowering phase while least in untreated tree (12.67%). Reducing sugar (*RS*) was found maximum with PBZ treated tree (11.3 %) followed by KNO_3 (11.30%). Like *TS*, the *RS* was also least in untreated tree during FBD (9.04) and flowering stage (7.93 %). According to Singh *et al.* (2017), the total CHO and RS content increases from FBD stage to flowering stage in litchi.

Nitrogen (N) is one of the most important manipulating nutrients. Thus the period of vegetative dormancy before flowering should be considered as a low N requirement. According to Chen *et al.*, (2011), the relative proportion of certain nutrients is the key to adequate blossoming and fruit bearing of the litchi tree. At the flower bud differentiation stage, litchi needs a lot of N, P and K (Guo *et al.*, 1992). Calcium is the second most abundant element of litchi leaves. In this investigation, the PBZ application recorded highest leaf N (1.94 %) followed by KH_2PO_4 (1.93 %) during FBD stage while least N content was recorded with *Spd* (1.66 %). The result showed significant effect during FBD and flowering stage. The leaf N content reduced during flowering as compared to FBD stage. The low N and K soil contents, and litchi trees with low K nutrition are some of the most significant reasons for low and unstable unit yields. Throughout the developmental period, the elemental contents in litchi leaves are in the descending order of N > K > Ca > Mg > P > S > B > Zn > Mo (Yang *et al.*, 2015). Similar to this investigation Yang *et al*. (2015) also found high N content of the leaves initially, which was significantly higher in the flower bud differentiation period than in other growth periods.

Devi and Tyagi (1991) also found similar results

which might be due to the utilization of N during FBD stage. Phosphorous (P) was found maximum with tree sprayed with *Spd* (0.38 %) followed by KH_2PO_4 (0.36%) and PBZ (0.36%) during FBD stage. At flowering phase, the highest P content with SA treated tree (0.29%) followed by *Spd* (0.28 %).

The maximum potassium (K) level was recorded with *Spd* (0.09%) followed by girdling and KH_2PO_4 (0.89%) during FBD stage (Table 2). At flowering phase, the highest K content (0.79 %) was recorded in *Spd* treated tress followed by control tree (0.78%). The K content was non-significant during FBD stage. Similar finding was recorded by Verma and Mishra (2005) who reported that polyamines involve in stabilization of biological membrane, helpful in modulating ion channels, binding to the antioxidant enzymes and elevate the K content in plants. With respect to the calcium (Ca) content, the PBZ ensured maximum amount of Ca (3.97%) followed (3.80%) by KH_2PO_4, while least in SA treated trees (3.47 %). During flowering phase, the maximum Ca content (2.90 %) was recorded with *Spd* followed by girdling and KH_2PO_4 (2.80 %), while least in PBZ applied trees (2.53 %). The highest magnesium (Mg) content was recorded with SA during FBD (0.54 %) and flowering stage (0.44%), while least in untreated tree (0.35%). Gunes *et al.* (2007) also reported that SA minimizes the Na uptake and raise the uptake of N, P, K and Mg in Maize. During FBD stage, the boron (B) content recorded highest in trees sprayed with KH_2PO_4 (66.33 ppm) followed by untreated tree (65 ppm), while least (50.67 ppm) in trees where PBZ was applied. During flowering phase, girdling brought highest B content (55.00 ppm) followed by PBZ (53.33 ppm). The girdling caused leaf N content, C/N ratio and carbohydrate to improved,

Table 1. Changes in leaf chlorophyll, total sugar and reducing sugar status affected by pre-FBD application of bearing regulating practices including girdling in litchi cv. China

Treatments	Chlorophyll a (mg g^{-1} FW)		Chlorophyll b (mg g^{-1} FW)		Total Chlorophyll (mg g^{-1} FW)		Total sugar (%)		Reducing sugar (%)	
	I	II	I	II	I	II	I	II	I	II
KNO_3 (2.0 %)	6.01	7.47	3.71	2.88	9.72	10.35	20.30	17.85	10.77	7.70
pro-Ca (0.5 g L^{-1})	7.31	7.38	4.72	4.57	12.04	11.95	18.67	18.02	8.73	6.80
PBZ (2.50 g)	7.68	7.87	4.07	4.18	11.75	12.04	20.28	19.26	11.30	9.63
Girdling	5.80	4.89	3.53	2.98	9.33	7.86	18.71	17.61	9.27	7.00
SA (2000 ppm)	8.36	10.39	4.89	3.63	13.25	14.88	23.38	22.19	10.73	8.33
KH_2PO_4 (0.5%)	6.91	7.90	4.58	4.60	11.49	12.49	14.63	12.12	10.10	9.23
Spd (0.01mM)	4.07	5.78	3.48	6.52	7.55	8.72	21.14	19.9	10.68	9.40
Control	3.51	8.36	3.72	2.92	7.22	14.02	12.67	10.37	9.04	7.93
$CD_{0.05}$	1.08	2.53	NS	NS	2.33	3.87	1.36	0.65	0.60	0.91
SE(m)	0.35	0.83	0.65	0.83	0.76	1.26	0.45	0.22	0.20	0.30
C.V.	9.81	19.07	27.45	35.78	12.82	18.98	4.13	2.16	3.37	6.23

Table 2. Effect of different bio-regulators and girdling on leaf nutrient status of leaves of cv. China

Treatments*	Nitrogen (%)		Phosphorous (%)		Potassium (%)		Calcium (%)		Magnesium (%)		Boron (ppm)	
	I	II	I	II	I	II	I	II	I	II	I	II
KNO_3 (2.0 %)	1.83	1.54	0.19	0.18	0.84	0.74	3.63	2.63	0.46	0.36	57	47
pro-Ca (0.5 g L^{-1})	1.86	1.63	0.35	0.25	0.85	0.76	3.67	2.65	0.5	0.4	56	49.33
PBZ (2.50 g)	1.94	1.58	0.36	0.26	0.87	0.77	3.97	2.53	0.48	0.37	50.67	53.33
Girdling	1.89	1.77	0.31	0.21	0.89	0.76	3.57	2.8	0.49	0.39	60.67	55
SA (2000 ppm)	1.73	1.57	0.4	0.29	0.88	0.75	3.47	2.7	0.54	0.44	57.67	47.67
KH_2PO_4 (0.5%)	1.93	1.74	0.36	0.26	0.89	0.76	3.8	2.8	0.47	0.37	66.33	49.67
Spd (0.01mM)	1.66	1.57	0.38	0.28	0.9	0.79	3.57	2.9	0.46	0.38	61	47.67
Control	1.83	1.79	0.37	0.27	0.87	0.78	3.77	2.6	0.47	0.35	65	45
$CD_{0.05}$	0.067	0.05	0.019	0.016	NS	0.025	NS	0.196	0.033	0.038	6.751	NS
SE(m)	0.022	0.016	0.006	0.005	0.015	0.008	0.122	0.064	0.011	0.012	2.204	2.123
C.V.	2.081	1.702	3.152	3.56	3.053	1.853	5.768	4.006	3.86	5.632	6.439	7.453

Table 3. Pearson correlation matrix showing effect of various bio-regulators and girdling on yield, flowering and physico-chemical attributes of cv. China during FBD stage

Characters	Yield (kg tree^{-1})	No. of female flower	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Total Sugar (%)	Reducing Sugar (%)
Yield (kg^{-1} tree)	-	0.508	0.464	0.201	0.416	0.756	0.513
No. of female flower panicle $^{-1}$	-	-	0.0708	- 0.174	0.010	0.183	0.157
Chlorophyll a (mg g^{-1} FW)	-	-	-	0.785	0.987	0.418	0.301
Chlorophyll b mg g^{-1} FW	-	-	-	-	0.874	0.131	- 0.358
Total chlorophyll (mg g^{-1}FW)	-	-	-	-	-	0.412	0.226
Total sugar (%)	-	-	-	-	-	-	0.591
Reducing sugar (%)	-	-	-	-	-	-	-

therefore, flowering and fruit set are increased (Table 2).

Correlation is significant at 0.05 probability level

Overall, the correlations between the studied characters (Table 3) showed positive correlations. Indeed, no. of female flower panicle^{-1} was negatively correlated with leaf chlorophyll b content. Likewise leaf chlorophyll b content was also negatively correlated with reducing sugar content. The total sugar content of leaves was highly correlated with the fruit yield; while, the no. of female flower panicle^{-1} was least correlated with the leaf total chlorophyll content. As expected, the leaf chlorophyll content was also partially correlated with no. of female flower panicle^{-1}. The highest positive correlation was observed between chlorophyll a and total chlorophyll.

The results of the present study revealed that the application of KNO_3, pro-Ca and practicing girdling after 100 days of harvesting was best for ensuring higher chlorophyll content, high sugars and reduced N status of the leaves which is highly conducive for flowering.

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