



Influence of chemicals and girdling on tree physiology and fruiting of litchi

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ABSTRACT

The present study was conducted to evaluate the effect of various chemicals (paclobutrazol, KNO₃, Prohexadione Ca, salicylic acid, KH₂PO₄ and spermidine) and girdling on plant growth, tree physiology, fruit yield and quality in litchi cv. China. Experimental results revealed least increase in the tree height (1.58%) after spraying of KNO₃. Girdling significantly increased the tree girth (3.55 %) and trunk cross sectional area (7.29%). All the treated trees had 20-50% more leaf relative water content (RWC) than the control trees. Paclobutrazol (PBZ) application and spray of KH₂PO₄ recorded the maximum value of the stomatal conductance (gs) and transpiration rate (E) at flower bud differentiation stage. The untreated trees had low stomatal conductance (gs), transpiration rate (E) and internal CO₂ concentration (Ci). The transpiration rate was higher during the flowering than at FBD stage. PBZ application showed the highest free proline and total phenol contents in leaves. The water use efficiency (WUE), mesophyll efficiency (ME) and carboxylation efficiency (CE) were found to be high during flowering compared to the flower bud differentiation (FBD) stage. The Pro-Ca showed the maximum WUE, followed by application of KNO₃. The highest number of floral panicles (3656) and yield (2427 fruits /tree & 55.42 kg/tree) were recorded with the spray of KNO₃ followed by girdling. The colour intensity of fruits was significantly improved regarding hue, value and chroma.

Keywords: *Litchi chinensis* Sonn., Bio-regulators, Girdling, Yield

INTRODUCTION

India is the second largest producer of litchi in the world after China with an annual growth rate of 4.30 % in area and 7.20 % in production in the country. Bihar as such accounts for 32 % in area and 42 % of production of litchi in the country. In litchi, yields are often irregular and suffer from alternate bearing. Limited data suggested that cultivars with early fruit ripening (like Shahi) had a lower alternate-bearing tendency than the late ripening cultivars (like China). To discourage the alternate bearing habit and to stabilize the yield, the flush growth should be restricted to 0.6 – 2.0 cm in October-November and the new leaves should be removed from flush growth (having > 10 cm in length), which is a tedious process.

Potassium compounds like KNO₃ and KH₂PO₄ have been found to be promising with regard to flower induction and improving bearing potential of litchi. Deficiency of potassium affects many metabolic processes viz. rate of photosynthesis and translocation. Paclobutrazol (PBZ) @ 5.50 - 8.25 g a.i. per tree (both as a soil drench and spray applications) are effective in suppressing vegetative growth with high total non-structural carbohydrate in their shoots (before flowering). PBZ also resulted in production of more floral shoots, panicles, percentage of hermaphrodite flowers, yield as well as quality of the fruit in mango

when compared with untreated control (Yeshitela *et al.* 17).

Besides PBZ, prohexadione-Calcium (calcium-oxide-3-propionyl-4-oxo-5 cyclohexene 3- carboxylate; Pro-Ca) is also becoming popular among growers to regulate the bearing habit of fruit crops. Although PBZ has been reported to be bringing regularity of bearing in litchi cv. China yet its alternative (Pro-Ca) is more popular, as it breaks down relatively rapidly with half-life of approximately 7 to 10 days whereas the PBZ residue persists in soil for longer period. Furthermore, Pro-Ca is translocated exclusively through xylem and is, therefore, unlikely to be accumulated in the fruit. Pro-Ca (when applied during the early stages of vegetative growth) reduces the levels of GA₁ (highly active) and increases the concentration of its precursor, the GA₂₀ (inactive) in plant tissues. Besides this, Pro-Ca also plays an important role in increasing uptake of nutrients by increasing root growth at the cost of shoot growth (Javaid and Misgar, 7). Salicylic acid (SA) has been reported to play an important role in regulating number of physiological process including synthesis of auxin and/or cytokinin, and enhances the fruit yield (Feriduddin *et al.* 5).

Girdling of branches having 3 to 4 cm in diameter with hardened flush in May or foliar application of 0.5 g paclobutrazol+0.4 g of ethephon per liter promotes flowering in unproductive litchi cv. Tai So. However, fruiting is inconsistent on girdled branches. Erratic

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fruiting was also reported on branches treated with growth retardant (Ramburn, 13). Experiments on *Cucurbita pepo* L and *Ginkgo biloba* have also found that spermidine (Spd) predominates during flower bud differentiation. When Spd reaches a certain level, it can promote the initiation of a flowering gene, thereby triggering the synthesis of a special protein and finally the formation of the flower primordium (Quin *et al.* 12).

Considering the above point in view, an experiment was conducted to evaluate the influence of these bio-regulators/GA₃ inhibitors and girdling on yield and tree physiology during FBD and flowering in litchi cv. 'China'.

MATERIALS AND METHODS

The field experiment was conducted during the 2018-2019 and 2019-2020 years at the experimental farm of ICAR-National Research Centre on Litchi, Mushahari, Muzaffarpur, Bihar. The experimental site is situated at 26°5' 87" N latitude, 85°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 calcareous having pH 7.5 - 8.0. Twelve-year-old uniform sized trees of litchi cv. China spaced at 8m × 8 m were selected for this experiment. The experiment was laid out in Randomized Block Design (RBD), and replicated thrice. The experimental trees (3 trees of each treatment = 24 trees) were maintained under uniform cultural practices.

The details of treatment were, foliar spray of KNO₃ (2.0 %), pro-hexadione Ca (0.5 g a.i. /L) (Pro-Ca); paclobutrazol (2.5 g a. i. per m canopy diameter) (PBZ); girdling with 3 mm intensity practiced in 75 % branches; foliar sprays of salicylic acid @ 2, 000 ppm; KH₂PO₄ @ 0.5 % and spermidine (Spd) @ 0.01Mm. All the treatments were imposed during last week of September to 100 days of harvesting). Every solution was sprayed @ 5 litres per tree in the evening hours except PBZ. 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) and applied as per method suggested by Burondkar and Gunjate (3). Control trees were drenched with tap water. For girdling, a stripe of bark was removed (3 mm in width) in a circular fashion around the selected primary branch with the help of pruning saw. The phloem portion was removed carefully without damaging the xylem.

The tree height was measured vertically from the ground to the tip of the tree and expressed in meter. The trunk girth was measured at 30 cm above from the base and expressed in centimetre. The canopy height was taken from place of first branching of main stem

to the apex of the canopy and recorded in meter for estimation of TCA as per the following formula.

Trunk cross sectional area (TCA) (cm²) = {Trunk circumference (cm) x 0.16}² x 3.143

The leaf area (cm²) was measured with the use of scanner (Epson Perfection, V 700 Photo, Scanner, Seiko Epson Corporation, Japan). The leaf relative water content (RWC) was measured as per the method suggested by Pieczynski *et al.* (11). The leaf gas exchange parameters namely photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and internal CO₂ concentration (C_i) were recorded with PP System (CIRAS-2). The instantaneous leaf water use efficiency (WUE) was calculated as P_N/E , and the instantaneous carboxylation efficiency (P_N/C_i) was calculated as suggested by Silva-Marcelo de Almeida *et al.* (12). The mesophyll efficiency (ME) was determined using formula C_i/g_s . The quantum use efficiency (QUE) was calculated using formula P_N/Q , where, Q refers to the PAR (photosynthetically active radiation) absorption rate. To measure total phenol (TP) content in leaves, the methods suggested by Malik and Singh (8) was used. Proline content of the leaves was measured using rapid colorimetric method (Bates *et al.*, 2). Total number of flowers per panicle was estimated by counting floral shoots of tagged branches in individual trees. The fruits were harvested at commercial maturity for further analysis. The fruit yield was calculated by weighing the total fruits harvested from each tree and weight (in kg) was taken by open pan balance. External fruit colour was evaluated using Chroma Meter (Model CR-400, Minolta Corp., Japan). The data obtained during the course of present studies were analysed using SAS[®]9.2 statistical software.

RESULTS AND DISCUSSION

Girdling and chemical treatments tested in the present study significantly influenced the tree growth of 'China' litchi tree (Table 1). The highest increase in tree height was registered in trees with spray of spermidine @ 0.01mM (11.43%) followed by Pro-Ca sprayed trees (9.66 %) and control trees (9.29 %), while it was lowest (1.58%) in potassium nitrate sprayed trees. Mitali *et al.* (9) also found that potassium availability results resistance against various stresses in the plants and also has role in adjusting excessive vegetative growth by inhibiting nitrogen absorption and thereby encouraging floral initiation. Tree girth was also influenced due to the treatments and recorded highest increase with girdling (3.55%). The girdling of trees also tended to show the highest increase (7.23%) in trunk cross sectional area (TCA). No change was observed in tree girth and TCA in Pro-Ca sprayed trees. Various

Table 1. Changes in tree height, girth and trunk cross sectional area (TCA) in litchi cv. China.

Treatment	Tree height (m)		%	Tree girth (cm)		%	TCA (cm ²)		%
	I	II	Increase	I	II	Increase	I	II	Increase
Potassium nitrate (2.0 %)	3.16	3.21	1.58	61.00	61.66	1.08	299.16	305.70	2.19
Prohexadione Calcium (0.5 g per L)	3.83	4.20	9.66	68.00	68.00	0.00	371.91	371.91	0.00
Paclobutrazol (2.50 g)	3.58	3.86	7.82	58.00	58.66	1.14	270.78	276.89	2.26
Girdling	3.46	3.73	7.80	56.33	58.33	3.55	255.21	273.65	7.23
Salicylic acid (2000 ppm)	3.40	3.66	7.65	57.33	58.00	1.17	264.30	270.46	2.33
Potassium di-hydrogen phosphate (0.5%)	4.15	4.26	2.65	66.66	67.66	1.50	357.28	368.07	3.02
Spermidine @ 0.01mM	3.50	3.90	11.43	60.00	60.66	1.10	289.43	295.86	2.22
Control	3.23	3.53	9.29	59.00	59.66	1.12	280.03	286.3	2.24
CD _{0.05}	0.54	0.43	-	3.00	2.56	-	29.18	25.43	-
SE(m)	0.17	0.14	-	0.98	0.83	-	9.52	8.30	-

I: Before application of treatment; II: After two months of treatment

reports suggested that the branch girdling in litchi inhibit shoot growth, growth of new sprouts as well as downward translocation of photosynthates via phloem might be reason of increased TCA in this investigation. However, Basak (1) observed that prohexadione-Ca (75mg dm⁻¹) if applied twice, led to reduction in tree size and improved the structure of canopy in young apple trees of cv. Jonagold.

The data on leaf area, relative water content (RWC) and physiological parameters of 'China' litchi tree subjected to various treatments are presented in Table 2. At the stage of FBD, the leaf area was maximum due to spray of Pro-Ca (43.17 cm²) followed by potassium dihydrogen phosphate, salicylic acid and potassium nitrate. At same stage, the leaf area was minimum (26.33 cm²) in the trees received girdling (Table 2). This might be attributed to the utilization of the reserved starch in roots after girdling. The maximum retention of water in terms of RWC at FBD stage was recorded with spray of salicylic acid, KNO₃ and PBZ whereas the control trees retained least RWC (27.12 %). At flowering stage, the effect of treatments on leaf area and leaf relative water content (RWC) was insignificant. Our results were in congruence with findings of Singh *et al.* (16) who reported higher leaf RWC during August and October which drastically reduced during February month (flowering stage) after application of PBZ @ 2-3.0 g.

Perusal of date in Table 2 revealed that net photosynthetic rate (P_N) could not be significantly affected over time by any treatment. Singh *et al.* (15) also found that PBZ had only small effect on leaf gas exchange parameters but it significantly improved

chlorophyll content, reduced leaf N content (just prior to FBD) and improved yield (by 10-15-fold), pulp vitamin C and anthocyanidin content in litchi cv. China.

At the time of FBD, the highest g_s (33.33 m mol m⁻²s⁻¹) were recorded with application of PBZ followed by spray of potassium dihydrogen phosphate (29.00m mol m⁻²s⁻¹). The control trees recorded least g_s (0.13 m mol m⁻²s⁻¹). At flowering stage, potassium nitrate brought highest g_s followed by girdling. The E and C_i was also significantly affected at FBD stage due to various bearing regulated chemicals and E was recorded maximum due to PBZ application (0.73 m mol m⁻²s⁻¹) while Spd caused least E at both the stages. At flowering stages, the E was highest (1.80 m mol m⁻²s⁻¹) due to spray of potassium nitrate followed by girdling of branches. The C_i was recorded highest (339.00 m mol m⁻²s⁻¹) at FBD stage after application of salicylic acid and lowest due to Spd or spray of potassium nitrate. The untreated trees also recorded higher C_i than many other treatments at both the stages. A high C_i value is associated with a low stomatal conductance, which indicates a decrease in the P_n/C_i ratio in sugarcane (Silva-Marcelo de Almeida *et al.*, 14). Various treatments tested insignificant to influence the C_i at the time of flowering. Escalona *et al.* (6) also found reduction in C_i with parallel reductions in g_s in grapevines with increasing soil water deficit.

In this investigation, the WUE , QUE and CE were higher during FBD over flowering phase (a water stress phase). The leaf WUE was insignificant between the treatments during flowering stage. At FBD stage, carboxylation efficiency (CE) was

Table 2. Leaf area, RWC and gaseous exchange parameters, affected by bearing regulating chemicals and girdling in litchi cv. China.

Treatments	Leaf area (cm ²)		RWC (%)		P_N (μmol m ⁻² s ⁻¹)		g_s (m mol m ⁻² s ⁻¹)		E (mmol m ⁻² s ⁻¹)		C_i (m mol m ⁻² s ⁻¹)	
	I	II	I	II	I	II	I	II	I	II	I	II
Potassium nitrate (2.0%)	41.33	40.33	42.20	64.20	8.03	2.57	8.00	50.00	0.37	1.80	256.67	302.66
Prohexadione Calcium (0.5 g per L)	43.17	39.67	35.82	66.00	4.67	1.10	15.00	40.00	0.10	1.30	276.67	315.33
Paclbutrazol (2.50 g)	34.00	35.33	42.14	77.03	7.87	1.40	33.33	19.00	0.73	0.77	265.00	242.33
Girdling	26.33	39.33	39.69	72.24	3.23	0.97	6.00	41.67	0.17	1.70	279.67	339.00
Salicylic acid (2000 ppm)	42.17	36.33	47.74	71.86	4.07	0.87	15.67	18.00	0.30	0.67	339.00	264.33
Potassium di-hydrogen phosphate (0.5%)	42.67	34.67	33.07	55.72	4.30	1.17	29.00	17.67	0.40	0.53	288.00	271.63
Spermidine (0.01mM)	34.33	45.67	37.93	52.21	2.70	0.63	3.00	7.00	0.10	0.33	256.33	345.66
Control	39.00	31.00	27.12	85.41	3.13	1.30	0.13	19.67	0.20	0.63	302.33	262.00
CD _{0.05}	9.69	NS	9.59	NS	NS	NS	15.26	18.88	0.38	0.59	36.57	NS
SE(m)	3.17	3.88	3.13	7.28	1.46	0.63	4.98	6.17	0.12	0.19	11.94	36.17

*Observation taken at I: FBD stage (December-January); II: Flowering phase (February-March)

higher due to spray of KNO₃ at both the stages (Fig.1a-d). Escalona *et al.* (4) also found, a slowly induced drought (under field conditions) significantly promote A_{maxlight} and A_{maxCO2} reductions but without causing important changes in CE in grapevines. In this investigation, the quantum use efficiency (QUE) was higher at FBD stage in most of the treatment and least in untreated trees. The QUE is much lower than the FBD stage, as the litchi plant experiences water stress during flowering phase. At FBD stage PBZ recorded maximum QUE (Fig. 1c).

The pro-Ca spray led to highest WUE (at FBD stage) followed by trees which received potassium nitrate (Fig.1a). From a physiological perspective, a high WUE value is traditionally considered an improvement in the mechanism that improves the productivity and survival in dry environments which was shown by pro-Ca and potassium nitrate in this investigation and litchi trees could do better in water stress area if sprayed with Pro-Ca or KNO₃. During flowering stage, the control trees had highest ME over other treatments however the spray of potassium nitrate also enhanced ME over other bearing regulating chemicals (Fig. 1b). The spray of KNO₃ and PBZ was able to enhance CE to highest level at both the stages while control trees were found to have much lower values (Fig. 1d).

The free proline content was more in control trees during flowering over FBD stage. For the treatments, the reverse trend was observed i.e. leaf free proline content was higher at FBD over flowering stage. PBZ caused highest leaf free proline and total phenol

content at FBD stage, at flowering phase, KNO₃ and KH₂PO₄ improved the same (Fig. 2a). Girdling of litchi trees led to lowest total phenol content during flowering stage while spray of KNO₃ and Pro-Ca improved a lot in total phenol content (Fig. 2b). Besides PBZ, the spray of KNO₃, girdling and KH₂PO₄ also recorded higher free proline content in leaves over control trees at FBD stage.

As per data presented in Table 3, the no. of flowers per panicle was significantly highest (3656) due to spray of potassium nitrate (2.0%) followed by girdling, while it was lowest in control trees (108.33). Likewise, the application of potassium nitrate also excelled statistically in respect of number of fruits/tree (2427.66) and fruit yield (53.34 Kg/tree) over other treatments. Gawankar *et al.* (6) also found encouraging results related to the fruit yield in the girdled trees which showed a total fruit production increase with more than 50% over the non-girdled (control) trees. Singh *et al.* (14) observed in litchi cv. China that PBZ and KNO₃ applied during September-October brings no flushing or mild flushes during FBD stage that led to flowering in most of the branches. In mandarin cv. Balady, Mostafa and Saleh (10) also reported girdling (before blossoming in late Dec.) with spray of 2% KNO₃ (first in April and second in mid-June) had a positive effect on increasing fruit weight (157 g), number of fruits per tree (412) and yield (65 kg tree⁻¹). Mitali *et al.* (9) also reported that nitrate salt application stimulates bud break. Presumably, there is a threshold for nitrogen concentration that if exceeded, will allow the plant to flower. Potassium

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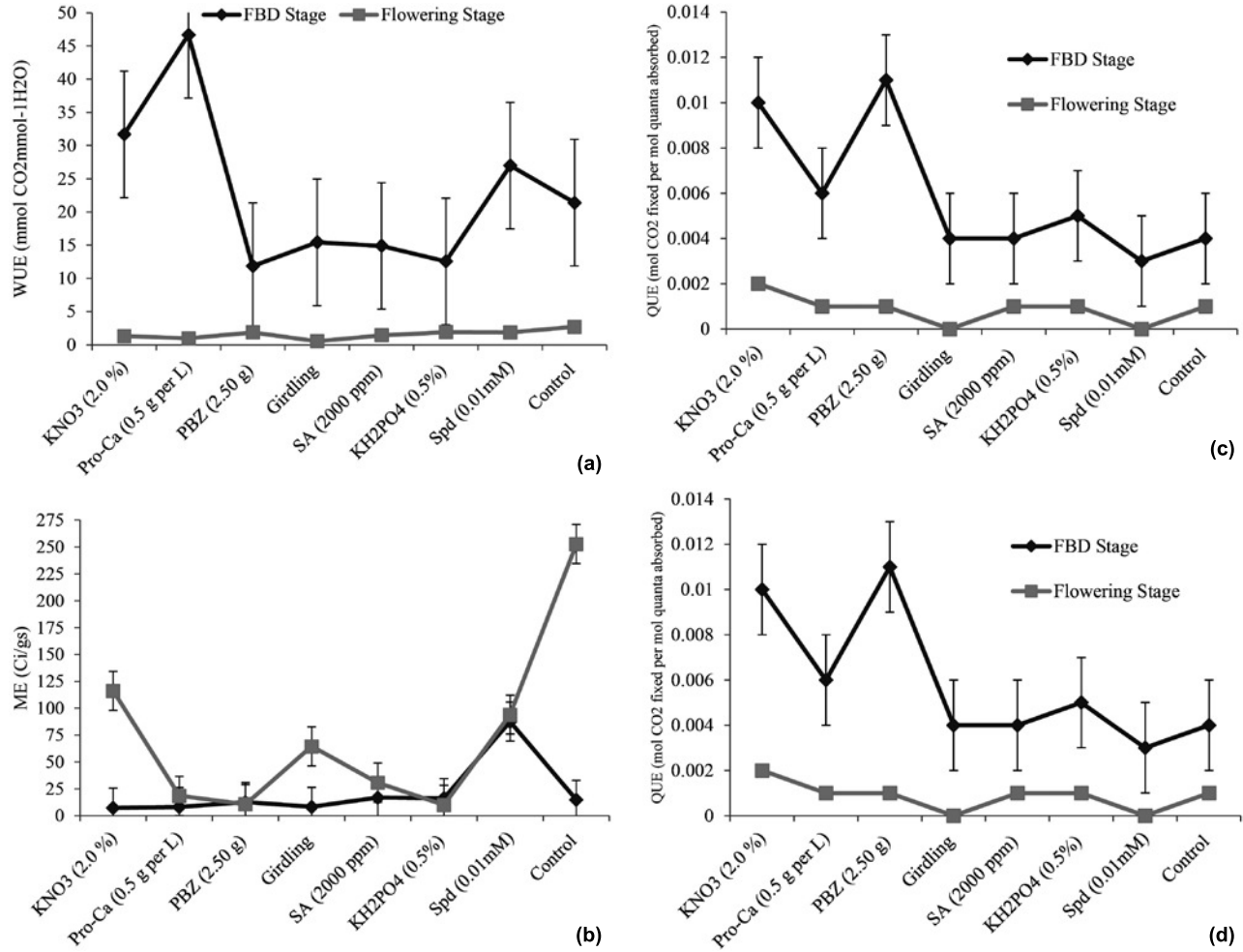


Fig. 1. (a) Leaf Water Use Efficiency; WUE (b) Mesophyll Efficiency; ME (c) Quantum Use Efficiency; QUE and (d) Carboxylation Efficiency; CE, affected by bearing regulated chemicals and girdling in litchi cv. China (Vertical bars are standard error (SE) of means).

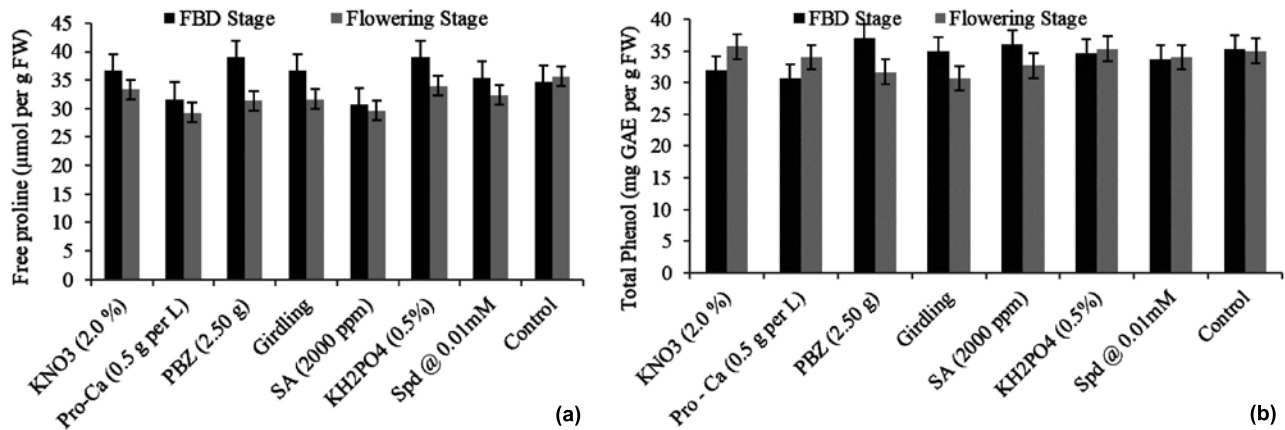


Fig. 2. Leaf proline (a) and total phenol content (b) affected by bearing regulated chemicals and girdling in Litchi cv. China [(vertical bars are standard error (SE) of means)].

Table 3. Flowering, fruit yield and colour intensity as affected by bearing regulating chemicals and girdling in litchi cv. China.

Treatment	Total no of flower/Panicle	No of fruits per tree	Yield Kg/Tree	Colour intensity*		
				L	A	B
Potassium nitrate (2.0 %)	3,656.00	2,427.66	53.34	36.77	20.02	13.23
Prohexadione-Ca (0.5 g per L)	681.67	2,006.66	33.97	33.75	17.28	12.16
Paclobutrazol (2.50 g)	80.00	40.667	0.77	34.72	28.07	11.32
Girdling	1,117.33	1,448.66	29.64	38.67	21.03	13.41
Salicylic acid (2000 ppm)	635.33	584.66	14.90	38.02	26.13	12.54
Potassium di-hydrogen phosphate (0.5%)	791.67	70.000	1.98	32.73	25.29	9.90
Spermidine (0.01mM)	157.33	67.66	1.13	37.44	27.57	12.47
Control	108.33	35.00	0.26	31.02	23.18	9.87
CD _{0.05}	324.32	898.29	13.18	4.38	4.63	1.75
SE(m)	105.90	293.31	4.305	1.50	1.59	0.60

Mean values of hue angle (L), luminosity (a), and chromaticity (b) of the peels on litchi fruits; L represent brightness and darkness; a* represent greenish to redness as the value increase (-greenness to + redness) and b* represent bluish and yellowish the lightness (-blueness to + yellowness),

nitrate probably acts by elevating nitrogen levels over a nitrogen threshold thereby synchronizing bud break from apices with existing floral initials.

The difference in pericarp colour intensity was improved a lot and all the values were recorded least in untreated trees (Table 3). The highest hue angle (L) [i.e., more red colour] was recorded after girdling and spray of SA. The poorest colour in terms of L and b was recorded in control trees. The chroma (b*) value of the fruits were highest in the girdled trees. The A value was maximum due to PBZ (28.07) followed by application of spermidine and SA.

Results of the present study revealed that the application of KNO₃, pro-Ca and practicing girdling was best for ensuring higher yield, better WUE, higher TCA, leaf area, net CO₂ assimilation rate with higher RWC, free proline and phenol content which ultimately improved the productivity of China litchi orchard. Pro-Ca can be better alternative to PBZ in litchi orchard but need some more investigation on yield and fruit quality regulating attributes.

AUTHORS' CONTRIBUTION

Conceptualization of research (SKS, SV, VN); Designing of the experiments (SKS, PK); Contribution of experimental materials (SKS); Execution of field/lab experiments and data collection (PK); Analysis of data and interpretation (PK, SKS, SV); Preparation of manuscript (SKS, PK)

DECLARATION

The authors declare that they have no conflict of interests.

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