Effects of $KClO_3$ and Girdling on Off-Season Flowering in Longan (*Dimocarpus longan* Lour.)

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Summary

Effects of potassium chlorate ($KClO_3$) and branch girdling on off-season flowering and changes of carbohydrates, total nitrogen and soluble protein contents in `Fenker´ longan trees during autumn was investigated. Result was shown that the application of $KClO_3$ (80 g/tree as soil drenching) and $KClO_3$ (80 g/tree) plus branch girdling were able to induce off-season flowering in longan.

$KClO_3$ plus girdling gave the best result on off-season flowering, girdling restricted vegetative flush and increased total soluble sugar and starch contents in shoots. The application of $KClO_3$ and $KClO_3$ plus girdling has positive effect on the increase in soluble protein and sucrose contents in shoots during floral initiation period. But it was found that $KClO_3$ treatment and $KClO_3$ plus girdling did not show any effect on total nitrogen content in shoot compared to untreated trees.

Introduction

Longan, like lychee, is a subtropical evergreen fruit tree and a member of Sapindaceae. Longan requires low temperature (below 22˚C) approximately 10 weeks for floral induction during cool season (Nakasone and Puall, 1998). Thus, a main problem in production is an irregular bearing (Choo, 2000) due to the fluctuation of temperature during cool season particularly in the tropical zone of Thailand. Since, a discovery of off-season floral induction in

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longan by potassium chlorate ($KClO_3$) (Manochai *et al*., 1999), this problem is solved by this chemical. $KClO_3$ could be applied as soil drenching at 8 g /m^2 (Manochai *et al*., 1999) or by foliar application at 1,000 ppm (Sritontip *et al*., 1999) for floral induction which leaf age should be grown in maturity stage (Hegele *et al*., 2004; Manochai *et al*., 2005). $KClO_3$ was not able to induce off-season flowering in longan when applied at young leaf stage. In addition, the earlier vegetative flush which occurred immediately before or after the application of $KClO_3$ also inhibited or reduced flowering in longan. This evidence is a serious problem for off-season production in Thailand during rainy season because longan trees are easy to produce new vegetative flush lead to less achievements of off-season production in longan by $KClO_3$ treatment.

Girdling has been reported on the reduction of vegetative growth in many fruit trees for example avocado (Ibrahim and Bahlool, 1979) and mango (Rabelo *et al*., 1999), and the increase of flowering or fruiting in citrus (Koller *et al*., 2000) and litchi (Menzel and Paxton, 1986). Although this technique is not widely used in longan production, it may improve flowering in off-season production.

Therefore, this study was conducted to investigate effects of $KClO_3$ and girdling on changes of carbohydrates, total nitrogen, soluble protein contents and off-season flowering in longan and hypothesize that these results may be beneficial on off-season production in longan leave as it is.

Materials and methods

1. Plant materials

This study was conducted at the Grape Center, National Chung Hsing University, Taichung, Taiwan, in 14-year old ˋFenkerˊ longan trees . Twenty trees with 3 m height and 3.4 m in diameter were selected in late September, 2009. Shoot pruning and a 200 g/tree of composite fertilizer (15N-15P_2O_5-15K_2O) were performed in August, 2009.

2. Methods and experimental design

Completely randomized design with 5 replications and 4 treatments was used in this study. Treatments were; (1) untreated control tree, (2) 80 g/tree $KClO_3$ (KC) by soil application, (3) spiral branch girdling (G), and (4) KC+G. The application of $KClO_3$ and spiral branch girdling were carried out on October 24, 2009. Fifty shoots per tree were selected for data collection and plant analyses. Leaf flushing, flowering and shoot growth were monitored weekly after treatment until flowering. Tagged shoots (10 cm in length) were collected weekly after treatment until flowering for determination of total soluble sugar, starch, total nitrogen and soluble protein content. For statistical analysis, the analysis of variance (ANOVA) and the comparison of treatment means with LSD ($P<0.05$) were analyzed by SAS program.

3. Plant analysis

3.1. Determination of total soluble sugar, starch, and sugar

For determination of total soluble sugar and starch content, shoots were dried and grounded, using a Willey Mill (40-mesh sieve). For analysis, the extraction and determination of total soluble sugar and starch were performed as described by Dubois *et al.* (1956). The absorbance values of sample solutions were read at a wavelength of 490 nm by a spectrophotometer. Standard curve was made with various concentrations of glucose solution.

For sugar analyses, dried sample (0.1 g) was extracted two times by 80% ethanol which each time added with 5 ml 80% ethanol and incubated in water bath at 70˚C for 20 minutes. Then, the sample solution was centrifuged at 4000 $\times g$ for 15 minutes and filtered through the Mira-cloth membrane. After the last extraction, supernatant was evaporated with vacuum evaporator (VAPOUR-MIX KC-12) at 50˚C. The concentrated sample was diluted with distilled water to 1 ml then, filtered through a 0.45 µm filter (Millex, Millipore). A glucose, sucrose and fructose standards were prepared at the same various concentration including 0, 0.05, and 0.2%. Both sugar standard and the filtrated samples were prepared in 1-ml syringe and injected into the HPLC column [Mightysil RP-18 GP 250-4.6 (5µm)] for analysis by using Hitachi HPLC System equipped with UV Detector L-7400 Model, Hitachi pump L-7100; and Hitachi D-2000 Chromato-integrator.

3.2. Determination of total nitrogen (Micro-Kjeldahl method)

Total nitrogen in dried shoots was determined by the Micro-Kjeldahl method described by Bradstreet (1965). In the process, 0.2 g of dried shoot sample was placed in a digestion tube, following by adding 1 g of catalytic agent (Merck 8030) and 4.5 ml of H_2SO_4 to the tube. The digestion tubes were then placed on the digestion apparatus, and digested at 410 ℃ for 2 hours. After the samples cooling to room temperature, 15 ml of distilled water and 20 ml of 12 N NaOH were added to the tube and transferred to device. The ammonia vapor were collected in 20 ml of indicator (19 M bromocresol green and 25 M methyl red in 2% boric acid) and titrated with 1/14 N H_2SO_4.

Results

This study has shown that shoot growth in length did not affect by any treatments within

35 days after treatment (Table 1). Thereafter, shoot length on 49th day after treatment of $KClO_3$ treated trees increased and greater than that in other treatments while the increase of shoots length in girdle trees were lowest.

The percentage of budbreak in $KClO_3$ treated tree (99%) was significantly higher than that in other treatments (Table 2). Girdling inhibited budbreak in longan (0%) therefore this result in $KClO_3$ plus girdling treated trees appeared the lower value than that in trees that treated $KClO_3$ alone. Percentage vegetative flush in $KClO_3$ treated tree was with highest value (30%) compared to untreated tree (12%). In contrast, it did not appear in girdle trees and $KClO_3$ plus girdling treated trees.

Table 1. Effects of $KClO_3$ and girdling on shoot growth of `Fenker´ longan tree in different time .

(mm)

Treatment	Day after treatment			
	7	21	35	49
Untreated	0.48a [z]	0.45a	0.25a	0.74c
$KClO_3$	0.08a	1.48a	2.42a	8.08a
Girdling	0.40a	1.09a	0.16a	0.34c
$KClO_3$+girdling	0.01a	0.30a	0.67a	3.65b

[z] Means are separated within columns by LSD test at $p \leq 0.05$.

Table 2. Effects of $KClO_3$ and girdling on off-season flowering and vegetative flush in `Fenker´ longan trees.

Treatment	Budbreak (%)	Vegetative flush (%)	Flowering (%)			Day to flowering
			Leafless panicle	Leafy panicle	Total	
Untreated	12c[z]	12b	0c	0b	0b	—
$KClO_3$ (KC)	99a	30a	37b	32a	69a	43.8
Girdling (G)	0c	0b	0c	0b	0b	—
KC+G	73b	0b	63a	10b	73a	46.4

[z] Means are separated within columns by LSD test at $p \leq 0.05$.

The application of $KClO_3$ and $KClO_3$ plus girdling induced off-season flowering with the percentage of flowering 69 % and 73 %, respectively. Although total flowering between $KClO_3$ treated trees and $KClO_3$ plus girdling treated trees was not significantly different, leafless panicle percentage was significantly different in $KClO_3$ plus girdling treated trees and higher than that in $KClO_3$ treated ones. However, both girdle trees and untreated trees did not show flowering. The first visible flowering occurred around 43.8 days (On December 7) after treatment for $KClO_3$ treated trees and 46.4 days (On December 9) after treatment for $KClO_3$ plus girdling treated trees, but the natural longan flowering in Taiwan occurred around early February (normal season).

Total soluble sugar in shoots of girdle trees showed highest level from 7th day to 21st day compared to other treatments (Table 3). Similarly, total soluble sugar in trees treated with $KClO_3$ plus girdling also increased on 7th day after treatment (10.86 %) greater than untreated trees (8.05 %), whereas, $KClO_3$ treatment only tended to increase total soluble sugar on 7th day after treatment (9.10 %) higher value than untreated one. The trend for total soluble sugar content in shoots of $KClO_3$ treated trees, girdle trees, and $KClO_3$ plus girdling treated trees was increased on 7th day thereafter decreased gradually from 14th day to 21st day after treatment. The change of total soluble sugar in untreated trees seemed to keep constant level from the beginning to 21st day.

Table 3. Effects of $KClO_3$ and girdling on total soluble sugar content in shoots of `Fenker´ longan trees.

(dw %)

Treatment	Day after treatment			
	0	7	14	21
Untreated	6.76a AB^{z}	8.05bA	7.36b AB	6.01abB
$KClO_3$	6.75a BC	9.10bA	7.52b B	5.49 b C
Girdling	6.94a C	10.96aA	9.25a B	6.42 a C
$KClO_3$+girdling	6.66a BC	10.86aA	7.61b B	5.94abC

^{z} Means are separated within columns by LSD test at $p \leq 0.05$.

Girdling did not increase sucrose and glucose contents in shoots on 21st day after treatment compared to untreated trees (Table 4). In contrast, $KClO_3$ alone or $KClO_3$ plus girdling treatment increased sucrose content in shoots on 21st day higher than that in both of girdle trees and untreated trees. In addition, glucose and fructose contents in shoots of trees treated with $KClO_3$ was also increased on 21st day and greater than those in girdle trees and untreated ones. However, contents of sucrose, glucose and fructose on 35th day after treatment were not significantly different among treatments.

Starch content in shoots of girdle tree was with highest level on 7th day (4.1 %), and followed by on 35th day (3.45 %) after treatment (Table 5). During 7th day to 21st day after treatment, $KClO_3$ or $KClO_3$ plus girdling treatment did not show any affect on starch content. However, $KClO_3$ plus girdling showed increase starch content on 35th day after treatment (2.53 %) higher than that in untreated trees (1.71 %) while starch content in $KClO_3$ treated trees (2.42 %) tended to increase higher than untreated ones. The trend for starch contents in each treatment had a similar result that starch content seemed to increase on 14th day after treatment.

Table 4. Effects of $KClO_3$ and girdling on the contents of sucrose , glucose and fructose in shoot of `Fenker´ longan trees.

(dw %)

Treatment	Day after treatment					
	21			35		
	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose
Untreated	0.17b[z]	0.23bc	0.83b	0.22a	0.25a	0.84a
$KClO_3$	1.04a	0.41ab	0.89b	0.72a	0.26a	0.66a
Girdling	1.14b	0.18c	0.64c	0.61a	0.29a	0.69a
$KClO_3$+girdling	1.22a	0.47 a	1.09a	0.75a	0.29a	0.75a

[z] Means are separated within columns by LSD test at $p \leq 0.05$.

Table 5. Effects of $KClO_3$ and girdling on starch content in shoots of `Fenker´ longan trees.

(dw %)

Treatment	Day after treatment				
	0	7	14	21	35
Untreated	3.02aB[z]	3.13bAB	3.66aA	2.78a B	1.71c C
$KClO_3$	2.63aB	2.99bAB	3.69aA	2.83aAB	2.42a B
Girdling	2.86aC	4.10aAB	4.46aA	3.19aBC	3.45aBC
$KClO_3$+girdling	2.92aB	3.03b B	3.95aA	3.10a B	2.53b B

[z] Means are separated within columns by LSD test at $p \leq 0.05$.

As shown in table 6, total nitrogen content in shoots did not affect by any treatments throughout an experiment with the average level between 0.57 to 0.81 %.

Soluble protein content in shoots of $KClO_3$ treated trees (37.06 mg/g fw) and $KClO_3$ plus girdling treated trees (31.61 mg/g fw) increased on 35th day after treatment and higher than that in girdling alone (21.66 mg/g fw) and untreated trees (18.81 mg/g fw), respectively (Table 7). However, there was no significant difference among treatments on 21st day after treatment.

Table 6. Effects of $KClO_3$ and girdling on total nitrogen content in shoot of `Fenker´ longan trees.

(dw %)

Treatment	Day after treatment		
	0	7	21
Untreated	0.57a^z	0.70a	0.71a
$KClO_3$	0.57a	0.68a	0.64a
Girdling	0.58a	0.81a	0.66a
$KClO_3$+girdling	0.61a	0.65a	0.67a

^{z} Means are separated within columns by LSD test at $p \leq 0.05$.

Table 7. Effects of $KClO_3$ and girdling on soluble protein content in shoots of `Fenker´ longan trees.

($mg \cdot g^{-1}$ fw)

Treatment	Day after treatment	
	21	35
Untreated	24.22a^z	18.81b
$KClO_3$	23.96a	37.06a
Girdling	26.38a	21.66b
$KClO_3$ + girdling	22.67a	31.61a

^{z} Means are separated within columns by LSD test at $p \leq 0.05$.

Discussion

Girdling treatment restricted vegetative flush in longan trees agree with previous reports in many fruit trees (Allan *et al*., 1993; Rabelo *et al*., 1999) lead to without budbreak in longan during the experiment. The reduction of vegetative flush, shoot growth, and budbreak may be caused by girdling restricted auxin transport as well as photosynthesis transport in phloem from shoots and leaves to roots (Goren *et al*., 2004). However, girdling alone did not induce off-season flowering in longan while $KClO_3$ or $KClO_3$ plus girdling induced off-season flowering in longan. The combination of $KClO_3$ and girdling improved flowering by increase of leafless panicle and flowering percentage compared to single treatment of $KClO_3$ that was due to girdling had effect on the inhibition of vegetative flush during flower initiation. Previously, Shao *et al*. (1998) reported that girdling increased flowering intensity in citrus which supported the result in this study. Although the girdling improved off-season flowering following $KClO_3$ treatment by the increase of leafless panicle, flowering percentage, and the reduction of earlier vegetative flush (within a few day after $KClO_3$ treatment), the restriction of budbreak after applying $KClO_3$ for 21 days was negative effect for off-season production. Because it could be reduced panicle number and delayed flowering time.

Girdling treatment was able to increase total soluble sugar content in shoots from 7th day to 21st day after treatment. Similarly, the combination of $KClO_3$ with girdling also increased total soluble sugar on 7th day after treatment. However, the inconsistence result of total soluble sugar content within tree after applying $KClO_3$ was due to the high variation in big trees.

Girdling increased the starch content with a peak on 7th day and 35th day after treatment. Therefore, the combination of $KClO_3$ with girdling also increased starch content in shoots higher than untreated trees on 35th day after treatment which this result was corresponding with floral initiation period. However, starch content in $KClO_3$ treated trees was not consistent. It suggested that girdling did not promote off-season flowering following $KClO_3$ treatment directly but it act as indirect effect on the accumulation of carbohydrates for flower induction process.

Interestingly, the application of $KClO_3$ alone or $KClO_3$ plus girdling clearly increased sucrose content in shoots during flower induction period higher which was than the girdling and untreated trees (none flowering). Although the results of total soluble sugar and starch in $KClO_3$ treated trees were not consistent, it was considered that carbohydrate is important for flower induction in tropical/subtropical fruit trees (Bower *et al*., 1990). In case of carbohydrates was supported with previous reports which noted that there was increase of total nonstructural carbohydrate and reducing sugar in shoots of longan during floral initiation after $KClO_3$

treatment in off-season (Kiatsakun and Tansuwan, 2004; Wangsin and Pankasemsuk, 2005). Moreover, sucrose content increased in all $KClO_3$ treated trees in this study may act as flowering signal similar to the result in adult mustard (*Sinapis alba*) (Bernier *et al*., 1993) which further studies need to conduct to clarify this evidence.

For total nitrogen in shoots, all treatments did not affect on the change of total nitrogen. It is indicated that girdling did not restrict nitrogen uptake within roots from soil. In addition, $KClO_3$ also did not induce flower induction in longan by the decrease of nitrogen in tree, although chlorate has been reported as analog of nitrate and a competitive inhibitor of nitrate reduction reaction (Nakagawa and Yawashita, 1986).

With a change of soluble protein found that soluble protein content in shoots of $KClO_3$ treated trees or trees treated with $KClO_3$ plus girdling (flowering) clearly increased higher than girdle trees and untreated trees (none flowering) during floral initiation period on 35th day after treatment. The increase of soluble protein in this period may associate on flowering process as flowering protein. However, there was no significant difference in the soluble protein content on 21st day after treatment during floral induction period. It was suggested that flowering protein was started to synthesize in leaves of longan (Sinlapasomboon and Pankasemsuk, 2006) thereafter transported to terminal shoots and functions during flower initiation period.

In conclusion, $KClO_3$ treatment induced off-season flowering in longan by its effect on changes of carbohydrates particularly sucrose which may act as flowering signal. Girdling can enhance off-season flowering induction by $KClO_3$ treatment had the effect on the reduction of vegetative flush, and the increase of total soluble sugar and starch content may be associated with flowering process in longan.

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References

Bernier, G., A. Havelang, C. Houssa, A. Petitjean, and P. Lejeune. 1993. Physiological signal that induce flowering. Plant Cell 5: 1147-1155.

Bower, J. P., C. J. Lovatt, J. G. M. Cutting, and M. M. Blanke. 1990. Interaction of plant growth regulation and carbohydrate in flowering and fruit set. Acta Hort. 275: 425-433.

Bradstreet, R. B. 1965. The Kjeldahl method for organic nitrogen. Academic Press. Inc. New York, USA.

Choo, W. K. 2000. Longan Production in Asia. FAO regional office for Asia and the Pacific, Bangkok, Thailand.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

Goren, R., M. Huberman, and E. E. Goldschmidt. 2004. Girdling: Physiological and horticultural aspects. Hort. Rev. 30: 1-25.

Hegele, M., D. Naphrom, P. Manochai, A. Chattrakul, P. Sruamsiri, and F. Bangerth. 2004. Effect of leaf age on the response of flower induction and related hormone changes in longan trees after $KClO_3$ treatment. Acta Hort. 653: 41-49.

Ibrahim, I. M. and S. E. D. Bahlool. 1979. The effect of girdling on flowering, fruiting and vegetative growth of avocado trees. Agr. Res. Rev. Hort. 57: 55-66.

Kiatsakun, D. and T. Tansuwan. 2004. Effect of potassium chlorate on nitrogen, phosphorus, potassiumand plant hormones content in longan roots. Kaset J. (In Thai) 20(1): 32-43.

Koller, O. L., E. Soprano, A. C. Z-da Costa, O. C. Koller, and O. K. Yamanishi. 2000. Flowering induction and fruit production in orange cv. `Shamouti´. Laranja 21: 307-325.

Leonardi, J., S. J. Blaikie, W. J. Muller, N. S. Scott, and E. K. Chacko. 1999. Effect of cincturing and chemical treatment on growth, flowering and yield of mango (*Mangifera indica* L.) cv. `Kensington Pride´. Aus. J. Exp. Agr. 39: 761-770.

Manochai, P., W. Suthon, W. Wiriya-alongkorn, N. Charasamrit, and S. Ussahatanon.1999. Effect of potassium chlorate on flowering of longan cvs. `E-Daw´ and `See Chompoo´. p.1-8. Plant Hormone for Off-Season Production in Fruit Crops Symp., Chantaburi, Thailand.

Manochai, P., P. Sruamsiri, W. Wiriya-alongkorn, D. Naphrom, M. Hegele, and F. Bangerth. 2005. Year around off season flower induction in longan (*Dimocarpus longan* Lour.) trees by $KClO_3$ application: potential and problem. Scientia Hort. 104: 379-390.Menzel, C.M. and B.F. Paxton. 1986. The effect of cincturing at different stages of vegetative flush maturity on the flowering of litchi (*Litchi chinensis* Sonn.). J. Hort. Sci. 61: 135-139.

Nakagawa, H. and N. Yamashita. 1986. Chlorate reducing activity of spinach nitrate reductase. Agric. Biol. Chem. 50:1893-1894.

Nakasone, H. Y. and R. E. Puall. 1998. Tropical Fruits. CABI Pub., HI, USA. p. 173-207.

Shao, L. H., L. Deng, and L. Y. Qing. 1998. Effects of floral promotion or inhibition treatments on flowering of citrus trees and protein fraction in buds. J. Trop. Subtrop. Bot., 6: 124–130.

Sinlapasomboon, W. and T. Pankasemsuk. 2006. Change of protein in longan leaves before flowering induced by potassium chlorate. Kaset Journal (In Thai) 22: 49-51.

Sritontip, C., Y. Khao-Sumeru, and S. Changenraja. 1999. Effect of potassium chlorate on flowering in longan cv. E-Daw. p. 30-37. Plant Hormone for off-season Fruit Crop Sym. Chantaburi, Thailand.

Sritontip, C., Y. Khaosumain, S. Changjaraja, and R . Poruksa. 2005. Effect of potassium chlorate, sodium hypochlorite and calcium hypochlorite on flowering and some physiological changes in `DO´ longan . Acta Hort. 665: 269-273.

Wangsin, N. and T . Pankasemsuk. 2005. Effect of potassium chlorate on flowering , total nitrogen, total nonstructural carbohydrate , C/N ratio and contents of cytokinin -like and gibberrellin-like substances in stem apex of `DO´ longan . Acta Hort. 665:255-258.

氯酸鉀及環刻對龍眼 (*Dimocarpus longan* Lour.) 開花調節之影響

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關鍵字：龍眼、氯酸鉀、環刻、開花調節

摘要：本研究探討氯酸鉀($KClO_3$)及主枝環刻對'粉殼'龍眼開花調節之影響及其母梢碳水化合物全氮含量之變化。結果顯示，在秋季施用氯酸鉀(80g/株)或氯酸鉀加環刻均能有效地誘使龍眼開花。

氯酸鉀加環刻呈現最佳的結果，環刻不僅能抑制營養生長並增加母梢的全可溶性糖及澱粉含量，另外亦發現其若配合氯酸鉀處理，在花芽分化期間會促進母梢可溶性蛋白質及蔗糖含量之作用。但是氯酸鉀或環刻處理並未影響母梢之全氮含量。

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